FINAL REPORT
OF SERDP PROJECT #770

SERDP Title: Development of Military IRIS System for the Hazard Identification and Risk Assessment/Characterization of Defense Related Pollutants

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Background

The Environmental Risk Assessment Program (ERAP) is a cooperative effort between the Department of Defense, Department of Energy, and the U.S. Environmental Protection Agency. The goal of the program is to improve the scientific methods and models for the performance and application of risk assessments. The project involved joint scientific reviews of pollutants of mutual concern and the development of new and improved methodologies for assessing risks related to federal facilities.

The Advisory and Coordinating Committee (ACC) along with three working groups; Material/Chemical Risk Assessment (MCRA) Working Group; Human Risk Assessment Methodology (HRAM) Working Group; and Ecological Risk Assessment Methodology (ERAM) Working Group were established in support of the ERAP.

The ACC is an interagency committee that serves as the direct oversight and communication link with the Working Groups and senior DOD, DOE, and U.S. EPA management. They serve as overall coordinators of SERDP/ERAP projects and research in the risk sciences, they prioritize and approve Working Groups' deliverables, and they nominate/select members of the Working Groups.

The MCRA Working Group's activities involve developing toxicity risk assessment values for materials and chemicals of mutual concern to DOD, DOE and EPA using alternate methodologies to current procedures when appropriate; developing uncertainty analyses of toxicity values; identifying data gaps and research needs; and the dissemination of toxicity values to the risk assessment community after appropriate peer review.

MCRA Working Group Goals:

- validate and approval of TNT cancer assessment
- finalize RfD for TNB
- finalize the TCE assessment pending outcome of Benchmark Dose Methodology evaluation
- develop and then evaluate the recently prioritized chemical agents (sulfur and nitrogen mustards, VS, Lewisite, cyanogen chloride, GA and GB)
- place additional materials/chemicals into the development pipeline for RfDs, RfCs and Cancer Assessments

Assessment of Environmental Hazards of 1,3,5-Trinitrobenzene - Attachment 1

The following preliminary drafts were completed by the MCRA Working Group: - Attachment 1 Toxicity Assessment for Cyanogen Chloride (CK); Diaminonitrotoluene; Ethylene Glycol (EG); JP-8, Lewisite; Nitrogen Mustard (HN2); The Nerve Agent Sarin (GB); The Nerve Agent Soman (GD); Sulfur Mustard (HD); Thiodiglycol (TDG)
Cancer Assessment of TNT (2,4,6-Trinitrotoluene

The HRAM Working Group's activities involve the review and evaluation of methodologies currently used in the human risk assessment process, developing strategies for methodology changes or alternative methods where appropriate; demonstrating the use of new methods with a selected list of candidate materials or chemicals; and recommending the incorporation of improved methodologies into the MCRA evaluation process when ready, and after appropriate peer review and publication.

HRAM Working Group Goals:

- establish a position regarding the consistent application of Benchmark Dose Methodology for application a Federal Facilities
- selection of curve-fitting procedures (e.g. logit, probit, etc.)
- selection of effect levels of concern
- selection of confidence levels
- develop methods addressing current data gaps or deficiencies in current methods

"Review of Human Health Risk Assessment Guidelines and Methodologies" - This report is a product of the HRAM Working Group and presents issues concerning risk assessment guidelines and methodologies established for evaluating cancer and noncancer hazards due to exposure to environmental substances. (Attachment 2)

The ERAM Working Group's activities involve the review and evaluation of methodologies currently used in the ecological risk assessment process; developing strategies for methodology changes or alternative methods where appropriate; demonstrating the use of new methods with a selected list of candidate materials or chemicals; and recommending the incorporation of improved methodologies into the MCRA evaluation process when ready, and after appropriate peer review and publication.

ERAM Working Group Goals:

- produce an inventory of recently evolving ecological risk methods
- establish and verify new methods in ecorisk as the needs arise
- establish data quality criteria, lists of indicator species, biomarkers for use in ecological risk assessments
- develop and/or evaluate models and assess uncertainties
- characterize specific ecosystems that are at risk

"Tri-Service Procedural Guidelines for Ecological Risk Assessments" - This report provides guidance for conducting ERAs for use by risk assessors at Navy, Air Force, and Army installations. (Attachment 3)

Fiscal Year 1991 Appropriations:

Funding was placed in an Interagency Agreement (# DW89935192-01) with the Dept. Of Energy, Oak Ridge Field Office

Evaluation of Toxic Effects and Development of Methodologies for Risk Assessment of Environmental Pollutants

Work and Accomplishments

Work related to the direct development and evaluation of material/chemical risk assessments, and development and evaluation/validation of risk assessment methods. These activities involve the use of SERDP IAG funds for support of working groups and development/defense of technical files/reports along with the donation of substantial FTE and resources of the Army, Navy, Air Force, DOE and EPA.

SERDP funds have been used through this IAG for the following work/accomplishments:

- A software precipitation data file preprocessor was developed for COMPDEP (version 93252), which are currently both available on the SCRAM Bulletin Board. The preprocessor was developed to convert precipitation data in the NCDC files into a format that could be used with COMPDEP. The output file will contain hourly precipitation data for a single selected year and site.

- Development/Application to Methodology for Assessing Health Risks Associated with Indirect Exposure to Combustor Emissions (EPA/600/6-90/003). The modeling of human exposure to pollutants emitted by combustion processes is an important element of risk assessments for combustors. The only guidance document, prior to this effort, was the US EPA 1990 Methodology Document on Indirect Exposure. At the completion of this task, guidance on predictive modeling for the atmospheric fate and transport of mercury should be available. Additionally, guidance on terrestrial and aquatic fate and transport of mercury as well as human and wildlife exposure should be available. The enhancements to the original models will be made publicly available. The models can be applied, after review by the HRAM and ERAM Working Groups, to any DOD/DOE or Superfund Site that utilizes combustion in clean-up.

Fiscal Year 1992 Appropriations:

Funding was used in-house for travel and placed in 1 contract, 1 Cooperative Agreement, 1 Interagency Agreement and 1 NRC Research Associateship.

Contract with A.T. Kearney

Work and Accomplishments

Funds were used to co-fund the development of the new air dispersion and deposition model, Industrial Source Complex 3 (ISC3). The intended uses include prediction of air concentrations and deposition estimates from air emissions sources.

Supplemental funding for Cooperative Agreement (CR817618) - Syracuse Research Corporation

Work and Accomplishment

Funds were used to co-fund ongoing investigations of generalized additivity models and methods for detecting additivity using graphical and statistical indicators. This work complements the Superfund supported research on additivity models but is oriented toward robustness and general applicability to diverse mixtures, including those with sparse data as is expected with mixtures of interest to the SERDP program. Several reports, presentations and journal articles have been completed under this cooperative agreement with only a small portion funded by SERDP.

Interagency Agreement (DW57936172-01-0) - Dept. of the Air Force, Toxicology Division, OEHD, Armstrong Laboratory, AL/OET, Wright Patterson AFB, OH

Interagency Environmental Health Council for the Development of Risk Assessment Research and Risk Characterization of Defense Related Issues and Site C

Work and Accomplishments

The work and accomplishments under this SERDP funded agreement are on 4 major projects: Total Petroleum Hydrocarbons (TPH); Perchlorate; Trichloroethylene (TCE); and Jet Fuel #8 (JP-8).

1. The TPH project co-supports the TPH Criteria Working Group, a consortium of government agencies, industry and academia to establish a realistic cleanup standard for TPH.
    - Technical support to the Analytical Technical Action Group of TPH Criteria Working Group.

- First TPH platform session at the annual Soils Conference, Amherst MA, 1/95, was partially funded with this SERDP effort.
- Report on TPH toxicity.
- Further follow-on studies have been proposed for assessing bioavailability and soils studies of weathered soils

2. The Perchlorate project is directed in support of the Perchlorate Study Group (PSG), a consortium of Air Force, EPA and industry to identify a NOEL for ammonium perchlorate. The current provisional RfD is below the detection limit. This is a joint effort with SERDP, Army and Air Force research funding.
   - Two PSG meetings have been held to discuss final details of a cooperative research agreement
   - Presented "Study design for the Toxicity Evaluation of Ammonium Perchlorate" at the Joint Army, Navy, NASA and Air Force (JANNAF) meeting 7/13/95 on Environmentally Sound Processing Technology

3. The TCE project is partially supported by this SERDP effort, as well as other SERDP and Air Force research funding. This is a major research project for the Armstrong Laboratory. Eleven poster presentations were given at the Society of Toxicology meeting in Baltimore, MD 36-9/95 as a result of the current jointly funded efforts.
   - Documentation of current information on non-cancer TCE risks were developed and supported under this project.

4. The JP-8 project is investigating the toxicity of JP-8 as DOD converts from JP-4 fuels to JP-8. This is a joint effort with funding also from the Navy.
   - Two documents have been prepared with partial SERDP support
   - A manuscript on 90-day oral gavage study in male rats dosed with JP-8 was prepared and submitted to Toxicology and Industrial Health journal 4/95
   - A poster was presented at the Society of Toxicology Meeting , Baltimore, MD 3/6/95
   - A manuscript on the teratogenic effects of JP-8 was accepted by the Journal of Applied Toxicology
   - Toxicity study of JP-8 plus additives was accepted and began 7/95

National Research Council Research Associateship(#CR815418) - Health Effects Research Laboratory/EPA, RTP

Evaluation of the Additivity Assumption for PAHs Using Strain A/J Mouse Lung

Work and Accomplishments

The objective of this project was to examine the validity of the additivity assumption f or carcinogenic risk using an in vivo mouse lung tumor model. The additivity assumption was tested in lung target tissues using tumor formation and DNA adducts profiles as endpoints and

using PAHs (both carcinogens and non-carcinogens) identified in DOD and Superfund Waste sites and from incinerator emissions.

SERDP funds were used to hire a postdoctoral fellow through the National Research Council Resident Associateship Program. Dr. Ken Strothcamp of Drew University was hired as a one year senior fellow to perform the lung DNA adduct mixture profile studies using the 32P-postlabeling assay.

The results of these studies will be directly applicable to the DOD site assessment process. If the results of these studies support the additivity assumption, then they will provide the scientific underpinnings for the use of additivity in cancer risk assessment for those classes of chemicals studied. If the results of these studies do not support the additivity assumption then they will provide the extent of the deviation from additivity to establish a plausible upper bound of uncertainty for the assessments.

Fiscal Year 1993 Appropriations:

Funds were used in-house for travel and placed on 1 contract, 3 Interagency Agreements and 6 Cooperative Agreements.

Contract with Syracuse Research Corporation

Work and Accomplishments

SERDP funds partially co-funded the development of the Methodology for Assessing Total Exposure to Combustor Emissions and the accompanying computer software.

Interagency Agreement (DW89936494-01-0) - Department of Energy, Air, Water and Radiation Division, Washington, DC

Interagency Environmental Health Council for the Development of Risk Assessment Research and Risk Characterization of Defense Related Issues and Site Clean-up

Work and Accomplishments

- "Methods and Tools for Estimation of the Exposure of Terrestrial Wildlife to Contaminants" - This report presents methods for estimating exposure of terrestrial wildlife to both chemical and radionuclide contaminants. (Attachment 4)

- Effects of Ionizing Radiation on Terrestrial Plants and Animals" - A Workshop Report (Attachment 4)

- "Ecological Screening Benchmarks Database" - Database containing benchmarks for aquatic organisms, wildlife, and sediments. Development of a computer down-

loadable DOS-based version and, most recently, making this database available interactively through the Office of Environmental Policy and Assistance (OEPA) web site. (Attachment 4)

Interagency Agreement (DW17936495-01-0) - Dept of the Navy, NCCOSC, RDTE Division, Marine Environmental Branch, San Diego, CA

Development of Methods/Procedures for Identifying, Characterizing and Assessing Potential Risks and Exposures to Aquatic Ecosystems Resulting from Defense Related Activities at Naval Bases and Shipyards

Work and Accomplishments

Studies:

- Benthic flux chamber modified to optimize sampling and physical-chemical control systems
- Range finding experiments conducted using the "comet" bioassay for quantifying DNA damage and repair and apoptosis levels in mussel tissues
- Intensive characterization of the six study sites including mapping of metals in bulk sediment, water column mapping of Zn and Cu, sediment flux measurements, and surveys using the REMOTS camera system to assess species diversity variations
- Transplantation of bivalves to collect bioaccumulation, growth and biochemical biomarker measurements. Field study began 6/16/95 and scheduled to end 7/31/95.

Interagency Agreement (DW17936493-01-0) - Dept. of the Navy, Naval Medical Research Institute Detachment (Toxicology), Wright Patterson AFB, OH

Interagency Environmental Health Council for the Development of Risk Assessment Research and Risk Characterization of Defense Related Issues and Site Clean-up"

Work and Accomplishments

- Support for NMRI/TD involvement in the MCRA and RAM Working Groups
- Support for on going and future work on TPH Criteria Working Group involving a consortium of government agencies, industry and academia to establish realistic clean-up standards for TPH in soils. Cooperative research with the Air Force's Armstrong Laboratory on JP-8 jet fuels and additive packages.
- Support for augmentation of in-house FTIR-spectrophotometer to be used for sample analyses in TPH and CFC replacement inhalation studies.

Cooperative Agreement (CR822757-01-0) - Colorado School of Mines

"Estimating the Absorbed Dose from Dermal Exposure to Environmental Pollutants: Development of Guidelines for the Acquisition, Interpretation and Use of *In Vivo* and *In Vitro* Data". The goal of the proposed research is to develop a consistent and scientifically base method for computing realistic estimates of absorbed dose from dermal exposure.

Work and Accomplishments - Attachment 5

Cooperative Agreement (CR822793-01-0) - Columbia University

"Human Bioavailability of Lead in Using Stable Isotope Dilution" Human volunteers would be used in a controlled study to determine extent to which soil type determines bioavailability of lead.

Work and Accomplishments - Attachment 6

- Soil characterizations for lead concentrations and stable lead isotopes in a number of soils have been completed
- Soils with the most promising stable isotope profiles have been analyzed for other trace elements and 200 priority pollutants to meet the requirements of the Research Risk In Human Subjects Review Board at Columbia University
- First test subject studies begun on 7/18/95 with the projected completion of clinical studies by 9/3095
- One manuscript has been submitted to Environmental Health Perspectives

Cooperative Agreement (CR822771-01-0) - Harvard School of Public Health

"Tumor Sites and Toxic Lesions: Concordance Across Sites and Species" - The objective of the proposal is to critically examine correspondence of tissue toxicity and tumor formation using data from the U.S. National Toxicology Program (NTP) Bioassays.

Work and Accomplishments - Attachment 7

This work entails the evaluation of any correlations between non-cancer organ toxicity and tumorigenicity. NTP/NCI cancer bioassay data are being used for these comparisons.

- "An Empirical Examination of Factors Influencing Prediction of Carcinogenic Hazard across Species" - Attachment 7

- "Anticarcinogenic Responses in Rodent Cancer Bioassays are Not Explained by Random Effects" - Attachment 7

Cooperative Agreement (CR822761-01-0) - Syracuse Research Corporation

"Methodology for improving Risk Assessment" - (a) Improved Risk Assessment of Inorganics from Analysis of Bioavailability, (d) Evaluation of Relative Cancer Potency Approach to Human Risk Assessment for PAH and (e) Development of a Surrogate Chemical Approach for RfDs

Work and Accomplishments - Attachment 8

The work under this agreement involves: improved risk assessments of Inorganics from analysis of bioavailability; exploration of the biological bases and consequences of joint action; an investigation of likelihood based methods for non-cancer risk assessment; and evaluation of relative cancer potency approach to human health risk assessment for exposure to PAHs.

Cooperative Agreement (CR822766-01-0) - Tulane University Medical Center

"The Use of the Japanese Medaka Fish *Oryzias latipes* for the Evaluation of the Carcinogenic Potential and Developmental Effects of Mixtures of Contaminants" - The purpose of this work is to develop a relevant, economical non-mammalian system for use in risk assessment of mixtures of hazardous materials.

Work and Accomplishments - Attachment 9

Cooperative Agreement (CR822758-01-0) - University of Colorado-Denver

"Use of Pharmacokinetic Allometric Scaling to Improve Risk Assessments and the Development of RfDs" - The proposed research will use toxicokinetics and allometric scaling methods to gain understanding to the way in which fundamental parameters such as biological half life and apparent distribution volumes are scaled across species.

Work and Accomplishments - Attachment 10

This work involves evaluating the allometric methods used for existing toxicokinetic data to obtain empirical estimates of the allometric exponents for a subset of environmental pollutants. Investigations are also under way investigating existing data regarding the effects of health status, age, gender, etc. on toxicokinetic parameters such as clearance to obtain estimates of intra-species uncertainty. Present methods will also be extended for allometric scaling which assume linearity to account for non-linear phenomena such as saturation.

Related ERAP Collaborative Efforts:

Fuels/Total Petroleum Hydrocarbon Criteria Working Group

- This activity is being headed up by the Air Force at Brooks AFB with participation by other DOD, EPA, industry and academia to establish realistic cleanup standards for TPH in soils and ground water
- EPA's Assistant Administrator for ORD (Dr. Huggett) was briefed June 16, 1995 on the Working Groups progress with discussions on future directions

Rocket Emissions Working Group (REWG)

- This activity is being headed up by the Air Force at Brooks AFB for the Air Force's Space Command with a charge to assess the emissions (HCL, nitrogen oxides, nitric acid and hydrazide fuels) from Titan 4 rockets during nominal launches and during catastrophic abort scenarios
- Work to date has resulted in the potential for a successful Titan 4 launch in August 1995 increasing from ~27% to 70-80%, which can result in a savings in excess of $1M/day for every day a launch is not delayed

Ammonium Perchlorate Working Group

- Current work has involved evaluations of the current toxicological data on perchlorates and the design of new studies to fulfill the data gaps that preclude our effective determination of a high confidence RfD for ammonium perchlorates

Attachment 1

ASSESSMENT OF ENVIRONMENTAL HAZARDS OF 1,3,5-TRINITROBENZENE

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The remedial investigation/feasibility studies conducted at certain Army installations showed a need to clean up contaminated sites, where high levels of ammunition chemicals such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), 1,3-dinitrobenzene (DNB), and their degradation products/metabolites were detected in surface soil and groundwater. TNB is a photodegradation product of TNT; it is not easily degraded, and persists in the environment. The toxicity data on TNB are scanty. Hence the U.S. Environmental Protection Agency in 1988 (U.S. EPA, 1997) developed a reference dose (RfD) for TNB (0.00005 mg/kg/d for chronic toxicity) based on the toxicity of DNB, which is structurally similar to TNB. Since then we have completed acute, subacute, subchronic, chronic, reproductive, and developmental toxicity studies and toxicokinetics studies. We have reviewed the mammalian toxicity data for TNB and have determined the no observed adverse effect levels (NOAEL) and low observed adverse effect levels (LOAEL) for subchronic, chronic, reproductive, and developmental toxicity. Based on the newly determined NOAEL and LOAEL values, we have now developed a new RfD for TNB (0.03 mg/kg/d), based on the chronic toxic effects on hematology and histopathological changes in testes and kidney.

1,3,5-Trinitrobenzene (TNB), a nitroaromatic compound, is prepared by the decarboxylation of trinitrobenzoic acid or by the oxidation of 2,4,6-trinitrotoluene. TNB is a Class A explosive that is less sensitive to impact but more powerful and brisant than 2,4,6-trinitrotoluene (TNT) (Budavari et al., 1989; Fedoroff et al., 1962). TNB is used pri-

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The views, opinions, and/or findings contained in this article are those of authors and should not be construed as official Department of the Army/EPA position, policy, or decision, unless designated by other official documentation.

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marily in explosive compositions and munitions and has had limited use in the vulcanization of rubber (Barnhart, 1981). However, most TNB contamination results from photodegradation of TNT. TNB has been detected as an environmental contaminant of surface water, groundwater, and soil near production waste disposal sites and certain Army installations. The wastewaters discharged from TNT manufacturing processes contain a large number of nitroaromatic compounds, including TNB. TNB is formed during the nitration step of TNT synthesis as a result of oxidation of methyl groups. Although the complete mechanism of TNB formation during TNT photolysis in the environment is unknown, it has been suggested that it is produced from decarboxylation of 2,4,6-trinitrobenzaldehyde, a major TNT photoproduct (Burlinson, 1980). TNB is also found in aquatic systems as a by-product of biotransformation and photolysis of TNT. TNB is not readily biodegradable; it persists in the environment and has a high propensity to leach out and contaminate groundwater near production waste disposal sites, and in soils at certain U.S. Army installations (Walsh & Jenkins, 1992) and hazardous waste sites (Garman et al., 1987).

Problem definition studies revealed that the toxicity data are limited on many of the nitroaromatic compounds related to production of munitions. The toxicity data on TNB are limited to a few abstracts describing oral LD50 values published in Russian (Timofievskaya & Rodinova, 1973; Korolev et al., 1977). Since there were no toxicity data on TNB, the reference dose (RfD) for TNB was derived in 1988 based on the toxicity of 1,3-dinitrobenzene, which is structurally similar to TNB (U.S. EPA, 1997). These values were used by the Army to clean up/remediate sites contaminated with TNB. The cost for this cleanup and remediation were found to be high. The estimated cost for 1 cubic yard (~1.5 ton) of contaminated soil to incinerate was about $500 to $1000 depending on other conditions (Charles Lechner, U.S. Army AEC, Aberdeen Proving Ground, MD, personal communication, U.S. EPA, 1990). In order to facilitate compliance with U.S. Environmental Protection Agency (EPA) regulations, the U.S. Army initiated the program to develop toxicity data on TNB to develop an RfD, which can be used to develop environmental and health effects criteria for clean-up and remediation.

METHODS

Chemical

1,3,5-Trinitrobenzene (CAS number 99-35-4, 99.83%) was prepared by Dr. W. Koppes, Naval Surface Warfare Center, Silver Spring, MD, and the purity of the compound was further confirmed by high-perfor-

mance liquid chromatography (HPLC) by the Army and EPA (T. V. Reddy et al., 1993).

Experimental

All studies—acute, subchronic, chronic toxicity, toxicokinetics, reproductive, and developmental toxicity—were conducted using TNB prepared from the same batch. These studies were conducted according to standard EPA health effects testing guidelines (40 CFR 798) in compliance with Good Laboratory Practices (GLP, 40 CFR 792). Toxicokinetics (absorption, distribution, and elimination) of ^{14}C-TNB was studied in Fischer 344 (F344) rats following a single oral dose (G. Reddy & Gunnarson, 1993). Rats were dosed with ^{14}C-TNB (152 mg/kg; 6–8 μCi) in dimethyl sulfoxide and placed in glass metabolism cages. ^{14}C-TNB levels in urine and feces were measured at 24, 48, 72, and 96 h after dosing. The expired ^{14}C-CO_2 was measured for 48 h. Tissue samples were taken at termination of experiments (after 4 d).

RESULTS

Acute Toxicity

Acute toxicity tests with TNB were conducted by FitzGerald et al. (1992a). They showed that TNB is not a dermal irritant and did not produce dermal toxicity (2 g/kg), but showed severe eye irritation potentials in rabbits and mild skin sensitization in guinea pigs. The acute oral LD50 values in male, female, and combined sexes of F344 rats were 298, 275, and 284 mg/kg, respectively. The oral LD50 values (mg/kg) for mice were >900 for male, 702 for female, and 804 for combined sexes. Mice appear to be less sensitive than rats. These values are comparable to the oral LD50 values (mg/kg) of 450 for white rats, 600 for white mice, and 730 for guinea pigs (Korolev et al., 1977). The toxicity to these animals was characterized by central nervous system and respiratory disorders and cyanosis. Timofievskaya and Rodionova (1973) also reported an oral LD50 of 572 mg/kg in mice. No details are reported in these abstracts published in Russian.

Toxicokinetics and Metabolism

Toxicokinetics The toxicokinetics (absorption, distribution, and elimination) results showed about 10% of the dose (^{14}C-TNB, 152 mg/kg; 6–8 mCi) was eliminated in urine of male and female rats in the first 24 h. About 21% and 36% of the dose appeared in the urine in male and female rats in 4 d, respectively. Excretion via feces was about 4% in the same period in both sexes. The expired [^{14}C]CO_2 comprised about 3% and 5% of the dose over 2 d in male and

female rats, respectively. Soft tissue analysis 4 d after treatment revealed radioactive residues of about 0.02–0.03%/g in liver, kidney, skin, and lungs, whereas other tissues showed substantially lower levels of residues, ≤0.001%/g. The results showed that a single oral dose of TNB in the rat was absorbed in the gut and was eliminated mainly in urine, with low levels in feces in 4 d. These results also showed low levels of TNB residues in tissues examined after 4 d (Reddy & Gunnarson, 1993).

Metabolism Bel et al. (1994) determined TNB and its metabolites in biological fluids of Sprague-Dawley rats fed diet containing TNB and identified reductive metabolites, 3,5-dinitroaniline (urine), 3-amino-5-nitroaniline (urine, feces, and blood), and 1,3,5-triaminobenzene (urine and feces). No TNB was found in the samples by a gas chromatography/mass spectroscopy (GC/MS) method. No details of dose or time were provided in the abstract. G. Reddy et al. (1996) studied the metabolism of ^{14}C-TNB (43 μg) in an in vitro rat liver microsomal system and observed that TNB is completely metabolized (within 5 min), and subsequently identified two metabolites as 3-amino-5-nitroaniline and 3,5-dinitroaniline by either spiking or coelution with authentic standards by HPLC. At least one additional, unidentified metabolite was also observed. These results suggest that TNB is metabolized in vivo and in vitro systems.

Subacute Toxicity

The subacute (14-d) toxicity of TNB was evaluated in male and female F344 rats (100–120 g; 8 wk old) by feeding diet containing TNB at dose levels of 0, 50, 200, 400, 800, and 1200 mg/kg diet (T. V. Reddy et al., 1994a, 1996a). The calculated average TNB intake for male rats was 4.41, 17.08, 34.30, 55.87, and 92.33 mg/kg body weight/d and for female rats 4.42, 17.72, 34.26, 59.19, and 79.68 mg/kg body weight/d. These studies were conducted to select suitable doses for subsequent subchronic (90-d) toxicity studies. The mean daily food consumption significantly decreased in female rats fed 34.2, 59.2, and 80 mg/kg/d and in male rats fed 56 and 92.3 mg/kg/d. Average daily water consumption was decreased in males fed the high dose (56 and 92.3 mg/kg/d). This decreased food intake and water consumption resulted in reduced body weight in the 92.3 mg/kg/d dose group.

Mean ratio of relative organ weights to final body weight showed significant changes in both sexes of rat. Mean relative brain weight (1200 mg/kg diet dose group) and spleen weight (400, 800, and 1200 mg/kg diet dose) for both sexes were significantly increased, while the relative thymus weight was significantly decreased in male rats (1200 mg/kg diet). In males the relative testicular weight was decreased significantly in rats fed 800 and 1200 mg/kg diet, while weights of kidneys increased significantly in male rats fed 200–1200 mg/kg diet. The

relative liver weight was increased in the 400 mg/kg group of males but decreased in the 50 mg/kg diet female group.

Hematology and clinical chemistry results showed a dose-related decrease in red blood cell count, hematocrit and hemoglobin levels, and alkaline phosphatase levels and an increase in methemoglobin concentrations as compared to control. There were no biologically significant changes of any analytes except for significantly decreased alkaline phosphatase levels. Histopathological analysis showed that TNB produced significant changes in the testis (seminiferous tubular degeneration), spleen (extramedullary hematopoiesis), and brain (hemorrhage and malacia) in groups treated with 400–1200 mg/kg. The kidneys of male rats in the 200, 400, 800, and 1200 mg/kg dose groups exhibited an increased incidence of cortical tubular hyaline droplet deposition.

Subchronic Toxicity

Rats Subchronic (90-d) oral toxicity studies of TNB in male and female Fischer 344 rats (120–130 g, 8 wk old) were conducted by T. V. Reddy et al. (1994b, 1994c). Rats were fed a diet containing 0, 66.67, 400, and 800 mg TNB/kg diet for 90 d. The average daily TNB consumption for females was 4.3, 24.7, and 49.3 and for males was 3.9, 22.7, and 44.2 mg/kg/d. Male and female rats receiving 400 and 800 mg/kg diet consumed less, which resulted in significant decrease in body weight. However, the water consumption in females in those groups was significantly increased. The mean relative organ weights (g/100 g body weight), liver and spleen weights (males and females), and brain weights (males) were increased significantly in rats receiving 400 and 800 mg/kg diet. The relative testicular weight was decreased significantly in males.

Subchronic exposure to TNB produced hematological effects in rats. A significant decrease in total red cell count in males (22.7 and 44.2 mg/kg/d) and females (24.7 and 49.3 mg/kg/d) was noted. Correspondingly, there was a significant increase in the percent of reticulocytes in males (22.7 and 44.2 mg/kg/d) and in all female groups. A decrease in hemoglobin content and a significant increase in methemoglobin were observed in both mid- and high-dose groups of males and females (400 and 800 mg/kg diet). Clinical chemistry results showed no significant changes in any of the analytes studied.

Histopathological analysis revealed a moderate to severe seminiferous tubular degeneration in testis of mid- and high-dose groups (22.7 and 44.2 mg/kg/d) and deposition of hyaline droplets in kidneys of all male rats receiving TNB diet. The spleen and bone marrow featured mild to moderate erythroid cell hyperplasia in both sexes of rats receiving mid and high doses (400 and 800 mg/kg diet).

Male rats receiving TNB at dose levels of 3.9, 22.7, and 44.2

mg/kg/d had an increased incidence of cytoplasmic droplets (hyaline droplets) in proximal cortical tubular epithelial cells of the kidney at all treatment levels. The severity of this change was dose dependent, ranging from moderate in the high-dose group (44.2 mg/kg/d) to mild in the low-dose group (3.9 mg/kg/d). These droplets were occasionally irregularly shaped and had angular contours but were more often spheroid. They stained positive with Mallory–Heidenhain protein stain. Further characterization of these droplets would require immunohistochemical staining. A diagnosis of alpha-2μ-globulin nephropathy was not deemed appropriate since there was no significant increase in single-cell necrosis, and no presence of granular casts or linear papillary mineralization or increased tubular hyperplasia. In addition to the deposition of hyaline droplets, the presence of early chronic progressive nephropathy was evident in both treated as well as control male rats. This change was characterized by an increased incidence of tubular degeneration and regeneration as well as mineralized foci. Tubular degeneration was the only change that appeared to be dose related, as noted by an increased severity (mild) in the high- and mid-dose groups.

From these subchronic toxicity studies, a no observed adverse effect level (NOAEL) of 4.3 mg TNB/kg/d was established for female rats. Because TNB produced toxic effects in the male kidney (deposition of hyaline droplets) at all doses tested, a low observed adverse effect level (LOAEL) of 3.9 mg/kg/d is suggested.

White-Footed Mice Subchronic (90-d) toxicity of TNB was evaluated in the white-footed mouse (*Peromyscus leucopus*), 8–12 wk old (T. V. Reddy et al., 1995). Animals of both sexes were fed diet containing 0, 150, 375, and 750 mg/kg diet. The average calculated TNB consumption was 20.16, 64.81, and 108.25 mg/kg/d for females and 23.50, 67.44, and 113.51 mg/kg/d for males. The only significant organ changes noted were increase in relative kidney weight (females) and in absolute and relative spleen weight (male) for mice of the 750 mg/kg group. Hematology analysis showed no significant changes in the females, while the males presented a significant increase in reticulocytes for 150 and 750 mg/kg diet groups and increase in white blood cells in the 750 mg/kg group. Histopathological analysis revealed treatment-related changes in spleen (erythroid cell hyperplasia) and in testis (seminiferous tubule degeneration) in the 750 mg/kg diet group. The only biological findings (histopathological changes) deemed significant were those present in the male 750 mg/kg group. From this subchronic toxicity study, a NOAEL of 20.1 mg/kg/d for female and 23.5 mg/kg/d for male mice is suggested. These results also show that the white-footed mouse is less sensitive to TNB than rats. The target organ toxicity profile for this species was similar to that observed in rats but at a higher dose.

Reproductive Toxicity

A modified screening information data set (SIDS) for a single-generation reproductive toxicity study of TNB was conducted in male and female Sprague-Dawley rats (Kinkead et al., 1994, 1995). Rats were fed a diet containing 30, 150, and 300 mg of TNB/kg diet. The calculated dose consumed was 2, 9, and 19 mg/kg/d for males and 3, 14, and 29 mg/kg/d for females. Male rats received TNB from 14 d prior to the mating and throughout the mating period for a total of 28 d. Female rats were exposed to TNB from 14 d prior to mating and during the mating, gestation, and postpartum (21 d) periods and also 4 wk postweaning for a total of 90 d. Pups were maintained on treated diet through 4 wk postweaning.

No mortality occurred in the parental animals during the study and no treatment-related differences were noted in absolute or relative organ weights in male rats necropsied following the mating period (28 d of treatment). However, an increase in absolute and relative spleen weight (300 mg/kg), relative liver weight (300 mg/kg), and relative kidney weight (150 and 300 mg/kg) was observed in females following 90 d of exposure. Male rats sacrificed at 28 d following mating showed sperm effects including reduced number and concentration of motile spermatozoa (300 mg/kg) and reduced percent of cells showing a circular motion pattern (150, 300 mg/kg). Histopathological evaluation revealed significant increase in splenic hemosiderosis (150 and 300 mg/kg) and presence of hyaline droplets (mild) in control and all treated animals after 28 d of treatment, which may not be treatment related.

TNB showed no adverse effects on reproductive indices, on mating index (100%), or on fertility index (92% in 300 mg/kg group and 100% in other groups). No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of offspring per litter. During the 21-d lactation phase, the mean body weights of the TNB-treated pups (male and female) were significantly less than the control group pups, except at the 14 d time point, when the weights of the 150 and 300 mg/kg group pups were equal to those of the control. These results showed that TNB produced organ toxicity at 150 and 300 mg/kg dose (males) but no adverse effects on reproductive indices in any dose groups. The calculated NOAELs based on reproductive toxicity endpoints such as reproductive indices (mating, fertility, and others given earlier) were 2 mg/kg/d for males and 3 mg/kg/d for females.

Developmental Toxicity

Developmental toxicity of TNB (in 1% agar in sterile water) in female rats (Sprague-Dawley Crl:CD BR) was studied by oral gavage at dose levels of 11.25, 22.5, 45.0, and 90.0 mg/kg/d (Cooper &

Caldwell, 1997). Animals were identified as sperm positive on d 0 and were given TNB on gestation d 6–15. A laparohysterectomy was performed on all surviving animals on gestation d 20. Evidence of maternal toxicity was expressed at a dose level of 90 mg/kg/d by significant decreases in body weight and in food consumption. Clinical changes consistent with toxicity included disorientation, shaking, unsteadiness, and hyperactivity observed in one animal (1/25) in the 90 mg/kg/d group. Developmental toxicity exhibited in the 90 mg/kg/d group included reduced mean fetal weight and crown–rump length and increased incidence of skeletal variation (in one animal). No maternal toxicity or developmental toxicity was observed at dose levels of 11.25, 22.50, and 45.00 mg/kg/d. Based on this study, a dose level of 45 mg/kg/d was considered to be the NOAEL for developmental toxicity.

Mutagenicity

Evaluation of available literature revealed that TNB is mutagenic in *Salmonella typhimurum* strains (McGregor et al., 1980; Spanggord et al., 1982; Kawai et al., 1987). However, TNB did not appear genotoxic in the DNA repair assay with *Escherichia coli* or in the mitotic recombination assays with *Saccharomyces cerevisiae* D5 (McGregor et al., 1980).

Chronic Toxicity/Carcinogenicity

Slaga et al. (1985) studied the carcinogenic activity of TNB in mouse skin and lung tumor assays. A single application of 10 or 50 mg TNB (in acetone) to the skin of mice increased the incidence of inflammation, epidermal hyperplasia, and dark cells. The response elicited by these dose levels was similar to the maximum response obtained with 12-*O*-tetradecanoyl phorbol 13-acetate (TPA, a potent promoter of two-stage carcinogenic tumors in the skin of SENCAR strain mice). However, TNB was tested negative in a skin initiation assay that employed TPA promotion for tumor expression. Górski (1969) studied the biological role of charge transfer complexes of aromatic hydrocarbon oxi-derivatives in chemical carcinogenesis in male BALB/*c* strain mice. He injected subcutaneously 3-methylcholanthrene (3-MC), its complex forms with TNB, and TNB itself (equivalent to 1 mg of 3-MC) in 0.4 ml paraffin oil. The charge-transfer complex from the oxidation product of 3-MC with TNB revealed carcinogenic activity on mice higher than for 3-MC by itself. Animals receiving TNB (1 mg equivalent of 3-MC) alone were in good condition and remained without tumors throughout the observation period (144 d). This showed that TNB alone is not tumorigenic in mice in this study.

Chronic (2-yr) toxicity study of TNB was conducted in male and female F344 rats (T. V. Reddy et al., 1996b). Rats were fed diet sup-

plemented with TNB at 0, 5, 60, and 300 mg/kg diet and were sacrificed at 3, 6, 12, and 24 mo after exposure. The calculated average TNB consumption over the 2-yr exposure period at different time periods is presented in Table 1. The calculated average TNB consumption at 2 yr for females was 0.23, 2.68, and 13.31 mg/kg/d and for males 0.22, 2.64, and 13.44 mg/kg/d. The body weight of both sexes decreased in the high-dose (300 mg/kg) groups. The relative spleen weights were decreased in both sexes, while brain weights were increased in females (300 mg/kg group). Hemoglobin and hematocrit levels were decreased in both sexes in the 300 mg/kg group over 12 mo (3, 6, 12 mo), but no significant difference was evident at the end of the study (24 mo). However, methemoglobin was increased in females (300 and 60 mg/kg groups) at 12 mo and in both sexes (300 mg/kg) at 24 mo. Thus, based on this study, histopathological examinations suggested that the susceptible organs for TNB toxicity were kidney (cytoplasmic droplets) in both sexes, spleen (erythroid cell hyperplasia/pigment deposition) in both sexes of the 60 and 300 mg/kg diet dose groups, and testis (seminiferous tubular degeneration) in the males treated with 300 mg/kg. During this period no other lesions related to carcinogenic activity were detected. The results of the final study (2 yr) indicated toxicological events, such as methemoglobinemia, kidney, and testicular lesions, due to TNB exposure in both sexes of rats. These toxic effects were also observed in the interim (1-yr) sacrifice, but severity was significant only in the high dose (300 mg/kg). The lesions observed in various organs were nonspecific and unrelated to TNB. From this chronic study a NOAEL of 2.64 mg/kg/d and a LOAEL of 13.31 mg/kg/d for both sexes of rats are suggested.

Structure–Activity Relationships

Previous studies have shown that the nitroaromatic compounds such as nitrobenzene (NB), dinitrobenzene (DNB), and TNT produced hematological effects (methemoglobinemia, anemia), testicular degenera-

TABLE 1. Estimated TNB consumption (mg TNB/kg/d)

	Female			Male		
Exposure duration (mo)	5 mg TNB/kg diet	60 mg TNB/kg diet	300 mg TNB/kg diet	5 mg TNB/kg diet	60 mg TNB/kg diet	300 mg TNB/kg diet
3	0.27	3.21	15.17	0.35	3.72	17.93
6	0.27	3.23	14.37	0.28	3.22	15.69
12	0.24	2.93	14.90	0.25	2.96	14.61
24	0.23	2.68	13.31	0.22	2.64	13.44

tion, and reproductive and central nervous system (CNS) effects in animals (Bond et al., 1981; Cody et al., 1981; Levine et al., 1984; Morgan et al., 1985; Philbert et al., 1987.) The oral LD50 values for combined sexes of rats were 59.5 mg/kg and 284 mg/kg for DNB and TNB, respectively (FitzGerald et al., 1992a, 1992b). The additional nitro group resulted in reduced toxicity. Comparative acute toxicological data indicate that DNB is considerably more potent than TNB. Since there were no toxicity data on TNB, EPA had developed an RfD on the basis of the subchronic toxicity on DNB (Cody et al., 1981). In this study a LOAEL was calculated based on increased spleen weights in Carworth Farm male rats treated with 8 mg/L (1.13 mg/kg/d) DNB. The corresponding NOAEL and LOAEL for TNB (mg/kg/d) were determined by multiplying the NOAEL and LOAEL of DNB by the molecular weight ratio of TNB/DNB (213.11/168.11 = 1.27). In the subsequent risk estimates (e.g., derivation of RfD) there were uncertainty factors of 10,000 (including a factor of 10 for subchronic to chronic exposure, 10 for interspecies extrapolation, 10 for sensitive members of the human population, and 10 for the derivation of RfD by analogy to structurally similar DNB).

Thus, the RfD for TNB, based on DNB toxicity, was calculated as follows:

NOAEL	3 mg/L DNB in drinking water, converted to 0.51 mg/kg/d TNB
Uncertainty factor	10,000
Modifying factor	1
Chronic oral RfD	5×10^{-5} mg/kg/d

INTERPRETATION OF DATA

The U.S. Army conducted several animal toxicity studies discussed earlier that have demonstrated adverse health effects (hematological and testicular) of TNB at high doses. The NOAEL and LOAEL for subchronic, reproductive, and developmental toxicities were estimated (given earlier). The susequant 2-yr toxicity studies conducted in F344 rats showed that TNB produced hematological, testicular, and kidney effects in rats exposed at high doses (~13 mg/kg/d) from 3 to 24 mo. The hematological effects such as methemoglobinemia were not exhibited at 24 mo, suggesting the animals may have compensated for these effects over the longer term. TNB effects on the kidney were observed at the 3, 6, 12, and 24 mo time points (T. V. Reddy et al., 1996b). These kidney effects are accumulation of cytoplasmic hyaline (protein) droplets in proximal tubule in male rats exposed to 2.64 or 13.44 mg/kg/d. These effects were also observed in female rats at 12 and 24 mo, but these effects were not observed in females earlier at

3 or 6 mo during the study. Immunohistochemical analysis of kidneys of rats exposed to TNB revealed the presence of alpha-2μ-globulin in some, but not all, hyaline droplets formed in proximal tubular epithelium in both sexes. Those droplets, which were treatment related, did not demonstrate strong positive staining for alpha-2μ-globulin in female rats. Older female rats (104 wk) may exhibit some characteristics associated with males due to age-related hormonal changes. The histopathologic and immunohistochemical findings from the present study (T. V. Reddy et al., 1996b) do not support the histopathology and lesion progression observed in typical reports of alpha-2μ-globulin-associated nephrotoxicity. Furthermore, in the present study, there was no increase in response to TNB treatment. Thus the pathological sequence of lesions associated with alpha-2μ-globulin nephropathy was not observed as described in EPA Risk Assessment Forum Guidelines (U.S. EPA, 1991). The typical lesions include single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia. These lesions were not observed in the present study; therefore, these results preclude consideration of TNB nephrotoxicity for this risk assessment.

Hyaline (protein) droplet accumulation in proximal tubules has been observed in male rats exposed to unleaded gasoline, 2,2,4-trimethylpentane, decaline, 1,4-dichlorobenzene, pentachlorobenzene, and *d*-limonene, the natural product found in citrus oils (Hard et al., 1993). This protein is not found in humans. TNB showed mutagenicity in a bacterial system, but in a short-term carcinogenic bioassay and in the chronic 2-yr studies (described earlier) revealed no carcinogenicity. None of the lesions observed in the 2-yr study appear to be attributable to TNB treatment. Therefore the doses of 2.64 and 13.31 mg/kg/d are considered as NOAEL and LOAEL, respectively for the derivation of an RfD in the present study. Thus, based on TNB toxicity studies (as outlined earlier), the following approach is proposed for calculation of the TNB RfD:

NOAEL	2.68 mg/kg/d (chronic toxicity studies of TNB)
Uncertainty factor	100
Modifying factor	1
Oral RfD	3×10^{-2} mg/kg/d

Data obtained from the chronic study clearly demonstrated TNB-induced toxicity to the hematopoietic system. Such effects have also as has been demonstrated for other nitroaromatics such as dinitrobenzene and trinitrobenzene. Application of an uncertainity factor of 100 (10 for animal to human extrapolations and 10 for human sensitive population) to the NOAEL of 2.68 mg/kg/d results in an RfD of 3 ×

10^{-2} mg/kg/d (as shown earlier). It is worth noting that the new RfD (0.03 mg/kg/d) is 600 times higher than the previously estimated RfD (0.00005 mg/kg/d) (based on DNB toxicity data). The uncertainty factor used in this was only 100, as the toxicity data obtained from TNB per se in the chronic toxicity study increased the confidence in the toxicological database. These studies are conducted under full GLP standards and contain considerable detail (e.g., animals were evaluated at 3, 6, 12, and 24 mo). These studies are further supported by the subchronic toxicity studies in F344 rats and in white-footed mice (second species), and with additional subchronic reproductive and developmental toxicity studies. Higher confidence is recommended for these data. Furthermore, the absence of neoplastic lesions in chronic toxicity studies and the kidney effects (alpha-2μ-globulins) observed in rats are considered not to be relevant for humans in the risk assessment.

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Attachment 1

TOXICITY ASSESSMENT FOR DIAMINONITROTOLUENE

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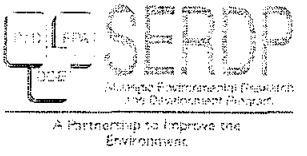

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PREFACE

This report assesses the potential non-cancer and cancer effects of diaminonitroluene (2,6-diamino-4-nitrotoluene, CAS No. 59229-75-3 and 2,4-diamino-6-nitrotoluene, CAS No. 6629-29-4). Information pertaining to non-cancer and cancer effects were previously assessed by U.S. Army Environmental Hygiene Agency in their 1994 study *Structure Activity Modeling of some Toxicological Properties of Munitions Degradation Products*.

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

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ABSTRACT

Data pertaining to the potential cancer/non-cancer effects of the two diaminonitrotoluene (DANT) isomers (2,6-diamino-4-nitrotoluene and 2,4-diamino-6-nitrotoluene) are reviewed. No subchronic or chronic exposure studies, for any route of exposure, were found in an extensive database search.

In occupational settings, DANT is considered a skin and eye irritant; no epidemiological studies of occupational exposure were found. The two isomers are urinary metabolites of oral exposure to 2,4,6-trinitrotoluene (TNT).

There is general agreement that the toxicological properties of DANT have not been thoroughly investigated. Insufficient data exist to derive oral or dermal RfDs or any cancer slope factor for DANT.

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1. SUMMARY OF TOXICITY INFORMATION

Diaminonitrotoluene (DANT; isomers 2,6-diamino-4-nitrotoluene and 2,4-diamino-6-nitrotoluene; CAS No. 59229-75-3 and CAS No. 6629-29-4, respectively; $C_7H_9N_3O_2$) is a degradation product of both TNT (2,4,6-trinitrotoluene, CAS No. 118-96-7, $C_7H_5N_3O_6$) and 2-amino-4,6-dinitrotoluene (CAS No. 35572-78-2, $C_7H_7N_3O_4$). 2-amino-4,6-dinitrotoluene is formed during TNT production and is released to the environment from munitions production and processing facilities; it is microbially degraded in the environment to the reduction products 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene (McCormick et al 1976; Hoffsomer et al 1978; Pennington 1988).

The following databases were searched for information on the diaminonitrotoluenes: TOXLINE 65, TOXLINE, MEDLINE 80, MEDLINE 85, MEDLINE 90, MEDLINE, HSDB, RTECS, and the NTIS database. No data on subchronic or chronic toxicity of these compounds were found. In addition, no data addressing reproductive or developmental effects, genotoxicity, toxicokinetics, or toxicity mechanisms could be located. Some information on mammalian excretory metabolism of DANT, as a biodegradation product of TNT, was identified; this material is summarized in Sect. 1.7.2 below.

A single study examining the structure-activity relationships of numerous munitions degradation products, including DANT, is summarized in Sect. 1.8. below.

There is general agreement that the toxicological properties of 2,6-diamino-4-nitrotoluene and 2,4-diamino-6-nitrotoluene have not undergone significant study to date.

1.1. EPIDEMIOLOGIC STUDIES

Pertinent epidemiologic studies for DANT are currently unavailable.

1.2. SHORT-TERM STUDIES

Short-term microbial toxicity assays indicate that, in solution mixtures of TNT, DANT and 2,6-dinitro-4-methylaniline (DNMA), TNT is 20 to 50 times more toxic on a mg/L basis than solutions of DANT or DNMA (Hankenson and Shaeffer 1991).

1.2.1. Human Toxicity. No information was found in the available literature describing the toxic effects of DANT on humans by any route of exposure.

1.2.2. Animal Toxicity. No information was found in the available literature describing the toxic effects of DANT on animals by any route or time period of exposure.

1.3. LONG-TERM STUDIES

No information was found in the available literature describing the chronic toxic or carcinogenic effects of DANT on animals or humans by any route of exposure.

By means of structure-activity analysis, Leach (1994) predicts that both isomers are carcinogens.

1.4. REPRODUCTIVE STUDIES

No information was found in the available literature describing reproductive effects in animals or humans by any route of exposure.

1.5. DEVELOPMENTAL STUDIES

No information was found in the available literature describing developmental effects in animals or humans by any route of exposure. By means of structure-activity analysis, Leach

(1994) predicted that 2,4-diamino-6-nitrotoluene would not be teratogenic, but that 2,6-diamino-4-nitrotoluene would be slightly (0.009) teratogenic.

1.5.1. Mutagenicity. No information was found in the available literature describing genotoxic effects in animals or humans by any route of exposure.

1.6. TOXICOKINETICS AND METABOLISM

1.6.1. Toxicokinetics. See discussion of urinary excretion in Sect. 1.7.2. below.

1.6.2. Metabolism. 2,4-diamino-6-nitrotoluene has been observed in human urine following oral exposure to TNT (DA 1986, Channon et al 1944, Lemberg and Callaham 1945; all as cited in ATSDR 1993).

The DANT precursor, 2-amino-4,6-dinitrotoluene was also found in the urine of TNT production workers and laboratory animals administered TNT orally (Hodgson et al 1977, El-hawari et al 1981) or dermally (Yinon and Hwang 1987). Further metabolic reduction of this dinitrotoluene is thought to take place, resulting in the urinary excretion of both DANT isomers in mammals administered TNT (Talmage 1996; ATSDR 1993).

1.6.3. Percutaneous Absorption. No information was found in the available literature describing percutaneous absorption of DANT in animals or humans.

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

As a means of obtaining estimated values for specific endpoints, toxicity modeling of structure-activity relationships has been performed for a number of munitions degradation products, including diamino-nitrotoluenes (Leach 1994). Estimates of acute oral LD_{50}, chronic LOAEL, carcinogenicity and teratogenicity were generated by means of the commercial software program TOPKAT® (a registered trademark of Health Designs, Inc., Rochester NY 14604).

1.8. MECHANISTIC STUDIES

No information was found in the available literature.

2. INTERPRETATION OF AVAILABLE INFORMATION

The isomer 2,6-diamino-4-nitrotoluene is considered an eye, skin and mucous membrane irritant in occupational settings, although there are no specific inhalation or dermal exposure criteria established by OSHA, ACGIH, or NIOSH. There are no human or animal toxicity data.

The two diaminonitrotoluenes evaluated in this assessment are mammalian urinary (and likely microbial) metabolites of TNT and 2-amino-4,6-dinitrotoluene. The two DANT isomers are found as reduction products in the urine of humans occupationally exposed to TNT, as well as that of both humans and laboratory animals receiving oral doses of TNT.

No experimental data addressing chronic or subchronic toxicity, carcinogenicity, reproductive or developmental effects, genotoxicity, structure-activity relationships or toxicity mechanisms were found in the extensive database search. A single structure-activity analysis predicts acute oral LD_{50}s, LOAEL, carcinogenicity and teratogenicity of both isomers.

Available data are insufficient to support development of RfDs, RfCs or slope factors.

3. DOSE-RESPONSE ASSESSMENT FOR NON-CANCER EFFECTS

3.1. INGESTION EXPOSURE

Available data are insufficient to support development of an oral Reference Dose (RfD) estimate for diaminonitrotoluene.

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- diaminonitrotoluene
CASRN -- 59229-75-3 and 6629-29-4
Last Revised -- No data

3.2. INHALATION EXPOSURE

Available data are insufficient to support development of a chronic inhalation exposure (RfC) estimate for diaminonitrotoluene.

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- diaminonitrotoluene
CASRN -- 59229-75-3 and 6629-29-4
Last Revised -- No data

3.3. DERMAL EXPOSURE

Available data are insufficient to support development of a chronic dermal exposure (RfD_d) estimate for diaminonitrotoluene.

REFERENCE DOSE FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- diaminonitrotoluene
CASRN -- 59229-75-3 and 6629-29-4
Last Revised -- No data

4. DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Data are insufficient to assess the carcinogenicity of DANT in humans or animals for oral or inhalation exposures.

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

DANT cannot be classified as to potential human carcinogenicity because of the lack of adequate data.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Not classifiable

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Attachment 1

TOXICITY ASSESSMENT FOR CYANOGEN CHLORIDE (CK)

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NOTICE

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PREFACE

This report assesses the potential non-cancer and cancer effects of cyanogen chloride (CK) (CAS Number 506-77-4). Information pertaining to non-cancer and cancer effects of CK was previously assessed by the United States Environmental Protection Agency (U.S. EPA) in a 1985 Drinking Water Criteria Document for Cyanide and by the Reference Dose (RfD) Work Group in 1987 (U.S. EPA, 1996b), as documented in the agency's Integrated Risk Information System (IRIS).

The fundings for this research were provided by the U.S. EPA, U.S. Army Center for Health Promotion and Preventative Medicine (CHPPM) and Strategic Environmental Research and Development Program (SERDP).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

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ABSTRACT

Information pertaining to the potential cancer/non-cancer effects of CK (cyanogen chloride) is reviewed. Directly relevant data are limited to short-term exposure studies in rats, cats and rabbits, which, supported by a limited suite of comparative metabolic information and by the severity of response, suggest a similar mechanism and level of toxicity to that of the cyanide anion. Thus, an oral lethal dose for CK in humans is considered to be in the range 50-300 mg/kg, with a derived oral LD_{50} of 6 mg/kg in rats and cats. CK vapor has been reported to be more irritating than that of hydrogen cyanide, but with similar symptoms and potentially, a similar mode of action. In the absence of longer term exposure studies in humans or laboratory animals for CK, toxicity values have been developed for the compound using epidemiological data and toxicological studies on sodium cyanide. In particular, a subchronic study featuring exposure of rats and mice to NaCN concentrations of up to 300 ppm in drinking water, caused alterations in reproductive parameters such as decreased relative and absolute weights of epididymis and testes, and reduced sperm numbers and motility, in male rats. In conjunction with an epidemiological study on human exposure to cyanide in Mozambique, a NOAEL of 4.5 mg/kg/day CN^- in the above study was used as a basis for deriving a chronic RfD for CK. Employing a molecular weight conversion factor and an uncertainty factor of 300 (10 to protect sensitive human subpopulations, 10 for animal to human extrapolation, and 3 for subchronic to chronic exposure extrapolation), an RfD for CK is estimated as 3E-2 mg/kg/day.

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1. SUMMARY OF TOXICITY INFORMATION

1.1. EPIDEMIOLOGICAL STUDIES

No epidemiological data on the effects of CK are available, save for an early report by Reed (1920) who recorded signs and symptoms of workers exposed to low concentrations of CK gas in a CK manufacturing plant. One particular worker, who was exposed daily for 8 months, experienced three episodes of heightened exposure to CK that resulted in dizziness, nausea, profuse lacrimation, blurring of vision, gasping, coughing, staggering, and prostration that lasted for several hours. There were also periods of irregular pulse that bore no relation to the acute exposure periods. The worker's weight decreased from 170 to 150 pounds during the 8 month exposure period, but rebounded to 160 pounds five weeks after cessation of exposure. Effects attributed to CK in other workers included chronic vomiting, diarrhea, frequent urination, persistent coughing, spasmodic pain in the respiratory muscles, cold perspiration, chronic dull headache, and weight loss.

Several reports of outbreaks of cyanide poisoning in regions of Africa may be relevant to the question of CK toxicity, based on the potential for the two compounds to feature common mechanisms of toxicity. Thus, chronic cyanide toxicity in humans has been implicated in various nerve disorders observed among people living in certain regions of Africa where cassava is a dietary staple. Cassava contains cyanogenic glycosides that release cyanide when metabolized *in vivo* (U.S. EPA, 1984; Westley, 1980). However, the studies lacked quantitative exposure information and failed to address such complicating factors as nutritional deficiencies and/or metabolic disorders which may also have been involved. Neurological findings were

generally correlated with increased thiocyanate levels in whole blood. Specific neuropathies (functional disturbances or pathological changes in the peripheral nervous system) observed included hyperreflexia (exaggeration of reflexes) of the upper limbs; spastic partial paralysis of the lower limbs; spastic dysarthria (imperfect articulation of speech); diminished visual acuity; changes in the cerebellum; and deafness (ATSDR, 1995).

A major outbreak of over 1000 cases of paraparesis (partial paresis of the lower limbs), affecting mostly women and children, was reported in Mozambique (Ministry of Health, Mozambique, 1984a,b). A prolonged drought in the area had exhausted most food resources except cassava, in which, because of the drought, the cyanide levels were particularly high. The normal process of detoxifying the bitter varieties by sun-drying may have been inadequate because of the general food shortage, and metabolic detoxification was probably reduced because of the absence of sulfur-containing amino acids in the diet. The raw cassava, which probably contained higher cyanide levels than those reduced by cooking, was eaten mostly by women and children. The estimated daily intake was 15-31.5 mg (approximately 0.2-0.45 mg/kg) in selected affected families chosen for the study. Clinical findings ranged from headache, vomiting, and slight weakness, to blindness and paralysis of all four limbs. Frequently, acute signs and symptoms were seen 4-6 hours after ingestion of meals. Although these signs and symptoms could not be definitively attributed to chronic cyanide intoxification because both patients and controls had similarly high thiocyanate levels, with no correlation of disease severity, the investigators concluded that the epidemiological features of the disease were suggestive of chronic cyanide intoxication due to cassava ingestion.

Chronic oral exposure to cyanide in humans who use cassava roots as their dietary staple has also been associated with thyroid effects. Ingestion of cassava, in combination with iodine deficiency, has been associated with a high incidence of goiter and cretinism in Zaire (U.S. EPA, 1985). Thyroid effects are generally attributed to thiocyanate, a metabolite that markedly inhibits the accumulation of iodine by the thyroid gland, thus decreasing the ability of the gland to maintain a concentration of iodine above that of blood (ATSDR, 1995).

Information derived from an occupational exposure study of hydrogen cyanide indicated that humans inhaling concentrations up to 10 ppm for 5-15 years may develop subjective signs and symptoms such as headache, weakness, changes in taste and smell, throat irritation, vomiting, effort dyspnea, lacrimation, colic precordial pain, and nervous instability (El Ghawabi et al., 1975). Also reported were mild to moderate thyroid enlargement and increased iodine uptake by the thyroid. Long-term exposure to cyanides in the occupational setting has also been associated with dermatitis, severe nasal irritation, nervous disorders, and EKG abnormalities (Hardy and Boylen, 1983; Carmelo, 1955).

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity. Very limited data are available on the short-term toxicity of CK in humans. Sittig (1985) reported that ingestion of a lethal dose of CK caused dizziness, rapid respiration, vomiting, flushing, headache, drowsiness, drop in blood pressure, rapid pulse, unconsciousness, and convulsions, with death occurring within 4 hours. The estimated lethal dose for adults reported in the Hazardous Substances Data Bank (HSDB) ranged from 50-300 mg/kg (HSDB, 1995).

CK has been described as being highly toxic by the inhalation, ocular, and dermal routes of exposure (Sax, 1984; Weiss, 1980). For example, although its toxic effects and mode of action are similar to those of hydrogen cyanide (HCN), CK is reported to be much more irritating, causing marked respiratory tract irritation with hemorrhagic exudates of the bronchi and trachea, and pulmonary edema (Hartung, 1994). HCN and CK were rapidly fatal to man at similar exposure concentrations (300-400 mg/m^3), for example. 50 mg/m^3 of HCN could be tolerated for about 30 minutes without immediate or delayed effects, whereas CK was an intolerable irritant at this concentration. Intermediate concentrations of CK may produce cyanide poisoning complicated by pulmonary edema (Hayes, 1982). Furthermore, based on extrapolation from animal data, the respiratory LCt_{50} in man has been estimated to approximately 11000 mg-min/m^3 (WHO, 1970). On this basis, CK would be less than half as toxic as HCN, which has an estimated LCt_{50} of 5000 mg-min/m^3. The casualty-producing concentration (causing significant injuries or incapacitation) has been estimated to be >7000 mg-min/m^3.

Due to its irritating properties, CK vapors cannot be tolerated even at very low concentrations. Hartung (1994) reported that the odor of CK was detectable at a concentration of 2.5 mg/m^3; and that exposure to 5 mg/m^3 for 10 minutes or to 50 mg/m^3 for 1 minute was intolerable. At higher concentrations, exposures to 120 and 400 mg/m^3 CK were fatal after 30 and 10 minutes, respectively. By contrast, one occupational study reported that concentrations as low as 1.8 mg/m^3 were unbearable, producing severe eye and nose irritation in exposed workers (ACGIH, 1991).

CK has caused severe injuries to the human eye following exposure to 100 mg/m^3 for 2 minutes (Sax, 1984). Exposure to this concentration produced immediate smarting of the eyes,

with severe blepharospasm and lacrimation (Grant, 1974). Liquid CK is also a severe skin irritant, causing second- and third-degree burns on short contact (Weiss, 1980).

1.2.2. Animal Toxicity.

1.2.2.1. Acute Toxicity —

1.2.2.1.1. Oral Toxicity. Information on the acute oral toxicity of CK in laboratory animals is limited to LD_{50} values for rats and cats of 6 mg/kg [U.S. Department of the Army, (DA), 1974: Registry of Toxic Effects of Chemical Substances (RTECS), 1995].

1.2.2.1.2. Non-Oral Toxicity. Values for the inhalation LC_{50}s of CK in a number of laboratory animals have been reported to range from 2750-6000 mg/m^3 [National Defense Research Committee (NDRC), 1946; Flury and Zernik, 1931]. Flury and Zernik (1931) considered the effects to be similar to those observed in humans. For example, at (unspecified) high concentrations, inhalation of CK induced effects that have been typically associated with cyanide poisoning (paralysis, unconsciousness), as well as severe irritation of the respiratory tract, with hemorrhage and edema of the lungs.

Exposure to lower concentrations for longer periods (specific data not provided) caused only respiratory irritation. Thus, Flury and Zernik (1931) compared the time for acute toxic responses to appear following exposure to CK and HCN in several animal species. Exposure to 0.3 mg/L (300 mg/m^3) produced complete paralysis and unconsciousness in mice 210 and 105 seconds after exposure to CK and HCN, respectively. A concentration of 1 mg/L (1000 mg/m^3) produced the same effects after exposure to CK and HCN, respectively.

Intravenous LD_{50} values were 4 mg/kg for rats [National Research Council (NRC), 1977], 2.5-3.2 mg/kg for rabbits (Aldridge and Evans, 1946: DA, 1974), and approximately 3 mg/kg for

dogs and goats (DA, 1974). Subcutaneous administration to rabbits, dogs, and pigeons yielded LD_{LO} values of 20, 5, and 9 mg/kg, respectively (RTECS. 1995).

1.2.2.2. Subchronic Toxicity — No data are available on the subchronic toxicity of CK in animals. However, if the analogy between the toxicity of CK and CN^- is valid, the subchronic drinking water study carried out by the NTP on NaCN in rats and mice may be relevant to the issue of CK toxicity. In this study, groups of F344/N rats and B6C3F1 mice (10/concentration/sex) were administered NaCN in drinking water at concentrations of 0, 3, 10, 30, 100, or 300 ppm for 13 weeks. The average doses of NaCN (based on average water consumption values for weeks 2-13 for all animals) were as follows: 0.3, 0.9, 2.7, 8.5, or 23.6 mg/kg/day (male rats); 0.3, 1.0, 3.2, 9.2, or 23.5 mg/kg/day (female rats); 0.5, 1.8, 5.1, 16.2, or 45.9 mg/kg/day (male mice; and 0.6, 2.1, 6.2, 19.1, or 54.6 mg/kg/day (female mice). Gross and histological examinations, sperm motility and vaginal cytology evaluations, and hematology, clinical chemistry, and urinalysis evaluations were performed on both species.

No deaths attributable to NaCN administration occurred in either species. In animals exposed to 300 ppm, male rats had slightly lower ($\leq$5%) final body weights and mean body weight gains than did the respective controls. Water consumption by rats and mice in the 100 and 300 ppm groups was 10% to 30% lower than that of controls; however, no clinical signs attributable to NaCN or to dehydration were observed. Poor palatability was suggested as the cause of the decreased water consumption. No gross or microscopic changes specifically related to cyanide toxicity occurred at any site in males or females of either species. Of particular note was the absence of changes to the brain or thyroid gland. Differences in absolute and relative organ weights between control versus exposed groups (with the exception of reproductive tissue

described in section 1.4) were minor, sporadic, and apparently not dose-related. Accordingly, the effects were thought to be incidental to treatment. Hematologic, clinical chemistry, and urinalysis evaluations of rat and mice revealed minimal changes that were also considered to be biologically insignificant, although the decreased urine volume and increased specific gravity observed in male rats were consistent with the observed decreases in water consumption. The concentration of urinary thiocyanate (the primary metabolite of cyanide) increased with increasing exposure concentrations at all time points.

Kamalu (1993) evaluated the effects of linamarin, a cyanogenic glucoside, in a diet containing cassava (*Manihot esculenta* Crantz), fed to growing dogs for 14 weeks. There were three groups of dogs (breeds not given), each comprising six animals. One group was fed cassava which was expected to release 10.8 mg HCN/kg of cooked food, a second group received a control diet to which enough NaCN was added at feeding time to release 10.8 mg HCN/kg of cooked food (in order to monitor the effects of HCN released from cassava), while a third group was fed a control rice diet containing no cyanide compounds. Each animal was given approximately 100 g diet/kg body weight. Although the body weight of the dogs was not reported, based on a default body weight estimation of 2.7 kg, the estimated dose was 1.08 mg HCN/kg/day. The biochemical variables investigated included plasma electrolytes, serum proteins, plasma-free amino acids, plasma enzymes, and the amount of protein in urine. Histopathological examinations of liver, kidney, myocardium, testis, and adrenal gland were performed. The cassava diet resulted an increased plasma thiocyanate concentration, increased urinary protein excretion, decreased levels of serum albumin, plasma potassium (K^+) and calcium (Ca^{++}). The NaCN diet caused increased plasma thiocyanate and urinary thiocyanate

excretion that was significantly ($p<0.01$) higher than that of dogs fed cassava. However, urinary protein ($p<0.01$) and serum albumin excretion were lower than that of dogs fed cassava, indicating that the amino acids were not utilized to the same extent as in the control (rice diet) group. Neither experimental diet had an effect on plasma gamma-glutamyl transferase, alanine transferase, isocitrate dehydrogenase activities, or on plasma sodium, magnesium or phosphorus concentrations. The cassava diet caused generalized congestion, hemorrhage, and periportal vacuolation of the liver; swelling, vacuolation, and rupture of the epithelial cells of the proximal convoluted tubules of the kidney; myocardial degeneration; and adrenal gland degeneration. The NaCN diet caused nephrosis, and adrenal gland hyperplasia and hypertrophy. Although both experimental diets produced testicular effects, as described in section 1.6., the differences in toxicological response were sufficient to allow the authors to conclude that not all of the observed changes induced by the cassava diet were due to the effects of cyanide.

Hertting et al. (1960) treated three dogs orally with NaCN in gelatin capsules for up to 14.5 months. As well as one control animal, the treatment protocol featured one dog receiving a daily dose of 0.27 mg CN^-/kg body weight for 13.5 months; another dog received a daily dose of 0.53 mg CN^-/kg for 16 weeks and then a daily dose of 2.2 mg CN^-/kg for 10.5 months; and the third dog received a daily dose of 1.1 mg CN^-/kg for 14.5 months. At daily doses of >0.53 mg CN^-/kg, there were signs of acute intoxication immediately after dosing, but with recovery occurring within <0.5 hours. In all treated dogs, histological examination revealed degenerative changes in ganglion cells of the central nervous system, and especially in Purkinje cells of the cerebellum (necrosis, reduced RNA content, and inflammation).

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. Information was unavailable on the carcinogenicity of CK in humans and animals. Similarly, the currently available evidence gives no indication that cyanides are carcinogenic. For example, although no studies specifically analyzing for cancer have been conducted, a chronic dietary study of rats exposed to HCN at estimated doses of 10.8 CN^-/kg/day (Howard and Hanzal, 1955; see section 1.3.2) found no tumors in various tissues examined histologically. The U.S. EPA assigned cyanide to Group D, not classifiable as to human carcinogenicity (U.S. EPA, 1996a). The International Agency for Research on Cancer (IARC) has not adopted a cancer classification for CK or cyanides (IARC, 1987).

1.3.2. Chronic Toxicity. Although information was unavailable on the chronic toxicity of CK in laboratory animals, two animal studies summarizing long-term oral exposure to cyanide have been identified. Howard and Hanzal (1955) maintained groups of 10 male and 10 female Carworth Farm rats for 104 weeks on diets that had been fumigated with HCN at nominal concentrations of 100 and 300 mg HCN/kg of diet. Though fresh rations were prepared every other day, the average daily low and high concentrations were about 76 and 190 mg HCN/kg diet (73 and 183 mg CN^-) due to the compound's volatility. This resulted in estimations for the average daily doses of 4.3 and 10.8 mg CN^-/kg body weight for low- and high-dose rats, respectively. Food consumption, growth rate, and survival of treated animals were comparable to those of controls, and there were no signs of gross toxicity, body or organ weight changes in either of the treated groups. Similarly, no histological lesions in the heart, lung, liver, spleen, stomach, intestine, kidney, adrenal, or gonads were identified in subsets of each group of treated

animals at autopsy. However, elevated thiocyanate levels were noted in the plasma, liver, and kidney in both treatment groups.

Philbrik et al. (1979) fed KCN in the diet to a group of 10 male rats (strain not specified) for 11.5 months at a concentration of 1500 mg KCN/kg of diet (600 mg CN^-/kg), corresponding to a daily dose of about 30 mg CN^-/kg body weight. Compared to controls, the treated rats had a 40% reduction in mean body weight gain, 535% decrease in plasma thyroxine levels, and a 68% decrease in thyroxine excretion rates. There were no definite histopathological lesions in the thyroid, sciatic, optic, nor to other neural tissues. However, some mild degenerative changes in the myelin of the spinal cord were observed.

1.4. REPRODUCTIVE STUDIES

Though no information has been identified concerning possible reproductive toxicity effects of CK, the NTP subchronic toxicity study on F344/N rats and B6C3F1 mice described in section 1.2.2.2 evaluated a suite of reproductive toxicity parameters. In male rats, decreases in epididymis weight (left), cauda epididymis weight (left), testis weight (left), and spermatid counts, decreased concentration-dependently, and achieved statistical significance. Similarly, sperm motility was lower than that in controls, although this effect could not be unequivocally ascribed to the treatment. Female rats in the 100 and 300 ppm treatment groups spent more time in proestrus and diestrus relative to estrus and metestrus than did control females. Time spent in estrus was slightly reduced (from 35% to 24.2% at 300 ppm), thereby reducing the time of receptivity in the female and possibly the rate of reproduction. In general, however, the investigators took the lack of a dose-response relationship between the onset of any of the

observed changes in female reproductive toxicity parameters and cyanide dose, as an indication that the changes could not be unequivocally attributed to NaCN exposure.

In male mice at 300 ppm, the left epididymal and left cauda epididymal weights were 90% and 82% ($p \leq 0.05$) compared to those of controls. However, no changes in sperm motility or spermatid density occurred in male mice and no significant changes on estrus cycle length were seen in female mice. The NTP investigators concluded that subchronic exposure to low doses of cyanide may produce mild, but possibly significant adverse effects on the male rodent reproductive system, but that the changes were probably insufficient to decrease reproductive performance. However, it has been speculated that, if the relative sensitivity of humans to changes such as those described were to be greater than that in rats (Working, 1988), a potential for adverse reproductive effects in human males following subchronic exposure to cyanide or cyanogenic compounds may exist.

Several studies have been conducted with animals exposed to cyanogenic glycosides through diets containing cassava meal, although, as noted by Tewe and Maner (1981a), the adverse effects of inorganic cyanides may not be the same as those from organic cyanide sources such as cassava.

In the 14-week study of Kamalu (1993), the effects of a cassava diet on spermatogenesis in growing male dogs were evaluated. One group was fed cassava that was expected to release 10.8 mg HCN/kg cooked food, a second group was fed a control diet to which enough NaCN had been added to release 10.8 HCN/kg cooked food, with a third group receiving the control diet only. The daily intake was calculated at 1.08 mg/kg/day. Occasional abnormal germ cells in the seminiferous tubules and occasional sloughing of germ cells (but with remnants of Sertoli cells)

were seen in the testes of animals fed the cassava diet. Spermatogenesis, however, appeared to be normal. The NaCN diet caused a significantly ($p \leq 0.01$) decrease in the relative frequency of testicular tubules containing spermatids in the lumen, and marked testicular germ cell sloughing and degeneration. Thus, the effect of the compound appeared to be on developing spermatids in the seminiferous tubules.

1.5. DEVELOPMENTAL STUDIES

Though no information has been identified concerning possible developmental toxicity effects of CK, the developmental toxicity effects of cyanide have been considered in a number of studies. For example, Singh (1981) administered a diet containing 50% or 80% cassava powder to female rats during the first 15 days of gestation. Though the doses of cyanide could not be determined from the report, the 80% cassava diet caused increased resorptions and a low incidence of developmental abnormalities (microcephaly, open eyes, limb defects, and growth retardation). In the group consuming 50% cassava, the only finding was lower fetal body weights. Maternal weight gain was lower in both treated group as compared to controls from day 6 onwards. The author speculated that the observed effects could have been due in part to the low protein content of the cassava diet.

Frakes et al. (1986) exposed Golden Syrian hamsters to cassava diets containing low and high levels of cyanide (approximately 0.6 mmol/kg and 7.9 mmol/kg, respectively) on days 3-14 of gestation. Body weight and food intake data were not provided, but a cyanide intake of 1.3 and 15.6 CN^-/kg/day were derived using default body weight and food intake values. Though no changes were observed in the number of implantations and resorptions, the low- and high-cyanide cassava-fed dams (both groups) gained significantly less weight than controls, and their

offspring showed signs of fetotoxicity through reduced body weight and reduced ossification.; In addition, the high-cyanide diet was associated with a significantly increased number of runts compared to the control group.

In a 2-generation study, Tewe and Maner (1981a) investigated the reproductive and developmental toxicity effects of cyanide in Wistar rats by the addition of KCN (500 CN^-/kg diet) to a cassava root flour-base diet containing approximately 12 mg HCN/kg diet. An equal number of control rats (20) received basal diet alone. Using default food consumption factors, the basal diet was considered to provide 1.2 mg CN^-/kg/day, with the KCN-supplemented diet providing 51 mg CN^-/kg/day. The diets were administered from about 20 days before gestation, during gestation, and through lactation and the post-weaning period. No significant differences were found between treated and control animals with respect to gestational weight gain, weanling weights, and offspring mortalities. However, the offspring that were continued on the cyanide diets during the post-weaning period consumed less food and grew at a significantly slower rate than the basal diet offspring, regardless of previous cyanide exposure (whether *in utero* and/or milk and/or diet). For those rats exposed in utero and fed cyanide during the post-weaning phase, an index of growth in comparison to food intake, the "protein efficiency ratio, was significantly reduced compared with basal diet rats.

In a similar study, Tewe and Maner (1981b) evaluated the reproductive performance of pregnant Yorkshire pigs (6/group) administered cassava diets containing 0, 250, or 500 mg CN^-/kg fresh cassava beginning on the day after breeding and continuing until parturition. The authors calculated the cyanide content of the basal diet to be 30 mg CN^-/kg diet, and that of the KCN-supplemented diets to be 277 or 521 CN^-/kg diet, approximating to daily intake values of

0.51, 4.6, and 9.16 mg CN^-/kg/day, respectively. There were no observed effects on litter size, litter size at weaning, birth weight, or in daily feeding intake for either sows or offspring. Small increases in maternal thyroid weight were seen at increased CN^- levels, and at the highest dose, pregnant sows had reduced thyroid activity. Proliferative changes in kidney glomeruli were seen in all three groups.

When pregnant hamsters were administered NaCN by subcutaneously implanted osmotic minipumps that delivered cyanide at a rate of 6.125-6.517 nmol NaCN/kg/hour beginning on day 6 of gestation through delivery, severe malformations were observed at all doses greater than 6.125 mmol/kg/hour. A dose of 6.517 mmol/kg/hour caused 100% fetal mortality and some maternal deaths (Doherty et al., 1982). Malformations included exencephaly, encephalocoele, non-closure of the neural tube, and microphthalmia.

1.5.1. Mutagenicity. No mutagenicity studies have been identified for CK, and genotoxicity studies with cyanides have yielded mostly negative results. For example, NaCN tested negative for gene reversion in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535, with or without S9 activation, at concentrations of up to 333 μg/plate (NTP, 1993). Other strains of *S. typhimurium* failed to display gene reversion in the presence of KCN (De Flora, 1981; De Flora et al., 1984). By contrast, HCN at concentrations of up to 5 mg/plate was considered to be marginally mutagenic to *S. Typhimurium* TA100 in the presence of metabolic activation, but not to strain TA98, with or without S9 (Kushi et al., 1983).

Negative response were obtained in a *rec* assay in *Bacillus subtilis* (Karube et al., 1981) and in a DNA repair test in *Escherichia coli* WP67, CM871, and WP2 (De Flora et al., 1984). Sodium cyanide did not induce DNA strand breaks in cultured lymphoma cells (Garberg et al.,

1988), and in an *in* vivo study, a single oral dose of KCN that provided 1 mg CN^-/kg did not induce testicular DNA synthesis in mice (Friedman and Staub, 1976).

1.6. TOXICOKINETIC STUDIES

1.6.1. Toxicokinetics. Although information on the toxicokinetics of CK is limited, the compound has been shown to be rapidly absorbed in the lung with subsequent metabolic conversion to the cyanide anion (Aldridge and Evans, 1946).

The potency of cyanide as an intracellular poison with blockade of electron transport and rapidly developing hypoxia in all tissues may obscure the mechanisms that exist for detoxifying sublethal concentrations of the compound. This occurs under the activity of the enzyme, rhodanese, which converts cyanide to the relatively benign thiocyanate ion, prior to elimination in the urine (Calabrese, 1991: U.S. Air Force, 1989).

1.6.2. Metabolism. CK is considered to be an irritant as well as a systemic poison when absorbed into the blood stream. Both *in vivo* and *in vitro* lines of enquiry support the hypothesis that the compound is rapidly converted to cyanide, through which it exerts its toxic effects. Aldridge and Evans (1946) detected cyanide in the blood stream of dogs immediately after inhalation of CK. Similarly, the same workers (Aldridge and Evans, 1946; Aldridge, 1951) demonstrated conversion of CK to CN in samples of whole rat blood or in isolated red blood cells. They considered these conversions to be dependent on the interactions of hemoglobin and glutathione with CK. However, since these conversion did not go to completeness, these workers estimated the conversion of CK to CN *in vivo* by comparing intravenous LD_{50} values for HCN and CK in rabbits, assuming that the systemic toxicity of CK is due entirely to the CN^- formed. Thus, the intravenous LD_{50} of 0.8 mg/kg for HCN and 2.5 mg/kg for CK would amount

to a 75% conversion of CK to CN, based on a molecular conversion factor of 26/61 derived from the compounds' molecular weights.

The cyanide ion is one of the most rapidly fatal poisons, with the central nervous system as the primary target organ. Its toxic effect is exerted by the formation of a complex with the ferric ion of mitochondrial cytochrome oxidase, the enzyme that catalyzes the terminal step in the electron transport chain, thereby preventing oxygen utilization. Since this metabolic function is a crucial step in the respiration of all cells, blockade of cytochrome oxidase results in uncoupling of oxidative phosphorylation and hypoxia of all tissues (ATSDR. 1995; Mitchell and Carroll, 1989; Hardy and Boylen, 1983). At sublethal doses, the metabolic processes that are in place to detoxify CN become evident. Thus, primarily in the liver, the enzyme rhodanese has the capability to convert CN to the less toxic thiocyanate ion, which is subsequently excreted in the urine (U.S. Air Force, 1989).

1.6.3. Percutaneous Absorption. Information on the rate and extent of absorption of cyanogen chloride through the skin is currently unavailable.

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

As discussed in earlier sections, the toxicity of CN^- has been used as a surrogate for that of CK, in view of the paucity of directly applicable information on the toxicity of the target compound, and through the body of evidence that implicates a similarity in toxicokinetic characteristics between the two compounds (Aldridge, 1951).

1.8. MECHANISTIC STUDIES

That CK has been shown to be rapidly to metabolized to CN^- *in vivo*, allows the suggestion that the toxicity of CK is mechanistically identical to that of CN^- (Aldridge and

Evans, 1946). This would implicate a mechanism involving blockade of cytochrome oxidase, with the consequent uncoupling of oxidative phosphorylation, resulting in hypoxia of all tissues (ATSDR. 1995; Mitchell and Carroll. 1989; Hardy and Boylen, 1983).

2. INTERPRETATION OF AVAILABLE INFORMATION

Cyanogen chloride (CK) (CAS No. 506-77-4), a halogenated cyanide with the chemical formula CNCl, is designated by the U.S. Army as a non-persistent blood agent (Fedoroff and Sheffield, 1962). CK is a colorless liquid or gas with a melting point of -6°C, a boiling point of 13.8°C (Hartung, 1994), and a vapor pressure of 1000 mg Hg at 25°C (DA, 1974). It is soluble in water (25 cm^3/mL at 20°C) and organic solvents (Hartung, 1994). CK is highly volatile (2,600,000 mg/m^3 at 12.9°C) (DA, 1974: Jacobs, 1942) and undergoes hydrolysis in water (Kononen, 1988). Hydrolysis half-lives range from one minute at 45°C to 10 hours at 5°C (Bailey and Bishop, 1970). CK undergoes considerable hydrolysis at alkaline pHs, forming cyanic acid (HOCN) and hydrochloric acid; the same products are found at a slower rate at acidic and neutral pH values (Clark, 1989). Because of its extreme volatility and relatively rapid hydrolysis in water, the chemical is not expected to persist in surface waters.

Toxicity data specific for CK are limited. The majority of available information for humans and animals deals with the effects of short-term exposure, with hardly any experimental data addressing the toxic effects of long-term exposure. As discussed in section 1, the systemic effects of CK are dependent on its rapid conversion to CN^- upon internalization, a quickly acting asphyxiant that rapidly induces death, as the body's detoxification mechanisms become saturated. In the absence of chemical specific subchronic or chronic studies for CK, an oral RfD has been derived based on results of experimental studies for HCN or other cyanides.

3. DOSE-RESPONSE ASSESSMENT

3.1. INGESTION EXPOSURE

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Cyanogen chloride (CK)
CASRN -- 506-77-4
Preparation Date -- September 1996

ORAL RfD SUMMARY

Critical Dose -- 8.5 mg/kg-day (NaCN) (NOAEL)
UF -- 300
MF -- 1
RfD -- 3E-2 mg/kg-day

Critical Study

Critical Effect -- Decreased epididymis, cauda epididymis, and testis weights, and decreased sperm counts

Study Type -- Rat Subchronic Oral Study

Reference -- NTP, 1993

NOAEL -- 8.5 (NaCN) mg/kg-day
NOAEL(ADJ) -- 10.6 (CK) mg/kg-day

LOAEL -- 23.6 (NaCN) mg/kg-day
LOAEL(ADJ) -- 29.4 (CK) mg/kg-day

Conversion Factors and Assumptions -- A molecular weight conversion factor of 26/49 is used to convert the NOAEL and LOAEL exposure rates for NaCN to CN^-, with a subsequent factor of 61/26 to convert CN^- to CK.

PRINCIPAL AND SUPPORTING STUDIES

NTP (National Toxicology Program). 1993. Sodium Cyanide (CAS No. 143-33-9) Administered in Drinking Water to F344/N Rats and B6C3F$_1$, Mice. NTP Toxicity Report Series No. 37. NIH Publication 94-3386. U.S. Department of Health and Human Services, Public Health Service.

In the experimental study, groups of F-344/N rats and B6C3F1 mice (10/concentration/sex) were administered five dose levels of NaCN (O. 3, 10, 30, 100, or 300 ppm) in drinking water for 13 weeks. The average doses of NaCN were as follows: 0.3, 0.9, 2.7, 8.5, or 23.6 mg/kg/day (male rats); 0.3, 1.0, 3.2, 9.2, or 23.5 mg/kg/day (female rats); 0.5, 1.8, 5.1, 16.2, or 45.9 mg/kg/day (male mice); and 0.6, 2.1, 6.2, 19.1, or 54.6 mg/kg/day (female mice). Endpoints evaluated for all dose levels were histopathology, clinical chemistry, hematology, and urine chemistry; reproductive toxicity was evaluated at 30, 100, and 300 ppm. Concentrations of 100 ppm and greater resulted in reduced water consumption that was attributed to poor palatability. No clinically significant body weight, organ weight (except for reproductive tissues), histopathologic, or clinical pathologic changes were observed in either species. Compared to controls, oral ingestion of sodium cyanide caused alterations in reproductive parameters. In male rats, these alterations included dose-related decreased absolute cauda epididymis weights ($p \leq 0.05$ at 2.7 and 8.5 mg/kg/day, $p \leq 0.01$ at 23.6 mg/kg/day); decreased absolute epididymis and testis weights at 23.6 mg/kg/day ($p \leq 0.01$); and decreased numbers of spermatid heads/testis at 23.6 mg/kg/day ($p \leq 0.05$) (see Table 2). Sperm motility was marginally lower in all groups of male rats. Statistically significant ($p \leq 0.05$) decreased absolute left cauda epididymis weights were also seen at the lower doses (2.7 and 8.5 mg/kg/day). Effects on reproductive parameters in female rats (more time in proestrus and diestrus relative to estrus and metestrus than control females) at 9.2 and 23.5 mg/kg/day could not be unequivocally attributed to cyanide treatment. In male mice at 45.9 mg/kg/day, left epididymal and cauda epididymal weights were lower ($p \leq 0.05$) compared with those of controls. No changes in sperm motility or spermatid density occurred in male mice and no significant changes on estrus cycle length was seen in female mice.

Based on the reproductive changes observed in male rats (decreased epididymis, cauda epididymis, and testis weights and decreased spermatid counts), 23.6 mg NaCN/kg/day is identified as the LOAEL and 8.5 mg NaCN/kg/day is identified as the NOAEL. The decreased epididymis, spermatid counts at 23.6 mg NaCN/kg/day implies a physiological change in rats that is of biological significance (decreases in sperm motility were not considered to be biologically significant). In the low- and mid-dose groups, the only statistically significant change was a decrease in left caudal epididymis weight. However, this organ weight change in the absence of changes in spermatid counts, is not considered to be an adverse effect. Using the molecular weight conversion factor of 26/49 (mol. wt. NaCN = 49; mol. wt. CN = 26), the LOAEL and NOAEL for CN^- is 12.5 and 4.5 mg/kg/day, respectively.

Studies by Aldridge and Evans (1946) indicate that CK may not release all of the available cyanide in the body and they suggested using a 75% conversion factor (based on a

comparison of intravenous LDso values for HCN and CK in rabbits, see Section 3.1). However, supporting studies are lacking to justify the use of a 75% conversion factor for the derivation of an RfD. Therefore, the RfD for CK assumes 100% conversion, and based on a maximum number of molar equivalents (1) of CN- being released. Thus, a molecular weight conversion factor of 61/26 (mol. wt. CK = 61; mol. Wt.. CN^- = 26) is used to convert the NOAEL of 4.5 mg/kg-day for CN^- to 10.6 mg/kg-day for CK. A total uncertainty factor of 300 was applied, 10 to protect sensitive human subpopulations, 10 for animal to human extrapolation, and 3 for subchronic to chronic extrapolation (a full factor of 10 was not used because a chronic oral study was available).

UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF -- 300

MF -- 1

The previous RfD of 0.05 mg/kg-day (U.S. EPA, 1996b) was based on the results of the chronic dietary study of rats exposed to HCN conducted by Howard and Hanzal (1955). In this study, male and female Carworth Farms rats were administered a diet fumigated with HCN at nominal concentrations of 100 or 300 ppm for 104 weeks. From the data reported on food consumption, body weight, and estimated CN content of food, the daily doses were 4.3 and 10.8 mg CN mg/kg-day for the low- and high-dose rats, respectively. There were no treatment-related effects on growth rate and no gross signs of toxicity. No histopathological lesions were seen in various tissues examined, including brain and reproductive organs. Because there were no adverse effects, the study provided a NOAEL (10.8 mg CN mg/kg-day), but not a LOAEL. Using a molecular conversion factor of 61/26 (mol. wt. CK = 61; mol. wt. CN- = 26), the NOAEL for CK was determined to be 25.3 mg/kg-day. Applying an UF factor of 100 (10 for species extrapolation, 10 tor sensitive populations) and a modifying factor (MF) of 5 (to account for the apparent tolerance to cyanide when it is ingested with food rather than when it administered in drinking water), the resulting RfD for oral exposure to CK was 0.05 mg/kg-day.

CO-PRINCIPAL STUDY

Ministry of Health, Mozambique. 1984a. Mantakassa: An epidemic of spastic parapesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. Bull. World Health Organization 62:477-484.

Ministry of Health, Mozambique. 1984b. Mantakassa: An epidemic of spastic parapesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic acid content in cassava. Bull. World Health Organization 62:485-492.

Although precise exposure data are lacking, an RfD can be also estimated from the human data reported by the Ministry of Health, Mozambique (1984a,b). A major outbreak of over 1000 cases of partial paresis of the lower limbs occurred in Mozambique, affecting mostly women and children. The investigators estimated that the daily CN^- intake derived from cooked and raw cassava was 15-31.5 mg (approximately 0.2-0.45 mg/kg/day, based on a 70 kg body weight) in selected families chosen for the study. The average intake of 0.33 mg/kg-day can be considered a LOAEL for cyanide, with nervous system toxicity as the critical effect. A NOAEL was not identified. A total uncertainty factor of 30 was applied, including 10 for LOAEL to NOAEL extrapolation and 3 for a less than chronic exposure situation. Using a molecular conversion factor of 61/26, the LOAEL for CK was 0.77 mg/kg/day, and an RfD of 0.026 mg/kg-day was derived.

Thus, considering the epidemiological (Ministry of Health, Mozambique, 1984a.b) and drinking water with rats (NTP, 1993) as cocritical studies, the RfD for CK is 0.03 mg/kg-day.

UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF -- 30

MF -- 1

ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

Subchronic studies: Kamalu, B.P. (1993) 14 week Subchronic Toxicity Study.

Testicular germ cell sloughing and degeneration and a decreased number of testicular tubules containing spermatids were observed in growing male dogs fed a NaCN-supplemented diet that was expected to release 10.8 mg HCN/kg food (Kamalu, 1993). Based on a food intake of 100 g food/kg body weight (value provided by author) and a reference body weight of 12.7 kg, the estimated daily dose is 1.08 mg HCN/kg or 2.5 mg CK/kg. The LOAEL derived from this study is 2.5 mg CK/kg/day, with reproductive toxicity as critical effect. This study was not selected for derivation of the RfD because the dosing protocol was not adequately described by Kamalu (1993). However, the RfD with appropriate uncertainty factors would be similar to that derived from the NTP study.

Subchronic studies: Philbrick et al, 1979) 11.5 month Subchronic Toxicity Study

Weight loss, thyroid effects, and myelin degeneration were reported in rats fed a higher dose (1500 mg KCN in the diet that provided approximately 30 mg CN-/kg day) for 11.5 months (Philbrick et al., 1979). Because only one dose level was tested a NOAEL could not be identified from this study.

CONFIDENCE IN THE ORAL RfD

Studies -- Medium to High
Data Base -- Medium
RfD -- Medium to High

Confidence in the experimental drinking water study by NTP (1993) is high. It was well designed with respect to exposure protocol, number of animals, and exposure duration, and identified a LOAEL and NOAEL. The confidence in the epidemiological study (Ministry of Health, Mozambique, 1984a,b) is medium, primarily because of uncertainties associated with cyanide consumption from cassava. Although the data base for CK is inadequate, the data base for cyanide contains subchronic and chronic bioassays in more than one species, and reproductive/developmental toxicity studies in more than one species. Confidence in the data base for cyanide is considered medium. Medium to high confidence in the RfD results.

EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source documentation --

Other EPA Documentation --

Agency Work Group Review --

Verification Date --

EPA CONTACTS (ORAL RfD)

Harlal Choudhury/NCEA-Cin -- (5134)569-7536

U.S. ARMY CONTACTS

3.2. INHALATION EXPOSURE

Pertinent information is not currently available.

3.3. DERMAL EXPOSURE

Pertinent information is not currently available.

4. DOSE RESPONSE ASSESSMENT FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Data are insufficient to assess the carcinogenicity of CK in humans or animals for oral or inhalation exposures.

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

CK cannot be classified as to potential human carcinogenicity because of the lack of adequate data.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Not classifiable

Basis -- Lack of adequate human or animal data

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Attachment 1

TOXICITY ASSESSMENT FOR ETHYLENE GLYCOL (EG)

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PREFACE

This report assesses the potential non-cancer and cancer effects of ethylene glycol (1,2-dihydroxyethane, 1,2-ethanediol, EG) (CAS Number 107-21-1). A summary of information pertaining to non-cancer and cancer effects of EG is provided by the United States Environmental Protection Agency (U.S. EPA) in the Integrated Risk Information System (IRIS) record for the subject compound (U.S. EPA 1996), in which a verified reference dose (RfD) value is provided.

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This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

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ABSTRACT

Data pertaining to the potential cancer/non-cancer effects of ethylene glycol (1,2-dihydroxyethane, 1,2-ethandiol, EG) are reviewed. The compound is an important industrial chemical with applications as an automotive and aviation antifreeze agent, a solvent, and a chemical intermediate. Thus, widespread potential exists for human exposure to EG, with the consequent possibility of significant health impacts. The Integrated Risk Information System (IRIS) (U.S. EPA (1996) reports a verified RfD value for EG of 2E+0 for EG, derived from a chronic feeding study by DePass et al (1986a) in F344 rats and CD-1 mice in which a range of frank effects, including increased mortality, kidney lesions, and hematological changes were observed at exposure levels greater than the No-Observed-Adverse-Effect-Level (NOAEL) of 200 mg/kg-day. However, data from other studies can serve as a basis for developing quantitative toxicity benchmarks for other toxicological effects of EG. For example, the use of a continuous breeding protocol in exposing CD-1 mice to concentrations of EG in drinking water of 0, 0.25, 0.5, and 1.0% EG (approximating to exposure levels of 0, 400, 800, and 1600 mg/kg-day, respectively) resulted in statistically significant developmental toxicity effects in the absence of maternal toxicity at the highest dose level, a justification for the use of the 800 mg/kg-day exposure level as a NOAEL (Lamb et al. 1985). This converted to a potential chronic (for fetal exposure) RfD of 8E+0 under the combined uncertainty factors of 10 for species to species extrapolation and 10 to protect sensitive subpopulations. Importantly, the potential for reducing uncertainty in the delineation of EG's subthreshold exposure levels may be confounded by the apparent duality of the compound's toxic effects, namely developmental toxicity and nephrotoxicity. In this report an oral RfD has been derived from a subchronic drinking water study in CD (Sprague-Dawley) rats (Robinson et al., 1990) in which, for males, histopathological lesions in the kidney and attendant clinical chemistry changes at exposure levels greater than 554 mg/kg-day justify the nomination of this exposure level to be the NOAEL. Application of a combined uncertainty factor of 1000 (consisting of 10 for extrapolation from subchronic to chronic exposure, 10 for interspecies extrapolation, and 10 to protect sensitive subpopulations) to this value resulted in a potential chronic oral RfD of 5E-1 mg/kg-day.

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1. SUMMARY OF TOXICITY INFORMATION

1.1. EPIDEMIOLOGICAL STUDIES

Though limited by a small sample size, an epidemiological study investigating renal cancer mortality in 1666 employees of a chemical plant reported no elevation in the odds ratio for the EG-receiving workers (Bond et al., 1985).

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity.

1.2.1.1. Oral Toxicity — A minimum lethal dose of 1.4 mL/kg of 95% EG has been suggested for adult humans (Parry and Wallach, 1974; Robinson and McCoy, 1989; Siew et al., 1975). Siew et al. (1975) reported the case of a 22-year-old man who consumed 300 mL of antifreeze resulting in a dose of 4071 mg/kg. This individual died approximately 48 hours after ingesting EG. In another incident a lethal dose of 7850 mg/kg was estimated for a 73-year-old male who died 68 hours after consumption of the compound (Gordon and Hunter, 1982). In another case, a man developed severe cranial nerve dysfunction including nerves VII, IX, and X, five days after ingesting 12,840 mg/kg of EG (Spillane et al., 1991).

1.2.1.2. Inhalation Toxicity — Nineteen men were exposed for 20 to 22 hours/day for 1 month to EG in an aerosol (Wills et al., 1974). The mean daily exposure concentrations of EG ranged from 3 to 67 mg/m^3. On three different occasions, the exposure concentration was elevated to 188, 244, or 308 mg/m^3 prior to the men entering the room. Subjects could tolerate an exposure concentration of 188 mg/m^3 for only 15 minutes, while the 244 mg/m^3 exposure concentration could be endured for only 1 or 2 minutes. The exposure concentration of 308

mg/m^3 could be tolerated for 1 or 2 breathes at the most. On other occasions. exposure concentrations of greater than 200 mg/m^3 resulted in unbearable pain from irritation of the respiratory tract. The average exposure concentration was 140 mg/m^3 for the 1 month exposure time. Hematological, serum, and urine parameters were monitored for these men as well as a control group, and no differences were noted. Slight headache and low backache were reported by the exposed individuals. A number of mental exercises were performed to establish whether any depression of the central nervous system had occurred, but no statistically significant differences between treated and control groups were found.

Siew et al. (1975) considered the potential inhalation hazard due to EG around hazardous waste sites to be low because of the compound's low vapor density.

1.2.2. Animal Toxicity.

1.2.2.1. Acute Toxicity —

1.2.2.1.1. Oral Toxicity. A lethal oral dose in cats was determined to be 4440 mg/kg with clinical signs including loss of reflexes, convulsions, central nervous system depression, and coma (Penumarthy and Oehme, 1975). Death occurred 20-36 hours after administration of EG. In dogs, the lethal oral dose was 4880 mg/kg (Beckett and Shields, 1971), and 4000 mg/kg for monkeys (Clay and Murphy, 1977). Monkeys drinking water with 0.25 to 10% for 6-13 days and receiving doses greater than 1388 mg/kg-day, had calcium oxalate crystals and evidence of necrosis in the kidney (Roberts and Seibold, 1969).

1.2.2.1.1. Inhalation Toxicity. The saturated vapor concentration of EG was calculated to be 79 ppm at 20°C (CMA. 1993), a concentration below the level that might result in any adverse response from acute exposure. In fact, a super-saturated vapor acute inhalation toxicity

study was conducted exposing rats to 200 ppm for approximately 4 hours/day for 1 week, and no deaths were reported (Browning, 1965).

1.2.2.2. Subacute Toxicity —

1.2.2.2.1. Oral Toxicity. No histological evidence of liver or kidney damage was present in mice gavaged with 1000 mg/kg-day for 14 days (Hong et al., 1988).

1.2.2.2.2. Inhalation Toxicity. Information on the toxicity of EG in animals exposed via inhalation was unavailable.

1.2.2.3. Subchronic Toxicity —

1.2.2.3.1. Oral Toxicity. In a 13-week feeding study using rats, Melnick (1984) reported that (1) 40% of the males died in the 5000 mg/kg-day group, (2) calcium oxalate deposits were found in brain blood vessel walls in the 5000 mg/kg-day group, (3) oxalate nephrosis, renal failure, and decreased body weight were present in the 2500 mg/kg-day group, and (4) no findings were observed in the 1250 mg/kg-day group. Mice in a 13-week feeding study gavaged with 1250, 2500, or 5000 mg/kg-day of EG developed mild nephrosis and hyaline degeneration of centrilobular hepatocytes in the 2500 mg/kg-day group.

Robinson et al (1990) administered EG in drinking water to Sprague-Dawley rats of both sexes for either 10 or 90 days. In the subchronic part of the study, 10 animals/sex/group were exposed to concentrations of 0, 0.5, 1.0, 2.0, or 4.0 % for females, and 0, 0.25, 0.5, 1.0 and 2.0 % for males. These approximated to exposure levels of 0, 554, 1108, 2216, and 4432 mg/kg-day respectively, for females, and 0, 227, 554, 1108, and 2216 mg/kg-day respectively, for males, based on an estimated drinking water intake of 100 mL/kg-day (data calculated by the authors). Mid and full-term body weights, and serial water and food consumption data were obtained. At

termination, blood samples for hematological and clinical chemistry determinations were obtained by cardiac puncture. Organ weights for all animals were obtained at necropsy, with histopathological determinations carried out on a wide range of tissues from half of the control group and from all survivors in the high-dose group. Histopathological examination of the kidney was carried out in all the surviving animals in the study.

At the highest dose, 8/10 female and 2/10 males rats died prior to term. At this exposure level also, the survivors displayed depressed body weights compared to controls, and comparatively reduced hemoglobin levels, hematocrit, and erythrocyte and leucocyte counts. For some clinical chemistry parameters, sporadic changes with apparent statistically significant differences to controls were also identified at varying dose levels, but, for the most part, the absence of any clearly defined dose-dependency suggested that these changes may have been incidental to treatment. The most consistent toxicological features relating to EG exposure were histopathological manifestations of kidney damage in male rats, with marked tubular dilation and/or degeneration and the presence of calcium oxalate crystals. Possibly associated with these lesions was a dose-dependent increase in kidney weights among the males, and changes in the plasma creatinine and blood urea nitrogen (BUN), that both rose dose-dependently to significantly higher levels than those of the control group. By contrast, the kidney lesions and their associated clinical chemistry parameters remained at control levels for female rats, a discrepancy mimicked by the differential responses to EG evident in those rats receiving the compound for only 10 days (the acute study). Taken together, the kidney and related clinical chemistry lesions identified in male rats would provide a NOAEL of 554 mg/kg-day; with 1108 mg/kg-day as the Low-Observed-Adverse-Effect-Level (LOAEL).

In a National Toxicology program (NTP) (1992) feeding study, mice were dosed with 1625, 3250, or 6500 mg/kg-day for 13 weeks. Males in the 1625 mg/kg-day group had decreased body weights. a potential LOAEL for EG, though possibly compromised as a point of departure for RfD development by being the lowest positive exposure level on the dose-response curve. In the 3250 mg/kg-day group, hyaline degeneration of the centrilobular hepatocytes, renal tubular dilation, and renal degenerative hyperplasia were observed.

1.2.2.3.2. Inhalation Toxicity. Information on the systemic toxicity of EG in animals exposed subchronically via inhalation was unavailable.

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. In a combined systemic toxicity/oncogenicity feeding study, with essentially lifetime exposure of CD-1 mice and F344 rats to 40, 200, or 1000 mg/kg-day for 12 months (DePass et al., 1986a). The results were negative regarding oncogenic effects. A 2-year dietary study in mice likewise had no carcinogenic effects (NTP, 1992).

1.3.2. Chronic Toxicity. DePass et al. (1986a) identified non-carcinogenic effects of EG in F344 rats and CD-1 mice that became evident over a 12 month dosing period. Thus, rats developed oxalate nephrosis (males), increased neutrophil count (male), decreased erythrocyte count, hemoglobin, and hematocrit, and fatty metamorphosis in the 1000 mg/kg-day group, allowing a NOAEL of 200 mg/kg-day to be established. This value was used as a basis for the development of the verified RfD value of 2E+0 presented in IRIS. For mice, no signs of toxicity were observed, and the NOAEL was 1000 mg/kg-day.

1.4. REPRODUCTIVE STUDIES

Oral doses of 40, 200, or 1000 mg/kg/day were administered to F344 rats for 3 generations, and no dominant lethal mutations or reproductive abnormalities were observed (DePass et al., 1986b). By contrast, in a study by Hong et al. (1988), mice gavaged with 200, 400, and 1000 mg/kg for 4 days had marked loss of spermatogenic epithelium in a portion of the seminiferous tubules. However, the study does not indicate if one or all doses resulted in this finding.

1.5. DEVELOPMENTAL EFFECTS

Schuler et al. (1984) reported that the administration of 11,090 mg/kg/day by gavage to CD-1 mice on gestation days 7-14 resulted in a 10% mortality rate in the dams and a significant reduction in the number of litters containing viable pups.

A continuous breeding study protocol was used by Lamb et al, (1985) to examine the developmental effects of EG in drinking water in CD-1 mice. Drinking water concentrations of 0, 0.25, 0.5, or 1.0% EG converted to exposure levels of approximately 0, 400, 800, or 1600 mg/kg/day, respectively. The findings were slight, but statistically significant decreases in the numbers of litters per fertile pair, live pups per litter, and live pup weight in the 1% group. Facial anomalies and skeletal defects (reduced size of skull bones, fused ribs, and abnormally shaped sternebrae and vertebrae) were observed in the 1% group. No defects were found in the 0.25 and 0.5% groups. No clinical signs of toxicity, significant body weight changes, or water consumption were noted. Two females died in the 0.5% group which may have been related to the oxalate crystal deposition in the kidney, however, no deaths occurred in the higher dose group.

Price et al. (1985) gavaged mice with 750, 1500, or 3000 mg/kg/day of EG on gestation days 6-15 and rats with 1250, 2500, or 5000 mg/kg/day of EG on gestation days 6-15. Fetal effects (decreased body weights, defects in the axial skeleton, and craniofacial defects) were observed for both mice and rats with all dose groups, and maternal effects in mice (decreased body weights) were observed in the middle and high dose groups, while maternal effects in rats (decreased body weights) occurred in all dose groups.

In one of a series of studies carried out by the Research Triangle Institute on behalf of the National Institute of Environmental Health Sciences (NIEHS), Marr et al (1992) examined developmental responses to EG in CD (Sprague-Dawley) rats. Pregnant females were gavaged during gestation days (GD) 6-15, with either 0 or 2500 mg/kg-day. Subgroups of seven dams and/or fetuses/pups were sacrificed on GDs 18 and 20, or on post-natal days (pnd) 1, 4, 14, 21, and 63. All were examined for maternal signs and/or developmental lesions.

Dams receiving EG at 2500 mg/kg-day during the major period of organogenesis showed no clinical, morphological or pathological signs of toxicity, a fact that allows the suite of fetal changes, abnormalities and variations described below to be unequivocally ascribed to the effects of developmental toxicity rather than a secondary consequence of maternal toxicity. For example, fetal weights per litter in the EG receiving group were reduced compared to controls at GDs 18 and 20. Pup body weight was reduced on pnd 1, but not at the later time points.

To examine the extent of skeletal lesions, variations and abnormalities, skeletons of sacrificed fetuses were stained with Alcian Blue and Alizarin Red S. Parts examined included the sternum, carpals, tarsals, phalanges, and vertebral cortex, with the extent of ossification noted.

The incidence of skeletal malformations in fetuses per litter increased at all time points except pnds 4 and 63. The extent of total ossification, most notably that of the sternebrae and vertebral cortex, was found to be reduced in EG-receiving groups on GD20 and pnds 1-21, but not on GD18 or 63. The incidence of malformations was likewise increased versus controls at all time points except pnd 63, the most common variations being the appearance of rudimentary rib(s) on lumbar arch I. The authors hypothesized that the developmental toxicity of EG may result in delays in skeletal ossification and/or increased alterations/malformations through pnd 21. However, the resolution of these lesions by pnd 63 might suggest that these abnormalities may not always be permanent.

In an abstract, the same group (Bates et al., 1990) reported the administration of, 0, 250, 1250, or 2250 mg EG /kg-day to pregnant CD (Sprague-Dawley) rats on GDs 6-20. Litters were evaluated for growth, viability, developmental landmarks, locomotor activity, and learning. By contrast to the data of Marr et al. (1992), both developmental and maternal toxicity were evident at the higher dose levels, an observation that might preclude a conclusion that EG causes the developmental effects unrelated to compound-related effects in dams in this study. Thus, live litter size, pup weight, and post-natal viability through pnd 4 were decreased at 2250 mg/kg-day, and a significant increase in axial skeletal malformations was evident in pups from the 2250 mg/kg-day group at pnd 22. However, there were significant decreases in dam weight on GD 20 at the highest dose level, with reduced fetal viability and pup weight.

In another study, the developmental toxicity NOAELs of EG were determined in CD rats and CD-1 mice (Neeper-Bradley et al., 1995). Dams were dosed with the compound on gestation days 6-15; rats receiving 0, 150, 500, 1000, or 2500 mg/kg/day and mice receiving 0, 50, 150,

500, 1500 mg/kg/day. Rat maternal effects in the 2500 mg/kg/day group were (1) increased water consumption and reduced body weights during the gestation period and (2) increased liver and kidney weights on gestation day 21. The maternal liver weights were also increased in the 1000 mg/kg/day group. For the fetuses, hydrocephaly, gastroschisis, umbilical hernia, fused, duplicated, or missing arches, centra, and ribs, poor ossification in thoracic and lumbar regions, and reduced body weights were observed in the 2500 mg/kg/day group. The fetuses from the 1000 mg/kg/day group had reduced body weights, duplicated or missing ribs, centra, and arches, and poor ossification. In mice no apparent maternal toxicity was noted. Fetal effects were (1) reduced body weights, fused ribs and arches, poor ossifications in thoracic and lumbar centra, and increased occurrence of an extra 14th rib in the 1500 mg/kg/day group and (2) slight reductions in fetal body weight and increased incidences of extra ribs in the 500 mg/kg/day group. The NOAELs for developmental toxicity were 500 mg/kg/day for rats and 150 mg/kg/day for mice, and the LOAELs for developmental toxicity were 1000 mg/kg/day for rats and 500 mg/kg/day for mice.

These values were similar to NOAELs specified in a study by Yin et al. (1985) in which rats (strain not stated) were given oral bolus doses of 253, 638, 858, 1078, or 1595 mg/kg/day on gestation days 6-15. The NOAELs for maternal and fetal toxicity were 1078 and 638 mg/kg/day, respectively.

Rabbits were the animal model in a developmental study of Tyl (1993). New Zealand White rabbits were gavaged with 100, 500, 1000, or 2000 mg/kg/day on gestation days 6-19. In the 2000 mg/kg/day group, 42% of the dams died, and four of the dams delivered early or aborted. No clear maternal toxicity signs were observed in the 1000 mg/kg/day group. Aside

from the maternal toxicity and aborted fetuses in the 2000 mg/kg/day group, the surviving fetuses exhibited no developmental effects. The lower dose groups were also without any fetal effects. The author concluded that the developmental NOAEL was greater than 2000 mg/kg/day in rabbits.

Tyl et al. (1995a) exposed CD-1 mice via inhalation (nose-only) to EG in an aerosol at exposure concentrations of 0, 500, 1000 or 2500 mg/m^3 on gestational days 6 through 15. In the dams, kidney weights were increased in the 1000 and 2500 mg/m^3 groups. The fetuses, however, in the 2500 mg/m^3 group had decreased fetal body weights/litter and increased incidences of fused ribs and skeletal variations. The NOAEL for developmental toxicity was 1000 mg/m^3.

1.5.1. Mutagenicity. *In vitro* mutagenicity studies using Salmonella typhimurium resulted in negative findings (Clark et al., 1979; McCann et al., 1975; Pfeiffer and Dunkelberg 1980; Zeiger et al., 1987). In another *in vitro* study, repair-deficient strains of Escherichia coli were exposed to EG which resulted in no growth inhibition due to deoxyribonucleic acid damage (McCarroll et al., 1981). Gene mutation in yeast, Schizosaccharomyces pombe (Abbondanolo et al., 1980), and aneuploidy induction in the fungus, Neurospora crassa (Griffiths 1979, 1981), were not observed after exposure to EG.

1.6. TOXICOKINETIC STUDIES

1.6.1. Toxicokinetics. Frantz et al. (1996) reported a detailed study of the rates and extent of absorption and excretion of ^{14}C-labeled EG when administered to male CD (Sprague-Dawley) rats via intravenous, oral, or dermal routes. Animals that had been implanted with a cannula in the jugular vein to collect blood samples, were housed in metabolic cages to facilitate the

collection of urine, feces, and expired CO_2. Labeled EG was mixed with unlabeled EG and used as a trace for dosing levels of 10 and 1000 mg/kg intravenously, 10, 400, 600, 800, and 1000 mg/kg by gavage, and 10 and 1000 mg/kg via the skin. In the gavage studies, four rats per group were dosed, then blood samples taken at intervals up to 96 hours. In the dermal studies, animals were dosed by means of a closed patch, then sacrificed after 96 hours, and the blood collected by cardiac puncture. Unmetabolized EG and its metabolites, glycolate, glycolaldehyde, glyoxylic acid, and oxalic acid were measured in plasma after resolution on an HPLC/ion exclusion column.

EG administered intravenously was cleared rapidly and monoexponentially from plasma up to 8 hours post-dosing. For oral administration, absorption was near complete with a $t_{1/2}$ of between 20-27 minutes depending on the dose. However, a biphasic mode of elimination was identified, with an initial $t_{1/2}$ of 1.42 hours. Unchanged EG was also evident in the blood after percutaneous administration of 1000 mg/kg EG, though some metabolism had occurred during the transfer. Of the EG metabolites identified, glycolate was the major component, though glyoxylate/glyoxal (unresolved in the chromatographic system employed) and glycolaldehyde were also identified. Interestingly, though no oxalate was identified in plasma, some counts appeared in the trichloroacetic acid (TCA) precipitate, indicating transfer of some of the labeled carbon moiety into protein.

The major route of elimination was as $^{14}CO_2$ via exhalation, with overall levels inversely related to dose. Thus, at higher concentrations, proportionally more (~50%) was excreted in urine, suggesting that the oxidative metabolic pathways are capable of saturation. The important labeled components of urine were EG itself and glycolate, although oxalate was detected at levels

during the first 12 hours after intravenous or oral dosing. During the 12-24 hour period, oxalate was detected at slightly higher levels after a 1000 mg/kg dose. In some animals receiving EG intravenously or by gavage, tissue distribution studies indicated >90% recovery of activity, with (after 96 hours) 41.2% exhaled, 27.3% in urine, 2.9 in feces, 8.5% in tissues, and 9.9% in the carcass.

1.6.2. Metabolism. Frantz et al. (1996) identified a number of metabolic products of EG, such as glycolate, glycolaldehyde, and glyoxylic acid, as discussed in section 1.6.1. Since the profound kidney lesions induced by EG are associated with oxalate formation, the identification of this metabolite also, appears to forge the link between metabolic activities and their histopathological consequences. However, the paper fails to explicitly state whether the oxalate was ^{14}C-labeled, thereby leaving unresolved the question of a direct precursor-product relationship between EG and oxalate.

1.6.3. Percutaneous Absorption. Because percutaneous absorption represents a common exposure route for humans to EG, Tyl et al (1995b) examined the compound's developmental toxicity when applied dermally to CD-1 mice. Groups of 30 copulation plug-positive females were exposed on GDs 6-15, 6 hours/day, to 0, 12.5, 50, or 100% EG in a closed patch (0.1 mL per patch, with nominal exposure levels of 0, 404, 1677, or 3549 mg/kg-day, as calculated by the authors). Dams were sacrificed on GD 18, with a full suite of developmental parameters examined in the fetuses. Though three out of 30 dams receiving 2250 mg/kg-day displayed minimal kidney tubular lesions, no developmental effects were evident in any treatment group. By contrast, in a positive control group of pregnant females receiving 3000 mg/kg-day on GDs 5-

15, the dams displayed characteristic kidney damage, and fetal body weights were reduced in a highly statistically significant manner.

The Frantz et al. (1996) study featured dermal exposure of a subset of male CD (Sprague-Dawley) rats to ^{14}C-labeled EG in a closed patch. Detection of radiolabelled EG in the blood allowed the conclusion that a proportion of the applied dose had been transported across the dermal barrier into the blood stream. However, the presence of other labeled products suggested that some metabolic changes could have occurred in transit.

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

Available data on EG are insufficient to evaluate the structure-activity relationships of this compound.

1.8. MECHANISTIC STUDIES

At present there are no studies on the mechanism by which EG causes toxicity.

2. INTERPRETATION OF AVAILABLE INFORMATION

Ethylene glycol (1,2-dihydroxyethane, 1,2-ethandiol, EG) (CAS No. 107-21-1) is a commercially-important chemical produced by the hydrolysis of ethylene oxide. It is soluble in aqueous and organic media, and finds utility as a solvent for cosmetics, pharmaceuticals, food additives, etc. Its application as a de-icing compound for the automotive and aviation industries ensures that considerable amounts of the compound will be lost to the environment. The compound is subject to the community right to know provisions of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), as amended by the Superfund Amendments and Reauthorization Act (SARA). It is listed as a hazardous air pollutant in section 112 of the Clean Air Act (CAA), in 40CFR parts, 152, 180, 185, or 186 persuant to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), in 29CFR 1910.119-App/A-92 of the Occupational Safety and Health Act (OSHA), and as a regulated compound by the State of California.

Though limited amounts of information are available on the compound's toxicological or pharmacological capacities, acute lethal dose values in the 4000-5000 mg/kg-day range have been identified for a number of animal species. Though the toxicological information available for EG is not extensive, the induction of kidney lesions described in this Toxicity Assessment is a well-documented consequence of EG exposure, to the extent that the compound is used as an experimental inducer of oxalate-related nephrosis in laboratory animals for application to pharmacological studies targeting amelioration of this condition. The other major toxic

consequence of EG exposure appeared to be the induction of developmental effects, as set forth in section 1.5.

No data addressing carcinogenicity, structure-activity relationships, or mechanisms of toxicity were found in the biomedical or pharmacotoxicological literature.

Available data are insufficient to support the development of a carcinogenic slope factor for the compound. However, IRIS reports a verified chronic oral RfD value for EG of 2E+O mg/kg-day to protect against chronic nephritis, altered hematocrit, "kidney hemoglobin", and increased mortality. Since a number of studies described here reflect EG's association with the induction of fetal abnormalities as well as kidney lesions, the need to further define a sub-threshold benchmark dose that could protect against both important toxicological consequences of the compound is emphasized.

3. DOSE-RESPONSE ASSESSMENT FOR NON-CANCER EFFECTS

3.1. INGESTION EXPOSURE

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Ethylene Glycol (1,2-dihydroxyethane, 1,2-ethanediol, EG)
CASRN -- 107-21-1
Preparation Date -- December 1996

ORAL RfD SUMMARY

Critical Dose -- 554 mg/kg-day
UF -- 1000
MF -- 1
RfD -- 5E-1 mg/kg-day
Confidence -- Medium

Critical Study

Critical Effect -- Dose-dependent incidence of kidney lesions, with increased plasma BUN and creatinine

Study Type -- Subchronic oral (drinking water)

Reference -- Robinson et al., 1990

NOAEL -- 554 mg/kg-day
NOAEL(ADJ) --

LOAEL -- 1108 mg/kg-day
LOAEL(ADJ) --

Conversion Factors and Assumptions --

PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Robinson, M. C.L. Pond, R.D. Laurie, et al. (1990) Subacute and subchronic toxicity of ethylene glycol administered in drinking water to Sprague-Dawley rats. Drug Chem. Toxicol. 13:43-70.

In the subchronic part of this study, 10 Sprague-Dawley rats/sex/group were exposed to concentrations of 0, 0.5, 1.0, 2.0, or 4.0 % for females, and 0, 0.25, 0.5, 1.0 and 2.0 % for males, in drinking water for 90 days. These approximated to exposure levels of 0, 554, 1108, 2216, and 4432 mg/kg-day respectively, for females, and 0, 227, 554, 1108, and 2216 mg/kg-day respectively, for males, based on an estimated drinking water intake of 100 mL/kg-day (data calculated by the authors). At the highest dose, 8/10 female and 2/10 males rats died prior to term. At this exposure level also, the survivors displayed depressed body weights compared to controls, and comparatively reduced hemoglobin levels, hematocrit, and erythrocyte and leucocyte counts. However, the most consistent toxicological features relating to EG exposure were histopathological manifestations of kidney damage in male rats, with marked tubular dilation and/or degeneration and the presence of calcium oxalate crystals. Possibly associated with these lesions was a dose-dependent increase in kidney weights among the males, and changes in the plasma creatinine and blood urea nitrogen (BUN), that both rose dose-dependently to significantly higher levels than those of the control group. By contrast, the kidney lesions and their associated clinical chemistry parameters remained at control levels for female rats, a discrepancy mimicked by the differential responses to EG evident in those rats receiving the compound for only 10 days (the acute study). Taken together, the kidney and related clinical chemistry lesions identified in male rats would provide a NOAEL of 554 mg/kg-day; with 1108 mg/kg-day as the LOAEL.

UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

Ten for protection of sensitive subpopulations, 10 for interspecies extrapolation, and 10 for subchronic to chronic exposure.

UF -- 1000

MF -- 1

ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

Developmental Study: Lamb et al., 1985:

The continuous breeding study used drinking water concentrations of 0, 0.25, 0.5, or 1.0% (exposure levels of 0, 400, 800, or 1600 mg/kg/day, respectively) to examine the developmental effects of EG in CD-1 mice (Lamb et al. 1985). The findings were slight, but statistically significant decreases in the numbers of litters per fertile pair, live pups per litter, and live pup weight in the 1% group. Facial anomalies and skeletal defects (reduced size of skull bones, fused ribs, and abnormally shaped sternebrae and vertebrae) were observed in the 1% group. No defects were found in the 0.25 and 0.5% groups. Though two females died in the 0.5% group (which may have been related to the oxalate crystal deposition in the kidney), in general no signs of maternal toxicity were evident at any concentration. Importantly, these data

demonstrated a suite of compound-related fetal abnormalities in the absence of maternal toxicity, thereby allowing the conclusion that, for CD-1 mice, EG can perturb organogenic systems directly while the general state of maternal well-being remained unaffected. The NOAEL for fetal abnormalities may be taken as 800 mg/kg-day, which converts to a chronic (for fetal exposure) oral RfD of 8E+0 under the combined uncertainty factors of 10 for species to species extrapolation and 10 to protect sensitive subpopulations.

The IRIS record for EG (U.S. EPA 1996) contains a verified RfD derived from a 2 year oral feeding study in CD-1 mice and F344 rats of 2E+0 mg/kg-day, in which the primary toxicological effect (rats only) was an increased incidence of crystal deposition in the renal tubule epithelium, with some changed hematological parameters. An uncertainty factor of 100 including 10 for interspecies variation and 10 to protect sensitive subpopulations was used to convert a NOAEL of 200 mg/kg-day to the 2E+0 value.

CONFIDENCE IN THE ORAL (RfD)

Study -- Medium
Data Base -- Medium
RfD -- Medium

U.S. EPA (1996) considered confidence in the verified RfD of 2E+0 mg/kg-day derived from the DePass et al. (1986a) study to be high, because of the study's apparent quality (large number of animals used, clear-cut effects and thorough documentation) and because of its potential to protect from teratogenic and reproductive effects. The latter conclusion is reinforced by the RfD (8E+0 mg/kg-day) derived from the data of Lamb et al. (1985) who demonstrated developmental toxicity effects unrelated to maternal toxicity.

The utility of the Robinson et al (1990) study for developing a chronic oral RfD for EG compared to that in U.S. EPA (1996) may be judged by (1) its lower and therefore more conservative estimate of a likely subthreshold exposure level to protect against the primary toxic effect (kidney lesions), and (2) the parallel statistically significant increases in clinical chemistry responses potentially related to these effects. That such a relationship exists is supported by the converse absence in female rats of histopathologically-defined kidney lesions, and of any dose-related increases in plasma BUN or creatinine.

The Robinson study is thoroughly documented, complete, and represents a significant addition to the database. Its demonstration of clinical chemistry changes that are directly related to the histopathological kidney lesions reinforces their potential utility as a marker for the onset of such lesions, a suggestion supported by the appearance of these effects in the high-dose male rats receiving EG in the DePass et al. (1986a) study.

By contrast, confidence in the Robinson et al. (1990) study may be partially diminished by the relatively small number of animals tested (10 per sex per group), and by the subchronic (90 day) dosing regimen. Though the derivation of a chronic oral RfD of 5E-1 mg/kg-day for EG from this study presented here has defaulted to an uncertainty factor of 10 for subchronic to chronic conversion, resulting in an overall uncertainty factor of 1000, the case for applying a lower (3 or zero) uncertainty component for this conversion should be evaluated. Either alternative would derive an RfD more closely similar to the verified value derived from the DePass et al. (1986a) study (U.S. EPA 1996). In any event, the potential chronic oral RfDs for EG presented here (5E-1, 2E+0, and 8E+0) represent a sufficiently narrow range of subthreshold exposure levels to allow the conclusion that, within our current understanding, the human impacts of exposure to EG can be regulated.

EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document -- U.S. EPA 1986. Health Effects Assessment for Ethylene Glycol: prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Emergency and Remedial Response, Washington, DC.

Other EPA Documentation --

Agency Work Group Review -- 3/19/97

Verification Data -- 3/19/97

EPA CONTACTS (ORAL RfD)

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U.S. ARMY CONTACTS

3.2 INHALATION EXPOSURE

Available data are insufficient to support development of a chronic inhalation exposure (RfC) estimate for EG.

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- Ethylene Glycol
CASRN -- 107-21-1
Last Revised -- No data

RfC SUMMARY

Critical Concentration --
UF --
MF --
RfC --
Confidence --

RELEVANT STUDIES (INHALATION RfC)

Tyl, R.W., Ballantyne, B., Fisher, L.C., Fait, D.L., Dodd, D.E., Klonne, D.R., Pritts, I.M., and Losco, P.E. 1995a. Evaluation of the developmental toxicity of ethylene glycol aerosol in CD-1 mice by nose-only exposure. Fundamental and Applied Toxicology 27:49-62.

The developmental toxicity of ethylene glycol was investigated via inhalation (nose-only) in CD-1 mice. Dams were exposed to 0, 500, 1000 or 2500 mg/m^3 on gestation days 6-15 for 6 hr/day. The average mass median aerodynamic diameter was 2.6 μm for the three ethylene glycol groups. In the 1000 and 2500 mg/m^3 groups, the dams had increased kidney weights but no renal lesions. The fetuses from the 2500 mg/m^3 group had decreased body weights/litter and increased incidences of fused ribs and skeletal variations. Since the maternal and developmental toxicity NOAELs were 500 and 1000 mg/m^3 respectively, these data were considered unsuitable for developing a chronic inhalation RfC for EG, because of the attendant uncertainty in ascribing the fetal responses to unequivocal effects of EG on organogenesis rather than to a potential secondary consequence of maternal toxicity.

3.3. DERMAL EXPOSURE

Available data are insufficient to support development of a chronic dermal exposure (RfD_d) estimate for EG.

REFERENCE CONCENTRATION FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- Ethylene Glycol
CASRN -- 107-21-1
Last Revised -- No data

4. DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Data are insufficient ro assess the carcinogenicity of EG in humans or animals for oral or inhalation exposures.

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

EG cannot be classified as to potential human carcinogenicity because of the lack of adequate data.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Not classifiable

Basis -- Lack of adequate human or animal data

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Attachment 1

TOXICITY ASSESSMENT FOR JP-8

United States Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment
and
National Exposure Research Laboratory

and

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Aberdeen Proving Ground, MD 21010

NOTICE

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PREFACE

This report assesses the potential non-cancer and cancer effects of JP-8, a kerosene-based aviation fuel. Information pertaining to non-cancer effects of JP-8 vapors was previously assessed by the Committee on Toxicology of the National Research Council (COT, 1996). An ATSDR Toxicological Profile for JP-5 and JP-8 is currently undergoing peer review (ATSDR, 1995a). An ATSDR Toxicological Profile for JP-4 and JP-7 is available (ATSDR, 1995b).

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This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

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ABSTRACT

JP-8 is a kerosene-based jet fuel consisting primarily of saturated alkanes (paraffins) and cycloalkanes (naphthenes), and not more than 25% by volume of aromatics. JP-8 may also contain additives such as antioxidants, static inhibitors, corrosion inhibitors, icing inhibitors, lubrication improvers, biocides, and heat stabilizers (ATSDR, 1995a). The exact composition of jet fuel varies depending on the crude oil from which it is refined and the types of additives used.

Acute exposures to high concentrations of JP-8 vapors are likely to affect the central nervous system and result in such symptoms as staggering gait, slurred speech, peripheral sensory loss, muscular weakness, decreased sensation to pain, headache, nausea, euphoria, memory deficits, and impaired hand-to-eye coordination, as has been observed in individuals exposed to high concentrations of JP-4 or JP-5 vapors. Epidemiological studies on workers exposed to jet fuel vapors have documented a high incidence of neurasthenic symptoms; however, it cannot be determined from the available data whether such effects resulted from chronic low-level exposures or intermittent exposures to peak concentrations above the permissible occupational exposure limit.

Subchronic exposure of rats to JP-8 vapors caused no adverse changes in pulmonary function and no damage to the lungs; however, in studies in which rats were exposed to JP-8 aerosols histopathological evidence of lung damage and pulmonary function deficits were recorded. Male rats exposed subchronically to JP-8 vapors, or dosed by gavage with liquid JP-8, developed kidney disease; however, the observed kidney effects were attributed to α2-microglobulin nephropathy which is specific to male rats and not relevant for human exposures. Although slight changes in liver weight and in liver enzyme activity have been observed in experimental animals exposed to JP-8, these changes were not accompanied by histopathological evidence of liver damage. In a developmental toxicity study conducted on rats, JP-8 was found to have no teratogenic activity.

Epidemiological studies evaluating the potential carcinogenicity of jet fuel vapors have not revealed any association between exposures and increased tumor incidences. The major components of jet fuels such as JP-8 are not known carcinogens and such fuels normally contain relatively small amounts of known carcinogens such as benzene and PAHs.

An oral RfD of 1 mg/kg/day was calculated for JP-8, based on a NOAEL of 3000 mg/kg/day for rats dosed by gavage. An inhalation RfC of 0.4 mg/m^3 was calculated for JP-8 vapors, based on a NOAEL of 1000 mg/m^3 for rats exposed to JP-8 for 90 days. No information is available on the dermal toxicity of JP-8 to derive a dermal RfD. Data are insufficient to assess the carcinogenicity of JP-8 in humans or animals.

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1. SUMMARY OF TOXICITY INFORMATION

JP-8 is a kerosene-based jet fuel consisting primarily of saturated alkanes (paraffins) and cycloalkanes (naphthenes), and not more than 25% by volume of aromatics (IARC, 1989). The benzene content of jet fuels is normally below 0.02% and polynuclear aromatic hydrocarbon (PAH) levels are very low (IARC, 1989). JP-8 may contain additives such as antioxidants, static inhibitors, corrosion inhibitors, icing inhibitors, lubrication improvers, biocides, and heat stabilizers (ATSDR, 1995a). The exact composition of jet fuel varies depending on the crude oil from which it is refined and the types of additives used.

The toxicity data for JP-8 are very limited; therefore, in the following sections supplemental information on the toxicity of JP-4 and JP-5 is also provided. According to COT (1996), the toxicity from the vapors of all these fuels is expected to be similar.

1.1. EPIDEMIOLOGIC STUDIES

Epidemiological studies have not been conducted specifically on JP-8; however, some information is available for jet fuels in general. The effects of chronic exposure to jet fuels in factory workers have been investigated by Knave et al. (1976, 1978, 1979). An initial evaluation of 29 aircraft factory workers exposed to jet fuel vapors revealed a high incidence of dizziness, respiratory tract symptoms, heart palpitations, a feeling of pressure on the chest, nausea, and headache (Knave et. al., 1976). These symptoms were reported in 13 of 13 heavily exposed workers and in 7 of 16 less exposed workers. Further studies revealed that, in comparison to a control group, workers exposed to jet fuel exhibited 1) significant differences in the incidence and prevalence of psychiatric symptoms, 2) significant differences in psychological tests

focusing on attention and sensorimotor speed, and 3) significant differences in electroencephalograms (Knave et al., 1978). Additionally, medical interviews and testing revealed that the workers exposed to jet fuel vapors had a higher occurrence of neurasthenic symptoms than controls ($p < 0.001$); particularly for fatigue, anxiety, mood changes, memory difficulties, and various psychosomatic symptoms (Knave et al., 1979). Information was not provided in the available abstracts as to whether exposure to other airborne chemicals contributed to the reported symptoms. It was estimated that the workers mean exposure duration was 17 years, and 300 mg/m^3 was calculated as a rough estimate of the time-weighted average workplace concentration, with peak concentrations ranging from 1200 to 3200 mg/m^3 (Knave, et al., 1978). The results of the studies of Knave and coworkers were considered questionable by COT (1996) for a number of reasons including weak and inconsistent evidence of impairment, inadequate methods of evaluation, inadequate consideration of confounding factors, small cohort of exposed workers, and lack of quantitative information on exposure levels (COT, 1996).

COT (1996) reviewed the results of several epidemiological studies conducted on military personnel and nonmilitary workers exposed to jet fuels. No significant increases in total neoplasms or site-specific neoplasms occurred in a cohort of 2,182 men exposed to jet fuel in the Swedish armed forces (Selden and Ahlbor, 1986, 1987). It was reported that jet fuel vapor concentrations to which this cohort was exposed exceeded 350 mg/m^3. IARC (1989) noted that this study was able to detect only large increased cancer rates because of the short follow-up period (6-7 years) and the young age of the participants. In another study of workers on a Swedish military base who were exposed to one or more of several jet fuel types (equivalent to JP-4, JP-1, Jet A-1, and a leaded synthetic fuel), Selden and Ahlborg (1991) could find not

statistical evidence of increased cancer morbidity or mortality rates. COT (1996) notes that this study was possibly limited by the short follow-up period (9-10 years) and by possible bias in the process for selecting the study participants. Siemiatycki et al. (1987) reported a significant increased risk of kidney cancer (odds ratio 2.5; C.I. = 1.1-5.4) among workers exposed to jet fuel (7 of 43). However, six of the 7 individuals were also exposed to aviation gasoline which was associated with kidney cancer at similar risk levels, and additional regression analysis of the data suggested that the kidney cancers were more likely due to exposure to aviation gasoline than to jet fuel.

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity. Information on the acute toxicity of JP-8 to humans was not found in the available literature. Acute inhalation exposures to other jet fuels such as JP-4 and JP-5 result in CNS disturbances resulting in staggering gait, slurred speech, peripheral sensory loss, muscular weakness, decreased sensation to pain, headaches, nausea, euphoria, memory deficits, and impaired hand-to-eye coordination (COT, 1996). Similar effects might be expected following exposures to high concentrations of JP-8 vapors.

1.2.2. Animal Toxicity.

1.2.2.1. Acute/Subacute Toxicity —

1.2.2.1.1. Oral Toxicity. Information on the acute oral toxicity of JP-8 to animals was not found in the available literature. [Additional information on the acute toxicity of JP-8 is available from Lt Cmdr. Warren Jederberg of the U.S. Naval Medical Research Institute].

1.2.2.1.2. Inhalation Toxicity. In short term tests conducted by Pfaff et al. (1995), changes in pulmonary function and substance P levels were seen in F344 rats exposed to an

aerosol of JP-8 equivalent to a concentration of about 500 mg/m^3. Exposures were nose only for 1 hr daily for 7 or 28 days. An increase in dynamic compliance ($p < 0.05$) was seen after 7-day exposure to 520 mg/m^3 but not after 28-days exposure to 495 mg/m^3. Pulmonary resistance was increased in both the 7-day and 28-day exposure groups ($p < 0.05$). Changes in pulmonary function were accompanied by a decrease in substance P concentrations from bronchoalveolar lavage fluid (BALF). Both exposed groups gained significantly less weight during the study period than the control groups ($p < 0.05$). Wet lung/body weight ratio was significantly increased in animals exposed to JP-8 aerosol for 7 days ($p < 0.05$), but not in those exposed for 28 days. Alveolar clearance of technetium-labelled diethylenetriamine pentaacetate was increased in the exposed groups. Light microscopy revealed no evidence of lung injury. According to Pfaff et al. (1995), the recovery of pulmonary compliance and relative lung weight in the 28-day exposure group indicated that the exposed rats were developing a tolerance to the jet fuel. As part of the same test program, male F344 rats were exposed to 500 or 1000 mg JP-8 aerosol/m^3, 1 hr per day for 7 or 28 days (Parton et al., 1993). Liver, kidney and spleen weights were significantly increased in both exposure groups ($p < 0.001$), and pathological lesions were seen in the kidney and spleen, but not in the liver. Plasma ALT activity was normal (Parton et al., 1993).

In related studies reported by Hays et al. (1995), changes in lung permeability and lung histology were evaluated in F344 rats exposed to a JP-8 aerosol at a concentration of 500 or 813-1094 mg/m^3, 1 hr per day for 7, 28 or 56 days. Lung epithelial permeability was measured by alveolar clearance of technetium-labelled diethylenetriamine pentaacetate. Clearance rates for exposed rats was dependent on both JP-8 concentration and exposure duration. Clearance values

were not significantly increased in the 7-day exposure group but were increased in both 28-day exposure groups ($p < 0.05$). In the 56-day exposure groups, clearance was significantly increased ($p < 0.05$) in the group exposed to 813-1094 mg/m^3, but not in the group exposed to 500 mg/m^3. Changes in lung histopathology in the exposed animals could be correlated with the changes in lung permeability. The lungs of animals in all exposed groups exhibited interstitial edema resulting from endothelial damage. There was also an apparent thickening of the alveolar septa and activation of the alveolar macrophages. Extensive endothelium damage was seen in the 7-day and 28-day exposure groups, but the damage appeared less severe in animals exposed for 56 days, suggesting that some recovery had taken place. However, Hays et al. (1995) noted that exposure to JP-8 aerosol may have induced a fibrotic response in animals exposed for 56 days.

In a pilot study conducted on specific pathogen-free New Zealand White rabbits, Whitten et al. (1990) found that exposure to a aerosol/vapor mixture of JP-8 resulted in increases in BALF cell counts and substance P levels, and an increase in pulmonary epithelial permeability as shown by clearance of technetium-labelled diethylenetriamine pentaacetate.

In rats treated with capsaicin and subsequently exposed to 497 mg/m^3 JP-8 (the physical form of the JP-8 was not reported) 1 hour per day for 7 days, a marked increase in sensitivity of the airways to histamine was observed (Witten et al., 1992a).

1.2.2.2. Subchronic Toxicity —

1.2.2.2.1. Oral Toxicity. Male Sprague Dawley rats (10/group) were dosed daily by gavage with 0, 750, 1500, or 3000 mg JP-8/kg/day for 90 days (Mattie et al., 1995). The test material was administered without a solvent vehicle. There were no clinical signs of toxicity in any of the exposed groups except for statistically significant and dose-related reductions in body

weight; however, food and water consumption were not measured and the extent that the animals were anoretic could not be determined. The major histopathological lesion seen in the test animals was α2-microglobulin-related nephropathy. This lesion is not considered to be relevant to human exposures (U.S. EPA, 1991). Clinical chemistry analyses revealed statistically significant changes in a number of parameters, but none were dose-related. A statistically significant decrease in glucose and statistically significant increases in total bilirubin, AST, and ALT occurred at all dose levels. Neutrophil counts were increased and lymphocyte counts decreased in all three dose groups, with the greatest changes seen at the highest dose. Absolute organ weights were not changed significantly at any dose level. Relative organ weights (brain, liver, kidney, spleen, and testes) were significantly ($p < 0.05$) increased in the high-dose group. Perianal dermatitis and gastritis occurred in animals in all dose groups but not in the controls. According to the investigators, "the most likely cause of the gastritis and perianal dermatitis is a contact irritation limited to squamous epithelium" (Mattie et al., 1995). The absence of clinical or histopathological signs except for the α2-microglobulin-related nephropathy indicate that the highest dose level of 3000 mg/kg/day can be considered a NOAEL for the purpose of developing an oral RfD.

1.2.2.2.2. Inhalation Toxicity. Fischer 344 rats (95 males and 75 females) and C57BL/6 mice (100 mice per sex) were exposed to JP-8 vapors (0, 500, or 1,000 mg/m^3) continuously for 90 days (Mattie et al., 1991). At termination of the exposure period, 15 rats and 25 mice/sex/dose group were sacrificed and necropsied. The remaining animals were observed for up to 21 months, with interim sacrifices at 2 weeks, 2 months and 9 months post-exposure. No biologically significant treatment-related changes in clinical chemistry parameters were

observed in the mice or rats. Liver enzyme changes reported earlier by McEwen and Venot (1985) for this same study were not verified. Male rats exposed to JP-8 exhibited a statistically significant decrease in body weight and statistically significant increases in absolute and relative kidney weight. Body weight and kidney weights of female rats, and liver and spleen weights of both males and females, were not affected by exposure to JP-8. At final sacrifice the only non-neoplastic lesion observed in female rats was an increase in splenic hematopoiesis. This change was statistically dose-dependent but usually of only minimal severity and, therefore, not considered to be treatment-related. Male rats exposed to JP-8 developed kidney lesions consisting of hyaline droplet formation in the proximal convoluted tubules, granular casts in the outer medulla, and exacerbated lesions of chronic progressive nephrosis. These kidney lesions are specific to male rats and not considered relevant to humans. Male rats also exhibited a dose-related increase in incidence and severity of epithelial cells in the urine. The only other non-neoplastic lesion seen in male rats was hepatic basophilic foci which occurred in 11% of the control group, 35% of the low-exposure group, and 31% of the high exposure group. The increases were of statistical significant, but of uncertain biological significance (Mattie et al., 1991). Most non-neoplastic lesions seen in the mice exposed to JP-8 (inflammatory skin lesions, splenic hematopoiesis, eosinophilic degeneration of nasal epithelial cells, inflammatory changes of the anus, liver, and kidney, focal mineralization in the brain, and tooth malformations) were considered incidental since incidences were comparable among groups. Deposition of amyloid material in the ileum of male mice (4 of 28 in the high dose group only) was considered as possibly, but not likely related to the exposure to JP-8 (Mattie et al., 1991). Compared to controls, a significantly higher mortality rate occurred in male mice; however, Mattie et al.

(1991) noted that much of the mortality was due to necrotizing dermatitis that resulted from fighting. Because of the absence of any clear dose-response for any reported effect, the high exposure of 1000 mg/m^3 is considered to be a NOAEL for rats and mice exposed to JP-8.

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. Long-term carcinogenicity studies have not been conducted on JP-8. No treatment-related tumors were found in F344 rats (95 males and 75 females) or in C57BL/6 mice (100 males and 100 females) exposed to JP-8 vapor concentrations of 500, or 1,000 mg/m^3 continuously for 90 days and then held for observation for 20 or 21 months (Mattie et al., 1991). In a chronic exposure study in which F344 rats and C57BL/6 mice were exposed to JP-4 (6 hr/day, 5 days/wk for 12 months), the only observed neoplastic lesion was benign hepatocellular adenomas which occurred at a slightly increased incidence in female mice exposed to 5000 mg/m^3; however, the trend was reversed in male mice. No neoplastic changes were seen in rats or mice exposed to 1000 mg/m^3 (Bruner et al., 1993).

According to IARC (1989), the major components of jet fuels such as JP-8 are not known carcinogens and such fuels normally contain relatively small amounts of known carcinogens such as benzene and PAHs. Epidemiological studies evaluating the potential carcinogenicity of jet fuel vapors have not revealed any association between exposures and increased tumor incidences (see section 1.1); however, most the available data are from studies with relatively short follow-up periods.

1.3.2. Chronic Toxicity. Long-term chronic exposure studies have not been conducted on JP-8. however, some information is available for related fuels. In a chronic exposure study in which F344 rats and C57BL/6 mice were exposed to JP-4 (1000 or 5000 mg/m^3, 6 hr/day, 5 days/wk)

for 12 months, no significant target organ pathology was observed except for male rat specific α2-microglobulin nephropathy (Bruner et al., 1993).

1.4. REPRODUCTIVE TOXICITY STUDIES

Information on the reproductive toxicity of JP-8 were not found in the available literature. A reproductive toxicity study is currently being undertaken (D. Mattie, pers. com.).

1.5. DEVELOPMENTAL TOXICITY STUDIES

In a developmental toxicity study conducted on Sprague-Dawley rats, JP-8 was found to have no teratogenic effects (Cooper and Mattie, 1996). Female rats (30 animals/group) were dosed with 0, 500, 1,000 or 2,000 mg/kg/day by gavage on days 6-15 of gestation. The number and type of fetal malformations and variations were not significantly different between dose groups. Significant decreases in body weight gain were observed in dams in the 1,000, 1,500 and 2,000 dose groups. Fetal body weights were significantly reduced in the two highest dose groups.

1.5.1 Mutagenicity. The mutagenicity of JP-8 was evaluated by Brusick and Matheson (1978) in a series of bioassays. JP-8 did not induce mutations in *Salmonella typhimurium* when tested in the Ames assay, nor did it induce mutations in L5178Y mouse lymphoma cells at the TK locus; however, it did cause a moderate increase in unscheduled DNA synthesis in WI-38 cells. JP-8 was also found to be negative in dominant lethal assays using rats and mice (Brusick and Matheson, 1978).

1.6. TOXICOKINETICS AND METABOLISM

1.6.1. Toxicokinetics. JP-8 is a complex mixture of hydrocarbons whose toxicokinetics are determined to a great degree by the tissue partitioning coefficients for each individual component

(COT, 1996). Chemicals with a high solubility in blood (a large blood/air partition coefficient) will be absorbed to a greater extent into the body than chemicals with a low solubility. Components of jet fuel vapors that have a high fat/air or fat/blood partitioning coefficient will more easily be sequestered in adipose tissue during exposure, and may then be slowly released from such tissues after the exposure has ceased. Toxicokinetics may be a key factor in determining the type and extent of toxic effects elicited by the volatile hydrocarbon components of jet fuels. Maximum CNS effects may result from peak exposure concentrations due to high blood levels, whereas long-term effects may result from sequestering in adipose tissue of those components having a high fat/blood partitioning coefficient.

1.6.2. Metabolism. The first step in the metabolism of volatile organic hydrocarbons such as those in JP-8 is oxidation of the liphophilic chemical to an oxidized or more water soluble chemical (COT, 1996). Metabolism is achieved through the cytochrome P-450-dependent monooxygenase pathway which can involve aliphatic hydroxylation, aromatic hydroxylation, epoxidation, *n*-dealkylation, *o*-dealkylation, deamination, sulfoxidation, or *n*-oxidation. Oxidation is often followed by a phase II reaction which conjugates the oxidized hydrocarbon to a water-soluble substrate such as sulfate or glucuronic acid (COT, 1996). Information is available on the metabolism of individual components of jet fuel vapors, the most important of these being benzene, n-hexane, toluene, xylene, trimethylpentane, and methoxyethanol (added to fuels as a deicer); however, as noted by COT (1996), the metabolism of one component can be enhanced or inhibited by the presence of one or more of the other components.

2. INTERPRETATION OF AVAILABLE INFORMATION

The limited data available indicate that JP-8 vapors are relatively low in toxicity. Although exposure to JP-8 vapors results in adverse kidney effects in male rats, these effects are not relevant to humans. According to COT (1996), all volatile hydrocarbons are CNS depressants and can produce anesthesia or asphyxia at high concentrations. Although epidemiological studies suggest that CNS effects can result from exposure to jet fuel vapors, it is likely that these effects will only occur at very high concentrations. Slight changes in liver weight and in liver enzyme activity have been observed in experimental animals exposed to jet fuel vapors; however, no histopathological evidence of liver damage has been observed in animals dosed orally with JP-8 or exposed to JP-8 vapors. Although long-term studies have not been conducted on JP-8, the available subchronic data are sufficient to calculate an oral RfD for JP-8 liquid and an inhalation RfC for JP-8 vapors.

3. DOSE-RESPONSE ASSESSMENT FOR NON-CANCER EFFECTS

3.1. INGESTION EXPOSURE

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- JP-8
CASRN -- CAS No. not assigned
Last Revised -- Prepared 9/30/96

ORAL RfD SUMMARY

Critical Dose -- 3000 mg/kg/day
UF -- 3000
MF -- 1
RfD -- 1 mg/kg/day
Confidence -- Medium

Critical Study

Critical Effect -- No adverse effects identified in critical study

Study Type -- Oral subchronic study in rats

Reference -- Mattie et al., 1995.

NOAEL -- 3000 mg/kg/day (7 days per week)

NOAEL(ADJ) -- 3000 mg/kg/day

LOAEL -- Not determined

LOAEL(ADJ) -- Not determined

Conversion Factors and Assumptions -- No conversion is necessary because the test animals were administered the dose daily 7 days/week.

PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Mattie, D.R., G.B. Marit, C.D. Flemming. 1995. The effects of JP-8 jet fuel on male Sprague-Dawley rats after a 90-day exposure by oral gavage. Tox. Indust. Health 11 (4):423-435.

Male Sprague Dawley rats (10/group) were dosed daily by gavage with 0, 750, 1500, or 3000 mg JP-8/kg/day for 90 days. The test material was administered without a solvent vehicle. There were no clinical signs of toxicity in any of the exposed groups except for statistically significant and dose-related reductions in body weight; however, because food and water consumption were not measured, there is no evidence to show that the animals were anoretic. The major histopathological lesion seen in the test animals was α2-microglobulin-related nephropathy. This lesion is not considered to be relevant to human exposures. Clinical chemistry analyses indicated statistically significant changes in a number of parameters, but none were dose-related. A statistically significant decrease in glucose and s.s. increases in total bilrubin, AST, and ALT occurred at all dose levels. Neutrophil counts were increased and lymphocyte counts decreased in all three dose groups, with the greatest changes seen at the highest dose. Absolute organ weights were not changed significantly at any dose level. Relative organ weights (brain, liver, kidney, spleen, and testes) were significantly ($p < 0.05$) increased in the high dose group. Incidences of perianal dermatitis and gastritis occurred at all dose levels but not in the controls. According to the investigators, "the most likely cause of the gastritis and perianal dermatitis is a contact irritation limited to squamous epithelium" (Mattie et al., 1995). The absence of clinical or histopathological signs except for the α2-microglobulin-related nephropathy indicate that the highest dose level of 3000 mg/kg/day can be considered a NOAEL for the purpose of developing an RfD.

UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF -- 3000
MF --1

A total uncertainty factor of 3000 has been used to account for intraspecies and interspecies extrapolation, subchronic-to-chronic extrapolation, and for data base deficiencies.

ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

In a developmental toxicity study, JP-8 was determined to be non-teratogenic (Cooper and Mattie, 1996). Sprague-Dawley rats (30 animals/group) were dosed with 0, 500, 1,000 or 2,000 mg/kg/day by gavage on days 6-15 of gestation. The number and type of fetal malformations and variations were not significantly different between dose groups. Significant decreases in body weight gain were observed in dams in the 1,000, 1,500 and 2,000 dose groups. Fetal body weights were significantly reduced in the two highest dose groups.

CONFIDENCE IN THE ORAL RfD

Study -- High
Data Base -- Medium
RfD -- Medium

The study is a well-conducted subchronic oral bioassay. The data base is lacking chronic oral studies, a developmental toxicity study with a second species, and a multigeneration reproductive toxicity study.

EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document --None
Other EPA Documents --
Agency Work Group Review --
Verification Date --
EPA Contacts --

3.2. INHALATION EXPOSURE

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- JP-8
CASRN -- CAS No. not assigned
Last Revised -- Prepared 9/30/96

RfC SUMMARY

Critical Concentration -- 1000 mg/m^3
UF -- 3000
MF -- 1
RfC -- 0.4 mg/m^3
Confidence -- Medium

Critical Study

Critical Effect -- Critical effect not determined

Study Type -- Subchronic inhalation study in rats

Reference -- Mattie, et al., 1991.

NOAEL -- 1000 mg/m^3 continuously for 90 days

NOAEL(ADJ) --1000 mg/m^3

NOAEL(HEC) -- 1180 mg/m^3

LOAEL -- Not determined

LOAEL(ADJ) --Not determined

Conversion Factors and Assumptions --

NOAEL(HEC) = 1000 mg/m^3 x 1.18 where 1.18 is the ratio of blood/gas partitioning coefficients for JP-10 (Gargas et al., 1989); see USEPA, 1991 for methodology for Type III gas.

PRINCIPAL AND SUPPORTING STUDIES (INHALATION RfC)

Mattie, D.R., C.L. Alden, T.K. Newell, et al. 1991. A 90-day continuous vapor inhalation toxicity study of JP-8 jet fuel followed by 20-21 months recovery in Fischer 344 rats and C57BL/6 mice. Toxicol. Pathol. 19(2):77-87.

Fischer 344 rats (95 males and 75 females) and C57BL/6 mice (100 mice per sex) were exposed to JP-8 vapors (0, 500, or 1,000 mg/m^3) continuously for 90 days (Mattie et al., 1991). At termination of the exposure period, 15 rats and 25 mice per sex/dose group were sacrificed and necropsied. The remaining animals were observed for up to 21 months, with interim sacrifices at 2 weeks, 2 months and 9 months post-exposure. No biologically significant treatment-related changes in clinical chemistry parameters were observed in mice or rats. Liver enzyme changes reported earlier by McEwen and Venot (1985) for this same study could not be verified. Male rats exposed to JP-8 exhibited a statistically significant decrease in body weight and statistically significant increases in absolute and relative kidney weight. Body weight and kidney weights of female rats, and liver and spleen weights of both males and females, were not affected by exposure to JP-8. At final sacrifice the only non-neoplastic lesion observed in female rats was an increase in splenic hematopoiesis. This change was statistically dose-dependent but usually of only minimal severity and, therefore, not considered to be treatment-related. Male rats exposed to JP-8 developed kidney lesions consisting of hyaline droplet formation in the proximal convoluted tubules, granular casts in the outer medulla, and exacerbated lesions of chronic progressive nephrosis. These kidney lesions are specific to male rats and not considered relevant to humans. Male rats also exhibited a dose-related increase in incidence and severity of epithelial cells in the urine. The only other non-neoplastic lesion seen in male rats was hepatic basophilic foci which occurred in 11% of the control group, 35% of the low-exposure group, and 31% of the high exposure group. The increases were of statistical significant, but of uncertain

biological significance (Mattie et al., 1991). Most non-neoplastic lesions seen in the mice exposed to JP-8 (inflammatory skin lesions, splenic hematopoiesis, eosinophilic degeneration of nasal epithelial cells, inflammatory changes of the anus, liver, and kidney, focal mineralization in the brain, and tooth malformations) were considered incidental since incidences were comparable among groups. Deposition of amyloid material in the ileum of male mice (4 of 28 in the high dose group only) was considered to be possibly, but not likely to be related to the exposure to JP-8 (mattie et al., 1991). Compared to controls, a significantly higher mortality rate occurred in male mice; however, the Mattie et al. (1991) concluded that much of the mortality was due to necrotizing dermatitis that resulted from fighting. Because of the absence of any clear dose-response for any reported effect, the high exposure of 1000 mg/m^3 is considered to be a NOAEL for rats or mice exposed to JP-8.

UNCERTAINTY AND MODIFYING FACTORS (INHALATION RfC)

UF -- 3000
MF -- 1

A total uncertainties factor of 3000 has been used to account for intraspecies and interspecies extrapolation, subchronic-to-chronic extrapolation, and for data base deficiencies.

ADDITIONAL STUDIES/COMMENTS (INHALATION RfC)

In studies reported by Hays et al. (1995), changes in lung permeability and lung histology were evaluated in F344 rats exposed to a JP-8 aerosol at a concentration of 500 or 813-1094 mg/m^3, 1 hr per day for 7, 28 or 56 days. Lung epithelial permeability was measured by alveolar clearance of technetium-labelled diethylenetriamine pentaacetate. Clearance for exposed rats was dependent on both exposure concentration and exposure duration. Clearance values were not significantly increased in the 7-day exposure group but were increased in both 28-day exposure groups ($p < 0.05$). In the 56-day exposure groups, clearance was significantly increased ($p < 0.05$) in the group exposed to 813-1094 mg/m^3, but not in the group exposed to 500 mg/m^3. Changes in lung histopathology in the exposed animals could be correlated with the changes in lung permeability. The lungs of animals in all exposed groups exhibited interstitial edema resulting from endothelial damage and there was an apparent thickening of the alveolar septa and activation of the alveolar macrophages. Extensive endothelium damage was seen in the 7-day and 28-day exposure groups, but the damage appeared less severe in animals exposed for 56 days, suggesting that some recovery had taken place. However, Hays et al. (1995) note that the 56-day exposure to JP-8 may have induced a fibrotic response in animals exposed for 56 days.

CONFIDENCE IN THE INHALATION RfC

Study -- High

Data Base -- Low
RfD -- Medium

The study is a well-conducted subchronic inhalation bioassay. The data base is lacking chronic toxicity studies, a developmental toxicity study in a second species, and a multigeneration reproductive toxicity study.

EPA DOCUMENTATION AND REVIEW OF THE INHALATION RfC

Source Document -- None
Other EPA Documents --
Agency Work Group Review
Verification Date
EPA Contact --

3.3. DERMAL EXPOSURE

REFERENCE DOSE FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- JP-8
CASRN -- Not assigned
Last Revised -- No data

RfD SUMMARY

Information is not available on the dermal toxicity of JP-8 to derive a dermal RfD.

4. DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Data are insufficient to assess the carcinogenicity of JP-8 in humans or animals for oral or inhalation exposures.

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

JP-8 cannot be classified as to potential human carcinogenicity because of the lack of adequate data.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Not classifiable

Basis -- Lack of adequate human or animal data.

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Attachment 1

TOXICITY ASSESSMENT FOR LEWISITE

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and
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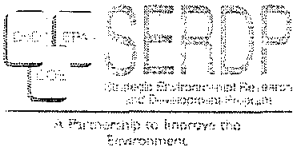

NOTICE

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PREFACE

This report assesses the potential non-cancer and cancer effects of chemical agent Lewisite (CAS Number 541-25-3).

The funding for the research was provided by the U.S. EPA, U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) and Strategic Environmental Research and Development Program (SERDP).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

This document was written by Dr. Robert A. Young, Health Sciences Research Division, Oak Ridge National Laboratory, Oak Ridge, TN. Internal peer review was provided by Dr. Robert Young, Dr. Annetta Watson, and Mr. Robert Ross. External review of the toxicity data was provided by Dr. Thomas J. Bucci, Integrated Services, White Hall, AR and Dr. I. K. Ho of the U. of Mississippi Medical Center, Jackson, MS. External review of the derivation of the RfDs was provided by Drs. Michael Dourson and Susan Velazquez of Toxicology Excellence for Risk Assessment, Cincinnati, OH, and By Dr. William Hartley of Tulane Medical Center, New Orleans, LA. Additional reviews were provided by Mr. Joe King, Dr. Jack Heller, Ms. Veronique Hauschild, Ms. Bonnie Gaborek, Mr. Maurice Weeks, Maj. Robert Gum, and Mr. Kenneth Williams of the U.S. Army.

PREAMBLE NOTICE

This document was produced under the auspices of the Environmental Risk Assessment Program (ERAP), which has its genesis in the DOD/DOE Strategic Environmental Research and Development Program (SERDP) that was established through Public Law 101-510 (10 United States Code 2901-2904). ERAP was established as a cooperative effort of DOD, DOE, and EPA to improve health and ecological risk assessments and to foster consistence in risk assessments across federal agencies. The program has three working groups chartered under its mission which are the Materials/Chemicals Risk Assessment (MCRA) Working Group, Human Risk Assessment Methodology (HRAM) Working Group, and the Ecological Risk Assessment Methodology (ERAM) Working Group. The program also has an Advisory and Coordinating Committee (ACC) that oversees the program and the working group's activities.

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ABSTRACT

Data pertaining to the potential cancer/non-cancer effects of the chemical agent Lewisite [dichloro(2-chlorovinyl)arsine] are reviewed. Lewisite is a lethal and systemic poison. Lewisite may be lethal following inhalation or dermal exposure, or by ingestion. Generally, the toxic effects of Lewisite are of rapid onset and result from acute exposures. Being lipophilic, percutaneous absorption of Lewisite is rapid and may be associated with systemic toxicity characterized by pulmonary edema, diarrhea, agitation, weakness, hypothermia, and hypotension.

For the derivation of an RfD for Lewisite, both a 90-day study and a multigeneration study in rats were used to identify effect levels. Data from the 90-day study identified a lowest observed adverse effects level (LOAEL) of 1.0 mg/kg/day based upon gastric lesions. The highest dose (0.6 mg/kg/day) from the multigeneration study is the most valid no observed adverse effects level (NOAEL) and is the best value for establishing an RfD. The route of administration of the Lewisite dose in these studies was by gavage or gastric intubation. This method of administering the dosage results in artificially increased exposure of the forestomach in comparison to exposure from environmental media such as drinking water. Therefore, an RfD based upon available data is tenuous and difficult to verify. The Material/Chemical Risk Assessment (MCRA) Working Group agreed that the critical toxic effect observed in Lewisite studies (forestomach lesions) appears to be an artifact of administration, and the overall database for Lewisite is not robust. Although it was recognized that the structure of Lewisite might imply toxic activity differing from inorganic arsenic, it was the consensus of the MCRA Working Group that the Lewisite RfD be considered not verifiable due to data deficiencies, and that the existing RfD for inorganic arsenic (3E-04 mg/kg/day) be used as a surrogate.

Data are inadequate to quantitatively assess the potential carcinogenicity of Lewisite. There are inadequate human and inadequate animal data regarding the carcinogenic potential of Lewisite. There is general agreement that the toxicological properties of Lewisite have not been completely investigated.

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1. SUMMARY OF TOXICITY INFORMATION

1.1. EPIDEMIOLOGIC STUDIES

Pertinent epidemiologic studies for Lewisite are currently unavailable.

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity.

1.2.1.1. Acute Toxicity — Liquid Lewisite applied by eye-dropper to the forearms of men caused blanching and discoloration of the skin followed by extensive erythema within 15 to 30 minutes and vesication within 12 hours or less (Wardell, 1941, as cited in Goldman and Dacre, 1989). The pain associated with these dermal exposures reportedly occurred within two minutes and considerable discomfort persisted for about one week. Other tests with human subjects and clinical reports also indicate a similar temporal sequence of events. Exposure to Lewisite vapor (0.06 to 0.33 mg/L) caused discoloration and blistering with the maximum effect occurring by 36 to 48 hours after exposure (Wardell, 1941). At a concentration of 0.01 mg/L, Lewisite vapor caused inflammation of the eyes and swelling of the eyelids after 15 minutes of exposure, and inhalation of 0.5 mg/L for five minutes is considered to be potentially lethal.

1.2.2. Animal Toxicity.

1.2.2.1 Acute Toxicity — Short-term exposure (10 to 30 minutes) of dogs to Lewisite vapor (0.05 to 0.12 mg/L) produced vomiting, urination, defecation, and severe respiratory distress that resulted in the death of 80 % of the dogs within 3 to 48 hours (Goldman and Dacre, 1989).

Acute oral toxicity values for Lewisite have been summarized by Watson and Griffin (1992). The only available oral LD_{50} is for the rat (50 mg/kg). Lethality values for other routes of exposure indicate some species variability but the values differ by less that an order of magnitude for any particular exposure route.

1.2.2.2. Subchronic Toxicity — A drinking water exposure study in rats was reported by Leitch et al. (1941). In this study, 10 rats were administered Lewisite in drinking water (10 or 16 mg/L) for 19 weeks (133 days). The treatment did not affect consumption of food or water and had no effect on animal growth. Additionally, there were no treatment-related histopathological findings. Based on this report, a Lewisite concentration of 16 mg/L drinking water would represent a no observed adverse effects level (NOAEL). However, this study has some deficiencies, as noted by Daniels (1990). The study neither defined an effect level, nor monitored the actual concentration of Lewisite in drinking water consumed. Additionally, the report did not provide information regarding water consumption by the test animals. These data would be critical in determining an actual or estimated dose of Lewisite. It is also possible that the consumed concentration may have varied from the target concentration because of test article degradation. Daniels (1990), however, suggested that these data would probably provide an estimate of a 7-day NOEL equivalent to 1.4 mg/kg.

In a dose range-finding study for a teratology study in CD rats and New Zealand White rabbits, Lewisite was administered by gavage to rats (10 per group) on gestation days 6-15 at doses of 0, 0.5, 1.0, 2.0, or 2.5 mg/kg, and by gastric intubation to rabbits (8 per group) on gestation days 6-19 at doses of 0, 0.5, 1.0, 1.5 or 2.0 mg/kg (Hackett et al. 1987). For rats, deaths attributed to Lewisite occurred in the 2.5 mg/kg group (2/10) and in the 2.0 mg/kg group

(1/10). Dosing trauma deaths were also reported (1/10, 2/10, and 1/10 in the 1.0, 2.0, and 2.5 mg/kg groups). For rabbits, deaths attributed to Lewisite were reported in the 1.0 mg/kg group (6/8), 1.5 mg/kg group (5/8), and 2.0 mg/kg group (8/8). Dosing trauma deaths were also noted: 1/8 and 3/8 in the 1.0 mg/kg group and 1.5 mg/kg group, respectively.

A 90-day subchronic toxicity study of Lewisite in Sprauge-Dawley rats was conducted by Sasser et al. (1989a). In this study, groups of 10 male and 10 female rats were given Lewisite in sesame oil by gastric intubation at doses of 0.01, 0.1, 0.5, 1.0 and 2.0 mg/kg. Dosing protocol was 5 days per week for 13 weeks or approximately 65 dosing days. Vehicle controls received sesame oil at a dose of 1.67 ml/kg. Deaths were observed in the three highest dose groups; three males and seven females of the 2.0 mg/kg dose group, eight males and six females of the 1.0 mg/kg dose group, and two males and three females of the 0.5 mg/kg dose group. Although all of the deaths occurred in the three highest dose groups, the response was not dose-dependent. Forestomach lesions were observed in the two highest dose groups (8/10 males and 4/10 females in the 2.0 mg/kg group, and 1/10 males in the 1.0 mg/kg group) and were attributed to the test article. These lesions were characterized by necrosis of the stratified squamous epithelium accompanied by infiltration of numerous neutrophils and macrophages, hemorrhage, and edema. In some instances, hyperplasia of adjacent areas was noted. There was no evidence that the lesions were precancerous, but the duration of exposure and observation was insufficient to assess carcinogenic responses. Lesions were also present in the glandular stomach but to a lesser degree. The presence of the lesions was consistent with the irritant effect of Lewisite. No lesion were observed in the lower dose groups.

Some of the animals died without exhibiting any clinical signs of toxicity: drooling or wetness around the mouth and chin, and labored respiration were noted among other rats immediately preceding death. Gross pathology findings attributed all deaths, except one to severe inflammatory lesions characterized by edema and epithelial necrosis of the respiratory tract. Respiratory lesions were most likely due to aspiration of the test material or induced reflux of stomach contents into the pharynx with subsequent aspiration into the airways. Inflammatory lesions observed in the respiratory tract of surviving rats were also indicative of accidental deposition or induced reflux of the test material. No significant treatment-related effects on body weights of organ weights were observed for any of the dose groups.

Clinical chemistry evaluations revealed a significant ($p<0.05$) decrease in total serum protein, serum creatinine, and serum SGOT, and SGPT in male rats of the highest dose (2.0 mg/kg) group at 13 weeks. Lowered serum enzyme activity was also observed in male rats of the other Lewisite dose groups. Females of the highest dose group exhibited significantly increased lymphocyte and platelet counts; the former at 6 weeks but not at 13 weeks and the latter only at 13 weeks. The biological/toxicological significance of these findings is, however, uncertain. The investigators noted that the no-effect dose was greater than 0.5 mg/kg and less than 1.0 mg/kg. The 0.5 mg/kg dose may be considered an estimate of the NOAEL for short-term oral exposure to Lewisite.

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. In a long-term follow-up study, Krause and Grussendorf (1978) reported the formation of a malignant lesion at the site of contact eight years following a single, acute dermal exposure to Lewisite. A German soldier had been accidentally exposed to liquid

Lewisite on his lower right leg in 1940. In 1948, the lesion was diagnosed as malignant. Thirty-eight years after exposure, the area around the contact site was still ulcerated and diagnosed as Bowen's disease (intradermal squamous cell carcinoma). Bowen's disease was also diagnosed in workers at a Japanese facility that produced Lewisite. These latter findings, however, were not conclusive because these workers were exposed concurrently to diphenylcyanoarsine and mustard agent and no quantitative estimates of dose or exposure rates were available (Inada et al., 1978).

There is only anecdotal evidence for the potential carcinogenicity of Lewisite. These data are not definitive and do not support classifying Lewisite as a suspected carcinogen. As such, quantitative assessment of the potential carcinogenicity of Lewisite is not currently possible. Although the available evidence is not of sufficient quality to label Lewisite a suspected carcinogen, the position maintained by CDC (CDC, 1988) that "some evidence suggests that Lewisite *might* also be a carcinogen" seems tenable. However, for environmental exposure and remediation concerns, the arsenic component and /or arsenic-containing degradation products would warrant concern.

Although the carcinogenicity of Lewisite *per se* is equivocal and cannot be assessed quantitatively, several of its degradation products are known carcinogens. Lewisite combustion produces the inorganic arsenicals arsenic trichloride and arsenic trioxide, as well as vinyl chloride. Inorganic arsenic is carcinogenic in humans and animals and is classified as a Group A carcinogen for both oral and inhalation exposure (U.S. EPA, 1989). Arsenic trioxide and vinyl chloride are both considered Group A carcinogens by the U.S. EPA (U.S. EPA, 1984, 1988) and Group 1 carcinogens by IARC (IARC, 1987). Additionally, compounds such as arsenic

trichloride, sodium arsenite (a Lewisite hydrolysis product), arsenic oxychloride, and inorganic arsenicals in general are of concern to EPA as potential carcinogens (U.S.EPA, 1988). However, there are no human epidemiological data or data from animal studies that show organic arsenical to be carcinogenic. A review by the World Health Organization (WHO, 1981) stated that "There is no conclusive evidence that any of the organoarsenic compounds tested for carcinogenicity in laboratory animals are carcinogenic." IARC (1987) concluded that adequate data were not available for evaluating the carcinogenicity of organic arsenic compounds.

1.3.2. Chronic Toxicity. No human or animal studies examining the effects of Lewisite following chronic exposure were located in the searched literature.

1.4. REPRODUCTIVE STUDIES

In a teratogenicity study by Hackett et al. (1987), Lewisite was administered by gavage to pregnant rats on gestation days 6 through 15 at doses of 0.5, 1.0, and 1.5 mg/kg and by gastric intubation to pregnant rabbits on gestation days 6 through 19 at doses of 0.07, 0.2, and 0.6 mg/kg. For rabbits, the mortality rates were 13%, 46%, and 69% for the 0.07, 0.2 and 0.6 mg/kg dose groups, respectively. These mortality rates were corrected for death from other causes (e.g. , dose-delivery trauma, accidental delivery of the dose to the lungs, handling trauma, pregnancy complications unrelated to the test article) and therefore, represent a significant dose-related frank effect. Surviving rabbits in the highest dose group exhibited decreased body weight gain relative to controls and other dose groups. However, the study authors noted more frequent incidences of anorexia in the high-dose rabbits when compared to controls and other dose groups. For those rabbits whose deaths were not attributed to the extraneous causes previously noted, gastric lesions (mucosal inflammation, edema, necrosis, and mucosal sloughing) were

observed at all dose levels. The only statistically significant developmental effects were a significant increase in the incidences of fetal stunting and supernumerary ribs in the high-dose group (0.6 mg/kg) group. Fetal weight and crown-rump length were somewhat lower in the 0.6 mg/kg dose group but these differences were not statistically significant. Maternal toxicity (23%) was also associated with the low-dose group, thereby indicating a NOAEL for this study to be <0.07 mg/kg/day for maternal toxicity and 0.2 mg/kg/day for developmental toxicity. The lowest observed adverse effect level (LOAEL) based on maternal effects is 0.07 mg/kg/day and for developmental effects is approximately 0.6 mg/kg/day. The increased mortality in the does (13%) and the occurrence of gastric lesion in the low-dose group (0.07 mg/kg/day) suggest that the rabbit is the most sensitive of the species for which data are available.

The use of increased mortality as the critical effect for derivation of a reference dose may be inappropriate. Furthermore, the intragastric intubation technique used for rabbits in this study concentrates the test article on the gastric mucosa more effectively than simple gavage administration thereby making the apparent increased sensitivity of rabbits more an artifact of administration than actual toxicodynamics. In the discussion of the study, Hackett et al. (1987) noted that the fetal toxicity observed in the rabbits appeared to be occurring at doses above those required to induce increased maternal mortality. The findings of this study are, however, statistically compromised by the low number of pregnant survivors (9/12, 6/11, 5/13, and 3/15 for the control, 0.07, 0.2 and 0.6 mg/kg groups, respectively). In a dose range-finding study for this experiment, significant mortality was observed in the 1.0 mg/kg group (6/8), 1.5 mg/kg group (5/8) and 2.0 mg/kg group (8/8).

1.5. DEVELOPMENTAL STUDIES

Hackett et al. (1987) investigated the potential teratogenicity of Lewisite in CD rats. In this phase of the study, no maternal toxicity or teratogenic effects were observed, thereby identifying 1.5 mg/kg as a NOAEL. However, it must be noted that in a dose range-finding study in rats (Hackett et al. 1987), doses of 2.0 mg/kg and 2.5 mg/kg resulted in 10% and 20% maternal mortality, respectively.

A two-generation reproductive study in rats was conducted by Sasser et al. (1989b). In this study, Lewisite (in sesame oil) was administered intragastrically at doses of 0.1, 0.25, or 0.6 mg/kg/day to groups of 25 male and 25 female Sprague-Dawley rats five days per week for 13 weeks prior to mating and 7 days per week during gestation (21 days), and at least four days per week during lactation (21 days). The doses were selected based upon the findings of the subchronic toxicity study by Sasser et al. (1989a) which identified a NOAEL between 0.5 and 1.0 mg/kg/day and those of the teratogenicity study by Hackett et al. (1987) in which 1.5 mg/kg/day was a NOAEL. In the dose range-finding phase of this report, 20% mortality (corrected for deaths due to dosing trauma) was observed in the 2.5 mg/kg/day group. At the time of birth of the F_1 generation, the F_0 male rats were sacrificed. Dams continued treatment (minimum of four doses per week) throughout lactation (3 weeks). A vehicle control group was given equivalent volumes of sesame oil (1.67 ml/kg). After weaning, 20 male and 25 female offspring were selected for the F_1 phase of the study. The treatment protocol for these animals was as described for the F_0 generation. Mortality was high among both the F_0 and F_1 females. The cause of death for most of these animals appeared to be associated with aspiration of the test article resulting in fatal respiratory tract lesions. Exposure of rats to Lewisite did not adversely

affect reproductive performance, fertility, or reproductive organ weights. The treatment had no significant effect on litter weights, sex ratio, mean pup weight, or offspring survival for either generation. Although this study revealed no toxic effects, arsenic is know to be embryotoxic and teratogenic, and the possibility exists that inorganic arsenic could be metabolically derived from Lewisite.

An unpublished USSR study analyzed by the U.S. Army Research Institute of Chemical Defense (Solana, 1992) provided data indicating that preconception maternal exposure of rats to 0.045 or 0.002 mg Lewisite/cm^3, 4 hours/day, 5 days/week for 4 months did not affect numbers of corpora lutea or implantations, number and physical dimensions or fetuses, increased intrauterine mortality or ossification of long bones. Approximately 140 litters of rats were used in this study.

Human data regarding reproductive/developmental effects due to Lewisite exposure are inconclusive because of confounding factors such as concurrent exposure to other agents such a sulfur mustards and incomplete exposure data. Yamakido et al. (1985) studied workers from the Okuna-jima (Japan) factory where mustard and Lewisite were manufactured in the World War II era, and noted no evidence of agent-induced mutations.

1.5.1 Mutagenicity. Data from genotoxicity studies do not indicate a carcinogenic potential for Lewisite. Genotoxicity studies in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 were negative with and without S9 activation at Lewisite concentrations, <1.0 μg/plate (Stewart et al., 1989). At 1.0 μg/plate and higher Lewisite was cytotoxic. Jostes et al. (1989) reported on the effects of Lewisite in one mutation assay (hypozanthine-guanine phosphoribosyl transferase [HGPRT] locus) and two cytogenetic assays (chromosomal aberration and sister

chromatid exchange [SCE]) using Chinese ovary cells. At concentrations ranging from 0.1 to 2.0 μM, the mutagenic response at the HGPRT locus was not significantly different from control values. The SCE assay resulted in a weakly positive response from 0.25 to 1.0 μM concentrations, but the values were not significantly different from control values. However, chromosome aberrations were induced a 0.50, 0.75, ad 1.0 μM that were significantly greater than control values. The investigators concluded that Lewisite was cytotoxic and clastogenic but SCE and mutation at the HGPRT locus was insignificant. Assays to determine sex-linked lethal mutations and chromosomal rearrangements in *Drosophila melanogaster* yielded negative results (Auerbach and Robson, 1946, 1947).

A dominant lethal study using CD rats was conducted by Bucci et al. (1993). In this study, male CD rats (20/group) were given Lewisite in sesame oil by gavage for five days at doses of 0.375, 0.75, or 1.5 mg/kg. Vehicle controls received an equivalent volume of the vehicle and positive controls were given the vehicle followed by 100 mg ethyl methanesulphonate/kg, i.p. on day five. Each male was mated with two females over the next 10 weeks. With the exception of positive controls, no significant differences were observed in reproductive indices and there were no histopathologic findings that could be attributed to Lewisite treatment. Under the conditions of this study there were no dominant lethal mutations resulting from exposure to Lewisite.

1.6. TOXICOKINETICS AND METABOLISM

1.6.1. Toxicokinetics. The distribution of arsenic depends upon the duration of administration and the particular arsenical involved (Gilman et al. 1990, as cited in HSDB). Arsenic is stored mainly in liver, kidney, and lungs. Much smaller amounts are found in muscle and neural tissue.

Because of the high sulfhydryl content of keratin, high concentrations of arsenic are found in hair and nails. It is also deposited in bone and teeth. Arsenic is eliminate by many routes although most is excreted in urine in man. The organic arsenicals are usually excreted more rapidly that are the inorganic forms.

The standard treatment of Lewisite poisoning is by chelation with British anti-lewisite (BAL) , dimercaptopropanol. Snider et al. (1990) investigated the effect of BAL treatment on the distribution of arsenic after Lewisite administration in rabbits. Lewisite was administered subcutaneously at the LD_{10} and LD_{40} of the compound. Without BAL treatment, arsenic was eliminated with a half-life in blood of between 55 and 75 hr and a blood clearance of 120 ml/hr/kg. Arsenic had a large volume of distribution of several L/kg, indicating extensive distribution in tissues. The highest tissue concentration were more than seven times blood concentration, and were found in the liver, lung and kidneys. These organs maintained an approximately constant concentration ratio with blood during the sampling period. Concentration in tissues with a blood to tissue barrier, such as the brain and the spinal cord, rose between 4 and 96 hours while blood concentration declined by more than four fold over the same time period. Treatment with BAL by four equal, maximally tolerated doses over 12 hr substantially reduced arsenic concentration in blood and tissues. For example, at 24 hr the concentration in brain and liver (target organs for arsenic toxicity) were reduced by 65 to 89% over the range of Lewisite doses administered. The total exposure of brain and spinal cord was reduced by more that two-thirds by BAL treatment. Further the blood clearance of arsenic was increased. Treatment with BAL enhanced the elimination of arsenic in two ways: by decreasing the tissue-to-blood

partitioning which mobilizes arsenic into the blood stream, and by increasing the clearance of arsenic.

1.6.2. Metabolism. No pertinent information on the metabolism of Lewisite was found in the literature searched.

1.6.3. Percutaneous Absorption. The vesicant properties of Lewisite result from direct skin contact: it has been estimated that as little as 2 ml to an adult human (equivalent to 37.6 mg/kg) can be fatal within several hours (Sollman, 1957). Being lipophilic, percutaneous absorption of Lewisite is rapid and may be associated with systemic toxicity characterized by pulmonary edema, diarrhea, agitation, weakness, hypothermia, and hypotension (IOM, 1993). The threshold for severe systemic effects in humans following dermal exposure to Lewisite is approximately 10 mg/kg (9.1 - 13.4 mg/kg) (Sollman, 1957). It has been hypothesized that fatalities following dermal exposure to Lewisite may be due to blood plasma loss resulting from extensive capillary damage (i.e., Lewisite shock) (Cameron et al., 1946). Ingestion of trivalent arsenicals may also cause death due to fluid loss resulting from intestinal epithelium damage. The vesicant properties of Lewisite are characterized by immediate onset of pain and, for ocular exposure, possible corneal necrosis. Studies in animals have shown that the target tissues and organs for systemic toxicity of Lewisite include the liver, gall bladder, urinary bladder, lung and kidneys (Cameron et al., 1946; Snider et al., 1990).

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

No human or animal studies examining structure-activity relationships of Lewisite toxicity were located in the searched literature.

1.8. MECHANISTIC STUDIES

The toxicological effects of Lewisite are ultimately due to its interaction with thiol groups of biologically active proteins such as enzymes. The interaction with sulfhydryl groups of enzymes may result in inhibition of enzyme function by the formation of stable cyclic structures with arsenic (As^{+3}) as a result of the reaction of the arsenic with the sulfhydryl groups of organic compounds such as those occurring in dihydrolipoic acid and in reduced keratin (De Bruin 1976). Dihydrolipoic acid is a dithiol cofactor active in several important enzyme systems (required for cellular respiration) including alpha-ketoacid oxidases such as pyruvate oxidase, 2-oxoglutarate oxidase, and aldehyde dehydrogenase. Lewisite combines with the dihydrolipoic acid to form stable six-member ring structures (cyclic thioarsenite complexes), thereby inactivating the enzymes. Overall, the end result of these interactions and the ultimate mechanism of Lewisite toxicity appear to be energy depletion which, in turn, results in cell death. Organochloroarsines, of which Lewisite is an example, are also potent alkylating agents; this feature suggests carcinogenic potential.

White New Zealand rabbits were used in a study to compare the acute intravenous toxicity of sodium arsenite and Lewisite, to provide a quantitative and qualitative model for future assessment of the treatment of Lewisite poisoning (Inns et al. 1988, as cited in Hazardous Substance Database [HSDB]). The medial lethal dose of Lewisite was 1.8 mg/kg. At 5 minutes following injection, rapid panting was observed, followed by prostration, with death occurring at about 4 hr. By 24 hr after injection the survivors appeared normal. The median lethal dose for sodium arsenite was 7.6 mg/kg with hypoactivity resulting 20 minutes after injection. On the basis of trivalent arsenic content, the toxicity of Lewisite was 6.5 times that of inorganic sodium

arsenite. The signs of poisoning and times of death and recovery differed between the two arsenicals. Severe pulmonary damage was documented in Lewisite treated animals by gross and histological appearance along with bilary epithelial necrosis. Neither of these conditions was found in arsenite treated animals. A marked difference was noted in the arsenic levels in all tissues tested, except the lung, between the two groups. It was concluded that Lewisite possessed a different type of toxic action from that of inorganic trivalent arsenic.

2. INTERPRETATION OF AVAILABLE INFORMATION

Lewisite is a lethal vesicant and systemic poison. The toxicology of Lewisite has recently been reviewed by Goldman and Dacre (1989), Watson and Griffin (1992), and Trammell (1992) and will, therefore, only be briefly discussed in this section. Lewisite may be lethal following inhalation or dermal exposure, or by ingestion. Its lethality is due primarily to vapor inhalation, although Lewisite is much less potent than neurotoxic chemical warfare agents. Generally, the toxic effects of Lewisite are of rapid onset and result from acute exposures. The vesicant properties of Lewisite result from direct skin contact: it has been estimated that as little as 2 ml to an adult human (equivalent to 37.6 mg/kg) can be fatal within several hours (Sollman, 1957). Being lipophilic, percutaneous absorption of Lewisite is rapid and may be associated with systemic toxicity characterized by pulmonary edema, diarrhea, agitation, weakness, hypothermia, and hypotension (IOM, 1993). The threshold for severe systemic effects in humans following dermal exposure to Lewisite is approximately 10 mg/kg (9.1 - 13.4 mg/kg) (Sollman, 1957). It has been hypothesized that fatalities following dermal exposure to Lewisite may be due to blood plasma loss resulting from extensive capillary damage (i.e., Lewisite shock) (Cameron et al., 1946). Ingestion of trivalent arsenicals may also cause death due to fluid loss resulting from intestinal epithelium damage. The vesicant properties of Lewisite are characterized by immediate onset of pain and, for ocular exposure, possible corneal necrosis. Studies in animals have shown that the target tissues and organs for systemic toxicity of Lewisite include the liver, gall bladder, urinary bladder, lung and kidneys (Cameron et al., 1946; Snider et al., 1990). It is

important to note that the gaps in knowledge regarding the toxic effects and dose response for Lewisite are extensive.

3. DOSE-RESPONSE ASSESSMENT FOR NON-CANCER EFFECTS

3.1. INGESTION EXPOSURE

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Lewisite [dichloro(2-chlorovinyl)arsine]
CASRN -- 541-25-3
Preparation date -- June 1996

Oral RfD SUMMARY

Critical Dose -- 0.44 mg/kg/day (Time weighted average, NOAEL)
UF -- 3000
MF -- 1
RfD -- 1E-04 mg/kg/day
Confidence -- Low

Critical Study

Critical Effect -- Forestomach lesions

Study Type -- Rat gavage, two generation subchronic exposure (Sasser et al. 1989b)
Rat gavage, gestational exposure (Sasser 1989a)

Reference -- Sasser et al. 1989a, 1989b

NOAEL -- 0.6 mg/kg/day
NOAEL(ADJ) -- 0.44 mg/kg/day

LOAEL -- 1.0 mg/kg/day
LOAEL(ADJ) --

Conversion Factors and Assumptions --

PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Sasser, L.B., J.A. Cushing, D.R. Kalkwarf, P.W. Mellick and R.L. Buschbom. 1989a. *Toxicology studies on Lewisite and sulfur mustard agents: Subchronic toxicity study of Lewisite in rats.* Final Report. Pacific Northwest Laboratory Report. PNL-6860. Richland, WA.

Sasser, L.B., J.A. Cushing, D.R. Kalkwarf, P.W. Mellick and R.L. Buschbom. 1989b. *Toxicology studies on Lewisite and sulfur mustard agents: Two-generation reproduction study of Lewisite in rats.* Final Report, Pacific Northwest Laboratory Report, PNL-6978, Richland, WA.

A two-generation reproductive study in Sprague-Dawley rats was conducted by Sasser et al. (1989b). In this study, Lewisite in sesame oil was administered by gastric intubation at doses of 0.1, 0.24, or 0.6 mg/kg/day to groups of 25 male and 25 female rats five days per week for 13 weeks prior to mating and 7 days per week during gestation (21 days), and at least four days per week during lactation (21 days). At the time of birth of the F_1 generation, the F_0 male rats were sacrificed. Dams continued treatment throughout lactation. A vehicle control group was given equivalent volumes of sesame oil (1.67 ml/kg). After weaning, 20 male and 25 female offspring were selected for the F_1 phase of the study. The treatment protocol for these animals was as described for the F_0 generation. Mortality was high among both the F_0 and F_1 females. The cause of death for most of these animals appeared to be associated with aspiration of the test article resulting in fatal respiratory tract lesions. Exposure of rats to Lewisite did not adversely affect reproductive performance, fertility, or reproductive organ weights. The treatment had no significant effect on litter weights, sex ratio, mean pup weight, or offspring survival for either generation. The highest dose (0.6 mg/kg/day) from this multigeneration study appears to represent the most valid NOAEL and would be the best value for deriving an RfD. However, because of the discontinuous exposure and variable dosing protocol, a time-weighted average dose must be calculated. This adjustment will provide a NOAEL adjusted for discontinuous exposure ($NOAEL_{adj}$) and is based on the following: rats were dosed at 0.6 mg/kg/day x 5 days/7 days for 13 weeks (91 days) = 0.43mg/kg/day for 13 weeks; females dosed daily (0.6 mg/kg/day) during gestation (21) days = 0.6 mg/kg/day for 3 weeks, and females dosed at least 4 days per week at 0.6 mg/kg/day during lactation (21 days) = 0.34 mg/kg/day for 3 weeks. The time weighted average (TWA) dose for this 133-day period is calculated as

TWA = Σ [0.43 mg/kg/day x 13 weeks)+(0.6 mg/kg/day x 3 weeks)+(0.34 mg/kg/day x 3 weeks)/19 weeks
TWA = 0.44 mg/kg/day

A 90-day subchronic toxicity study of Lewisite in rats was conducted by Sasser et al. (1989a). In this study, groups of 10 male and 10 female Sprague-Dawley rats were given Lewisite in sesame oil by gastric intubation at doses of 0.01, 0.1, 0.5, 1.0, or 2.0 mg/kg. Dosing protocol was 5 days per week for 13 weeks or approximately 65 dosing days. Vehicle controls received sesame oil at a dose of 1.67 ml/kg. Deaths were observed in the three highest does groups; three males and seven females of the 2.0 mg/kg dose groups, eight males and six females of the 1.0 mg/kg dose group, and two males and three females of the 0.5 mg/kg dose group. Although all the deaths occurred the three highest dose groups., the response was not dose-dependent. Forestomach lesions were observed in the two highest dose groups (8/10 males and 4/10 females in the 2.0 mg/kg group, and 1/10 males in the 1.0 mg/kg group) and were attributed to the test article. These lesion were characterized by necrosis of the stratified squamous epithelium accompanied by infiltration of numerous neutrophils and macrophages, hemorrhage.

and edema. In some instances , hyperplasia of adjacent areas was noted. There was no evidence that the lesions were precancerous, but the duration of exposure and observation was insufficient to assess carcinogenic responses. Lesions were also present in the glandular stomach but to a lesser degree. The presence of the lesion was consistent with the irritant effect of Lewisite. No lesions were observed in the lower dose groups. Additionally, some of the animals died without exhibiting any clinical sighs of toxicity; drooling or wetness around the mouth and chin, and labored respiration were noted among other rats immediately preceding death. Gross pathology findings attributed all deaths, except one, to severe inflammatory lesions characterized by edema and epithelial necrosis of the respiratory tract. Respiratory lesions were most likely due to aspiration of the test material or induced reflux of stomach contents into the pharynx with subsequent aspiration into the airways. Inflammatory lesions observed in the respiratory tract of surviving rats were also indicative of accidental deposition or induced reflux of the test material. No significant treatment-related effects on body weights or organ weights were observed for any of the dose groups. Clinical chemistry evaluations revealed a significant ($p<0.05$) decrease in total serum protein, serum creatinine, and serum SGOT and SGPT in male rats of the highest (2.0 mg/kg) dose group at 13 weeks. The biological and toxicological significance of these findings is, however, uncertain. The investigators noted that the no-effect dose was greater than 0.5 mg/kg and less than 1.0 mg/kg. Because the increased mortality was attributed to gavage error and/or reflux and subsequent aspiration of the test article, the 0.5 mg/kg dose may be considered an estimate of the NOAEL and the 1.0 mg/kg dose an estimate of the LOAEL for short-term oral exposure to Lewisite.

UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

Ten for protection of sensitive subpopulations, 10 for interspecies extrapolations, 10 for subchronic to chronic extrapolation, and 3 for a deficient database. No additional modifying factor is suggested.

ADDITIONAL STUDIES

Reproductive/Developmental Study: Hackett et al., 1987:

In a dose range-finding experiment for a teratology study in CD rats and New Zealand White rabbits, Lewisite (in sesame oil) was administered by gavage to rats (10 per group) on gestation days 6-15 at doses of 0, 0.5, 1.0, 2.0 or 2.5 mg/kg. Lewisite was also administered by gastric intubation to rabbits (8 per group) on gestation days 6-19 at doses of 0, 0.5, 1.0, 1.5, or 2.0 mg/kg. For rats, deaths attributed to Lewisite occurred in the 2.5 mg/kg group (2/10) and in the 2.0 mg/kg group (1/10). Dosing trauma deaths were also reported (1/10, 2/10 and 1/10 in the 1.0, 2.0, and 2.5 mg/kg groups). For rabbits, deaths attributed to Lewisite were reported in the 1.0 mg/kg group (6/8), 1.5 mg/kg group (5/8) and 2.0 mg/kg group (8/8). Dosing trauma deaths were also noted; 1/8 and 3/8 in the 1.0 and 1.5 mg/kg groups respectively.

A developmental toxicity study in rats and rabbits was conducted based upon these findings. Lewisite in sesame oil was administered by gavage to rats (0, 0.5, 1.0, or 1.5 mg/kg/day) on gestation days 6-15, and by intragastric intubation to rabbits (0. 0.07, 0.2, or 0.6 mg/kg/day) on gestation days 6-19. For rabbits the mortality rates were 13%, 46%, and 69% for the 0.07, 0.2, and 0.6 mg/kg dose groups, respectively. The mortality rates were corrected for death from other causes and, therefore, represent a significant dose-related frank effect. For those rabbits whose deaths were not attributed to extraneous causes, gastric lesions (mucosal inflamation, edema, necrosis, and mucosal sloughing) were observed at all dose levels. Maternal toxicity (13% mortality) was also associated with the low-dose group, thereby indicating a NOAEL of this study to be <0.07 mg/kg/day for maternal toxicity. The use of increased mortality as the critical effect for derivation of a reference dose is not appropriate. Furthermore, the intragastric intubation technique used for rabbits in this study concentrates the test article on the gastric mucosa more effectively than simple gavage, thereby making the apparent increase sensitivity of rabbits more an artifact of administration.

ADDITIONAL COMMENTS

Derivation of an RfD for Lewisite is complicated by the difficulty in identifying an appropriate critical effect. The key study (Sasser et al. 1989b) provides a free-standing NOAEL for rats that can be associated with a LOAEL from a companion 90-day study in rats (Sasser 1989a). Although the findings of the rabbit teratogenicity study by Hackett et al. (1987) suggest the rabbit as the most sensitive species, these latter data are compromised by the low number of animals and litters. In deriving an RfD for Lewisite, some consideration must be given to the potential effects of arsenic whch is known to be embryotoxic and teratogenic. The possibility exists that inorganic arsenic could be metabolically derived from Lewisite. However, the RfD for Lewisite (1E-04) mg/kg/day) is considerably lower than doses of arsenic and arsenic compounds associated with developmental/teratogenic effects (human TD_{lo}: 600 mg/kg [neonatal effects following arsenic trioxide exposure]; rat TD_{lo}: 0.58-0.62 mg/kg [fertility effects, musculoskeletal abnormalities following elemental arsenic exposure]: mouse TD_{lo}: 0.2 mg/kg [fertility effects following arsenic trioxide exposure]) RTECS, 1993). Additionally, the RfD for Lewisite is consistent with the 3E-04 mg/kg/day for inorganic arsenic (U.S. EPA, 1995).

The derivation of an RfD for Lewisite necessitated addressing several issues regarding the available data set: 1) interpretation of toxicity data from gavage/gastric intubation studies and 2) identification of the critical effect. The available data for Lewisite toxicity is limited to gavage and gastric intubation administration studies. Although these routes of administration allow for more precise control of the administered dose as opposed to drinking water or feeding studies, in the case of Lewisite (or any highly corrosive agent), they impart substantial caveats in data interpretation. Firstly, the use of a sesame oil vehicle and the gavage/gastric intubation administration result in the gastric mucosae being exposed to a bolus of material in a vehicle that limits normal dispersion of the test article in the stomach. The decrease in dispersion causes an increase in contact time between the corrosive agent and a limited surface mucosal area, thereby increasing the potential for inflammatory responses observed in the described studies.

Furthermore, the presence of an oil vehicle will likely affect the physicochemical interactions at the chemical /tissue interface by altering the solubility and distribution of the chemical. Secondly, within the context of the RfD, intake of a corrosive agent as a bolus/oil suspension would not be toxicologically or physiologically analogous to exposure to the chemical agent via environmental media such as water. The critical effect of orally administered Lewisite in animals appears to involve gastric lesions (rats and rabbits), possible developmental effects (rabbits), respiratory tract inflammation responses (rats and rabbits) and increased mortality (rats and rabbits). The increased mortality and respiratory tract inflammation responses reported in the available studies appear to be associated more so with dosing errors or simple reflux of the corrosive, irritating Lewisite. The reflux and subsequent respiratory tract response would be highly unlikely in an environmental exposure situation (i.e., drinking water contamination). Furthermore, the studies did not provide data affirming the respiratory responses to be a function of systemically mediated Lewisite toxicity. Therefore, an RfD based upon available data is tenuous and difficult to verify.

The proposed RfD for Lewisite underwent preliminary review (July 10-12, 1996) by the Material/Chemical Risk Assessment (MCRA) Working Group of the Environmental Risk Assessment Program (ERAP). The MCRA Working Group of ERAP represents multiagency (EPA, DoD, and DOE) input by individuals experienced in deriving and validating toxicity values. The MCRA Working Group agreed that the critical toxic effect observed in the Lewisite studies (forestomach lesions) appears to be an artifact of administration, and that the overall database for Lewisite is not robust. Although it was recognized that the structure of Lewisite might imply toxic activity differing from inorganic arsenic, it was the consensus of the MCRA Working Group that the Lewisite RfD be considered not verifiable due to data deficiencies, and that the existing RfD for inorganic arsenic (3E-04 mg/kg/day) be used as a surrogate. This is considered a valid and justifiable approach inasmuch as the inorganic arsenic RfD and the proposed Lewisite RfD are similar (3E-04 vs 1E-04 mg/kg/day, respectively), and the fact that Lewisite in environmental media will be degraded to inorganic arsenic.

CONFIDENCE IN THE ORAL (RfD)

Study -- Low
Data Base -- Low
RfD -- Low

3.2. INHALATION EXPOSURE

Available data are insufficient to support development of a chronic inhalation exposure (RfC) estimate for Lewisite.

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- Lewisite [dichloro(2-chlorovinyl)arsine]
CASRN -- 541-25-3
Last Revised -- No data

3.3. DERMAL EXPOSURE

Available data are insufficient to support development of a chronic dermal exposure (RfD_d) estimate for Lewisite.

REFERENCE DOSE FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- Lewisite
CASRN — 541-25-3
Last Revised -- No data

4. DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

Data are inadequate to quantitatively assess the potential carcinogenicity of Lewisite. There are inadequate human and inadequate animal data regarding the carcinogenic potential of Lewisite. Geonotoxic data are equivocal or negative.

It was recommended by the MCRA Working Group that for risk assessments, the carcinogenic potential of Lewisite degradation products be considered.

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Attachment 1

TOXICITY ASSESSMENT FOR NITROGEN MUSTARD (HN2)

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PREFACE

This report assesses the potential non-cancer and cancer effects of HN2 (bis(2-chloroethylmethylamine. mechlorethamine, nitrogen mustard),(CAS Number 51-75-2). Information pertaining to non-cancer and cancer effects has not been previously assessed by the United States Environmental Protection Agency (U.S. EPA).

The fundings for this research were provided by the U.S. EPA, U.S. Army Center for Health Promotion and Preventative Medicine (CHPPM) and Strategic Environmental Research and Development Program (SERDP).

This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Any toxicity values developed under this effort will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

PREAMBLE NOTICE

This document was produced under the auspices of the Environmental Risk Assessment Program (ERAP), which has its genesis in the DOD/DOE Strategic Environmental Research and Development Program (SERDP) that was established through Public Law 101-510 (10 United States Code 2901-2904). ERAP was established as a cooperative effort of DOD, DOE, and EPA to improve health and ecological risk assessments and to foster consistence in risk assessments across federal agencies. The program has three working groups chartered under its mission which are the Materials/Chemicals Risk Assessment (MCRA) Working Group, Human Risk Assessment Methodology (HRAM) Working Group, and the Ecological Risk Assessment Methodology (ERAM) Working Group. The program also has an Advisory and Coordinating Committee (ACC) that oversees the program and the working group's activities.

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ABSTRACT

Data pertaining to the potential cancer/non-cancer effects of HN2 (bis(2-chloroethylmethylamine, mechlorethamine, nitrogen mustard) are reviewed. Acute oral, subcutaneous, intraperitoneal and intravenous LD_{50}s have been reported for rats and mice, and, for percutaneous administration, in monkeys. The compound is considered to be a skin and eye irritant, though no epidemiological or occupational exposure studies were identified. Despite the marked cytotoxicity that forms the basis for HN2's potential application in chemical warfare (and for its inclusion in a "cocktail" of chemotherapeutic agents in cancer treatment), there are no data on the compound's subchronic or chronic toxicity. A limited database has provided evidence that HN2 itself may be carcinogenic, a concept consistent with the compound's alkylation properties. Teratogenic responses have also been described for HN2. Insufficient quantitative dose-response information exists to derive oral or dermal RfDs, an inhalation RfC, or a carcinogenic slope factor for HN2.

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1. SUMMARY OF TOXICITY INFORMATION

1.1. EPIDEMIOLOGICAL STUDIES

Pertinent epidemiological studies are currently unavailable.

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity. Mustard vesicants are acutely toxic by direct contact. Edema, ulceration, and necrosis of the skin and respiratory tract epithelium can occur, as well as conjunctivitis and blindness. General symptoms of systemic toxicity include nausea, vomiting, fever, and malaise (ITII, 1975). Delayed effects which may occur following acute exposures include, chronic bronchitis, and respiratory tract and skin cancers. However, information on adverse effects following long-term exposures to less-than acutely toxic concentrations of HN2 is limited. Health effects of mustard agents have been reviewed by ATSDR, (1990), Somani (1992), Sidell and Hurst, (1992) Watson and Griffin, (1992), and the Institute of Medicine (1993). HN2 can cause bone marrow suppression, and leukopenia, as well as hyperpigmentation of the skin and loss of hair and hearing (POISINDEX, 1993). The chemical has been associated with birth defects in humans and is also considered to be a carcinogen.

1.2.2. Animal Toxicity.

1.2.2.1. Acute Toxicity —

1.2.2.1.1. Oral Toxicity. Values from 10-85 mg/kg have been reported for the acute oral LD_{50} in rats and mice (NDRC, 1946, Fox and Scott,, (1980).

1.2.2.1.2. Inhalation Toxicity. Information on the acute inhalation toxicity of HN2 in humans or laboratory animals is presently unavailable.

1.2.2.1.3. Dermal Toxicity. Values ranging from 1.4-35 mg/kg for the acute lethality of HN2 following sub- or percutaneous application have been presented for rats, mice and monkeys (NDRC, 1946, Fox and Scott,, (1980).

1.2.2.1.4. Toxicity via other Routes of Administration. NRDC (1946) and Fox and Scott, (1980) reported values ranging from 1.1-4.4 mg/kg for the acute LD_{50} of HN2 when administered via either intravenous or intraperitoneal injection. In studies conducted by Conklin et al. (1965), FR mice given four intravenous injections of 2.4 mg HN2/kg at 14 day intervals exhibited reduced life span (490 days for animals surviving 30 days, compared to 632 days for controls). Decreased longevity was correlated with premature mortality due to various diseases associated with aging as well as with increased incidence of neoplasms. Premature development of lens opacities was also seen in the treated animals.

1.2.2.2. Subacute Toxicity — Information on the subacute toxicity of HN2 is currently unavailable.

1.2.2.3. Subchronic Toxicity — Information on the subchronic toxicity of HN2 is currently unavailable.

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. There is clinical evidence that patients treated with therapeutic doses of HN2 and other alkylating agents (as anti-neoplastic agents) for periods of weeks and months have an increased risk of developing acute non-lymphocytic leukemia (ANL) (see Institute of Medicine, 1993, for review). The peak time of onset of this leukemia is 3-9 years after treatment and the incidence rate is reported to be 3-5%, but may be as high as 30% in patients with prolonged or intensive treatment (Institute of Medicine, 1993).

Animal studies have also demonstrated the carcinogenicity of HN2 and HN2-hydrochloride, as well as the related compound nitrogen mustard N-oxide hydrochloride. Tumors in treated animals occurred in a number of sites, but particularly in the lungs (see IARC, 1975, Fox and Scott, 1980, for reviews). However, these studies used perenteral administration or dermal application and were considered inappropriate for quantitative assessment of carcinogenicity.

1.3.2. Chronic Toxicity. Information on the chronic toxicity of HN2 is currently unavailable.

1.4. REPRODUCTIVE STUDIES

Shimkin et al. (1966) reported testicular atrophy with decreased spermatogenic activity in A/J mice 39 weeks after intraperitoneal treatment with HN2-hydrochloride. Twelve thrice weekly injections were given resulting in total doses of 0.02, 1.1, 4.5 and 17.5 μmoles/kg.

1.5. DEVELOPMENTAL STUDIES

Sokal and Lessman (1960) reported than no abnormalities occurred in the offspring of four women with Hodgkin's disease who had been treated with HN2 during the first and third or second and third trimesters of pregnancy.

HN2 and its hydrochloride salt have been shown to be teratogenic in mice and rats. In mice, a single intraperitoneal dose equivalent to 1μg HN2-hydrochloride/g body weight, administered on the 10-12th day of pregnancy resulted in squat, edematous, exophthalmic embryos with practically no legs or tails (Danforth and Cater, 1954). Haskins (1948) injected albino rats with 0.55 or 1 mg HN2-hydrochloride/kg subcutaneously on either day 12, 13, 14, or 15 of gestation and all fetuses were removed at sacrifice on day 21. Six off 9 litters of the surviving females contained fetuses with abnormalities. Effects were most severe in animals

treated on day 12 of gestation. Abnormalities included receding lower jaws, cranial faults, cleft palate, deformed limbs, absence and fusion of digits, shortened tails, and unusual body proportions. Similar effects were reported by Murphy et al. (1958) for Wistar rats injected with HN2 on the 12th day of gestation. Teratogenic effects, consisting primarily of syndactylous paws and cleft palate, were seen at a dose level as low as 0.5 mg/kg. Sanyal et al. (1981) reported that the embryonic development and organogenesis of rat embryos maintained in culture were significantly inhibited when HN2-hydrochloride, at concentrations of 1 or 5 μg/mL, was added to the culture medium.

1.5.1. Mutagenicity. Information on any mutagenic effects of HN2 is currently unavailable.

1.6. TOXICOKINETICS AND METABOLISM

1.6.1. Toxicokinetics. There are no data in the biomedical or toxicological literature that describe the toxicokinetics of HN2 in humans or laboratory animals.

1.6.2. Metabolism. There are no data in the biomedical or toxicological literature that describe the metabolism of HN2 in humans or laboratory animals.

1.6.3. Percutaneous Absorption. The studies on the acute lethality of HN2 via dermal administration cited in section 1.2.2.1.3. are the only available data on percutaneous absorption of HN2 in laboratory animals or human beings.

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

Though no direct evidence exists to categorically ascribe structural-activity relationships between HN2 and other compounds, the fact that HN2's mechanism of toxicity closely resembles that of other mustard agents such as sulfur mustard (HD), is consistent with the concept that the

alkylating and vesication properties of these agents proceed through similar biochemical pathways and metabolic processes.

1.8. MECHANISTIC STUDIES

The acute toxic effects of mustard vesicants are usually ascribed to the consequences of alkylation reactions with organic compounds such as nucleoproteins and DNA. Alkylation reactions can result on physiological and metabolic disturbances as well as genotoxic effects. Several hypotheses have been advances concerning the primary cause of cell death following acute exposures. As reviewed by Papirmeister et al. (1991), the three major hypotheses are:

1. Poly(ADP-ribose) polymerase (PADPRP) hypothesis. In this theory DNA is the initial target of the mustard agent. Alkylated DNA purines undergo spontaneous and enzymatic depurination, leading to the production of apurinic sites which are cleaved ab apurinic endonucleases to yield breaks in the DNA. Accumulation of DNA breaks leads to activation of the chromosomal enzyme PADPRP which utilizes nicotinamide adenine dinucleotide (NAD^+) as a substrate to ADP-ribosylate and a variety of nuclear proteins, causing severe lowering of cellular $NAD^=$. Depletion of NAD^+results in the inhibition of glycolysis, and stimulation of the nicotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent hexose monophosphate shunt (HMS) pathway follows as a result of the accumulation of glucose-6-phosphate. Induction and secretion of proteases is stimulated as a result of enhanced HMS activity, and this leads to pathological changes in the cell.

2. Thiol-Ca^{++} peroxidation hypothesis. The first step in this process is thought to be the alkylation of glutathione (GSH) by the mustard agent. Depletion of GSH subjects protein sulfhydryl groups to damage from the agent or from reactive cellular oxidants. Depletion of GSH subjects protein sulfhydryl groups to damage from the agent or from reactive cellular oxidants. Proteins most susceptible to damage include Ca^{++} translocases (Ca^{++}-stimulated, Mg^{++}-dependent ATPase) which are dependent on thiol groups to maintain cellular Ca^{++} homeostasis, and microfilamentous proteins, where loss of sulfhydryl groups could result in disruptions of the cytoskeletal and the loss of structural integrity of the plasma membrane.

3. Lipid peroxidation hypothesis. In this hypothesis the mustard agent causes depletion of GSH which in turn leads to the buildup of highly toxic oxidants, usually through H_2O_2.-dependent reaction sequences. The oxidizing agents react

with membrane phospholipids to form lipid peroxides, initiating a chain reaction of lipid peroxidation which can lead to alterations in membrane fluidity, with consequent loss of membrane protein function and integrity.

2. INTERPRETATION OF AVAILABLE INFORMATION

Mustard vesicants are chemicals capable of causing severe skin and eye damage at very low concentrations. Agent HN2 (bis(2-chloroethyl)methylamine,, mechlorethamine) has the empirical formula $C_5H_{11}Cl_2N$. Much of the toxicity information for HN2 is based on studies using the compound's hydrochloride salt, with the presumption that the toxicity of the salt would be the same as that of HN2 alone.

Direct contact with HN2 to the skin results in edema, ulceration, and necrosis, while exposure to the lungs and eyes results in damage to the respiratory tract epithelium, conjunctivitis and blindness. Profound symptoms of systemic toxicity include nausea, vomiting, fever, and malaise (ITII, 1975), with delayed effects including chronic bronchitis, and respiratory tract and skin cancers.

By analogy to the chemical warfare agent HD (sulfur mustard), powerful alkylating agents such as HN2 might be expected to be carcinogenic as a consequence of direct interaction with the host genome. The limited evidence that is available on the carcinogenicity of HN2 is consistent with this concept, though the data lack the quantitative requirements necessary to serve as a basis for the development of numerical carcinogenicity benchmarks.

3. DOSE-RESPONSE ASSESSMENT FOR NON-CANCER EFFECTS

3.1. INGESTION EXPOSURE

Available data are insufficient to support development of a chronic oral RfD estimate for HN2 In addition, there are no ambient water quality criteria or drinking water standards for HN2.

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Agent HN2 ((bis(2-chloroethyl)methylamine,, mechlorethamine)
CASRN -- 51-75-2
Last Revised -- No Data

3.2. INHALATION EXPOSURE

Available data are insufficient to support development of a chronic RfC estimate for HN2.

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- Agent HN2 ((bis(2-chloroethyl)methylamine,, mechlorethamine)
CASRN -- 51-75-2
Last Revised -- No Data

3.3. DERMAL EXPOSURE

Available data are insufficient to support development of a chronic dermal exposure (RfD_d) for HN2.

REFERENCE DOSE FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- Agent HN2 ((bis(2-chloroethyl)methylamine,, mechlorethamine)
CASRN — 51-75-2
Last Revised -- No Data

4. DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Data are insufficient to assess the carcinogenicity of HN2 in humans or animals for oral or inhalation exposures.

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

HN2 cannot be classified as to potential human carcinogenicity because of the lack of adequate data.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Not classifiable

Basis -- Lack of adequate human or animal data

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Attachment 1

TOXICITY ASSESSMENT FOR THE NERVE AGENT SARIN (GB)

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PREFACE

This report assesses the potential non-cancer and cancer effects of the nerve agent Sarin, (agent GB, isopropyl methylfluorophosphate) (CAS Number 107-44-8). Information pertaining to non-cancer and cancer effects of GB has not been assessed previously by the United States Environmental Protection Agency (U.S. EPA).

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ABSTRACT

Data pertaining to the potential cancer/non-cancer effects of agent GB (methyl-phosphonofluoridate isopropyl ester, sarin) are reviewed. The compound is one of a number of organophosphorus nerve agents that are defined by their acute toxicity at very low doses, extremely rapid internalization, and the near-instantaneous onset of a suite of devastating symptoms that lead to death or incapacity within a short time. The group are characterized by their anti-acetylcholinesterase (AChE) activity, with consequent ACh overstimulation representing the trigger for profound clinical signs and physiological responses, preeminent among which are a general respiratory failure and the perturbation of the nervous system. Longer term studies of GB administration at concentrations below the threshold for acute effects have failed to provide any evidence for the carcinogenicity of agent GB, an observation supported by the absence of evidence of mutagenicity or of any increased tumor incidence in occupationally-exposed workers. Similarly, GB was not associated with the onset of developmental toxicity in either rats or rabbits, even at concentrations that induced toxicological responses in the dams. However, a subchronic study exposing rats to agent GB has been used to derive a chronic oral RfD, with inhibition of red blood cell (RBC) and plasma AChE activities representing the critical effects. An uncertainty factor of 2700 (consisting of 10 for inter-species extrapolation, 10 to protect sensitive subpopulations, 3 for subchronic to chronic extrapolation, 3 for LOAEL to NOAEL extrapolation, and 3 for deficiencies in the data base) was used to reduce an adjusted LOAEL of 0.054 mg/kg-day to a chronic oral RfD of 2E-2 μg/kg-day.

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1. SUMMARY OF TOXICITY INFORMATION

1.1. EPIDEMIOLOGICAL STUDIES

Information is currently unavailable on the human health effects of GB as revealed through epidemiological studies.

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity. Health and environmental impacts of nerve agents and related compounds (i.e., organophosphate insecticides) have been reviewed by O'Brien (1960), Matsumura (1976), Dacre (1984), Carnes and Watson (1989), Watson et al. (1989), and Munro et al. (1994). Nerve agents are acutely toxic by all routes of exposure. Initial symptoms of acute poisoning are fatigue, headache, mild vertigo, weakness, and loss of concentration. Moderate exposures result in miosis and excessive sweating, tearing, and salivation. Acidosis and hyperglycemia may also occur in addition to muscular weakness, muscular twitching, lacrimation, urination, and defecation. Acute poisoning can result in prostration, clonic convulsions (rapid repetitive movements) and tonic convulsions (limbs stretched and rigid) (Matsumura, 1976). Exposures sufficiently high to cause convulsions have resulted in brain lesions and cardiomyopathy in laboratory animals (Singer et al., 1987). In addition to the immediate toxicity of the nerve agents, there is concern that exposures may lead to chronic neurological effects similar to those reported for some organophosphate insecticides, (Savage et al., 1988; Gershon and Shaw, 1961; Mick, 1974; Rodnitsky, 1974; Wagner, 1983; Tabersaw and Cooper, 1966), although available data do not support the hypothesis for chronic

neuropsychological effects of nerve agents such as GB to occur in the absence of significant changes in blood cholinesterase.

Acute exposures to nerve agents are known to cause EEG changes (Grob and Harvey, 1958; Sidell, 1992) which may persist for long periods of time after exposure (Metcalf and Holmes, 1969; Burchfiel et al., 1976; Duffy et al., 1979; Duffy and Burchfiel, 1980); however, the reported changes have been considered to be clinically insignificant and not correlated with behavioral or physiological changes (DHHS,1988).

In tests on humans, Grob and Harvey (1958) found that a single oral dose of 0.022 mg GB/kg produced mild toxic effects including anorexia, nausea, heartburn, tightness in the stomach and chest, increased fatigue, nervous tension, anxiety, and other CNS responses including insomnia and excessive dreaming. An additional dose of 0.008 mg/kg within 8 hr resulted in moderate toxic effects including stomach cramps, vomiting, diarrhea, increased salivation and lacrimation, slightly decreased heart rate, and abnormal breathing. According to Thienes and Haley (1972), a single dose of 0.002 mg GB/kg caused excessive dreaming and talking during sleep and a dose of 0.020 mg/kg caused insomnia, excessive dreaming, withdrawal, and depression. At high exposures, brain damage may occur as a result of oxygen deprivation in brain tissue during GB-induced convulsions (Sidell, 1992).

Grob and Harvey (1958) reported that the first appearance of toxicity in humans occurred when red blood cell cholinesterase (RBC- ChE) activity was depressed 88% (to 12% of the baseline value) following a single oral dose of GB. The single dose oral ChE_{50} value was reported to be 0.01 mg GB/kg, and the lethal oral dose was estimated to be 0.14 mg/kg. In comparison, the single dose intra-arterial ChE_{50} was reported to be 0.003 mg/kg, and the lethal

intramuscular dose was estimated to be 0.03 mg/kg. Following intravenous (i.v.) administration, toxic effects occurred when RBC-ChE activity was depressed 40-50% (60-50% of baseline) indicating a more immediate effect on the nervous system than that caused by oral dosing (Grob and Harvey, 1958).

Grob and Harvey (1958) also administered multiple oral doses of GB to human volunteers over a period of 3 days (3-24 hr apart; average 7.5 hr). In two individuals, doses of 0.0005 or 0.005 mg/kg, totaling 0.007 mg/kg over the 3-day period, reduced RBC-ChE 33% and 27%, respectively, but neither produced toxic effects. Multiple doses of 0.008-0.016 mg/kg, totaling 0.088 mg/kg over the 3-day period, produced mild symptoms of toxicity. Similar incremental doses, totaling 0.102 mg/kg over 3 days, produced moderate symptoms of toxicity and >90% reduction in RBC-ChE activity. Grob and Harvey (1958) reported that exposure to GB had a cumulative effect that resulted in increased sensitivity to the chemical.

1.2.2. Animal Toxicity.

1.2.2.1. Acute Toxicity —

1.2.2.1.1. Oral Toxicity. Bucci et al. (1991) and Bucci and Parker (1992) conducted range-finding studies with GB Type I (GB containing tributylamine as a stabilizer) and GB Type II (GB containing diisopropylcarbodiimide as a stabilizer). The chemicals were administered by gavage once per day, 5 days per week for 3 weeks. These studies indicated that for both GB mixtures, the maximum tolerated dose was 0.3 mg/kg/day and a dose of 0.5 mg/kg/day was lethal to the test animals. These values are in line with earlier reported values for an acute oral LD_{50} for GB in rats of between 0.6-1.06 mg/kg/day (DA, 1974. Grob and Harvey, 1958).

1.2.2.1.2. Inhalation Toxicity. Anzueto et al., (1990) examined the acute inhalation toxicity of GB (and of Soman) in anaesthetized baboons, by measuring blood pressure, electrocardiogram (ECG) output, arterial/mixed blood gases, lung volumes, lung pressures, and efferent phrenic nerve activities in animals receiving GB vapor via a self-sealing breathing tube with attached delivery syringe. Controls received isopropanol via a similar system. In addition to the above parameters, bronchoalveolar lavage (BAL) was also used to evaluate the changes in composition of pulmonary epithelial cells occurring in response to GB challenge. Measurements were carried out pre-treatment, then after 4 hours, 4 days and 28 days post-treatment. Animals receiving either nerve agent displayed decreased blood pressure, and bradyarrhythmias, conditions found to be reversible by atropine administration. Hematological, hemodynamic and clinical chemistry parameters were perturbed, with both plasma and red blood cell RBC-AChE inhibited at 4 hours and 4 days, but with partial recoveries of activity after 28 days. However, BAL data showed no significant differences or trends in total cell count or viability between treated and control groups, and changes in the numbers of macrophages and neutrophils appearing in the BAL recovery fluid showed no consistent treatment-related responses.

1.2.2.1.3. Dermal Toxicity. LD_{50} values have been reported for the acute dermal toxicity of GB in a number of animal species, ranging from 1.08 mg/kg/day in mice to 115.9 mg/kg/day in pigs.

1.2.2.1.4. Toxicity Via Other Exposure Routes. In laboratory animals exposed to GB, single subcutaneous injections sufficiently high to cause convulsions resulted in brain lesions (Singer et al., 1987; see also McLeod, 1985). Brain lesions in the absence of convulsions have

also been reported in rats dosed by gavage with 0.3 mg GB Type I/kg/day for 90 days (Bucci et al., 1991).

As an example of the extensive library of studies that have addressed the potential for using chemical antidotes to protect against nerve agent attack, Koplovitz and Stewart, (1994) exposed pathogen- and atropinesterase-free New Zealand White rabbits to intramuscular injections of nerve agents such as GB at dose levels an order of magnitude higher than the 24-hour LD_{50}. The study sought to evaluate the combined ability of intravenous atropine (a cholinergic antagonist) and AChE-reactivating oximes such as pralidoxime chloride (2-PAM) or 1-2-hydroxy-iminomethyl-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI6) to protect against a GB challenge, in the presence or absence of pyridostigmine pretreatment. Injections of atropine and HI6 combined to protect against the acute lethal effects of the super-optimal dose levels of agent GB, in the presence or absence of pyridostigmine pretreatment. Atropine and 2-PAM were also protective in the presence of pyridostigmine, but failed to protect un-pretreated animals.

1.2.2.2. Subchronic Toxicity — In a subchronic study conducted by the National Center for Toxicological Research (NCTR), male and female CD rats were administered GB Type I (GB containing tributylamine as a stabilizer) or GB Type II (GB containing diisopropylcarbodiimide as a stabilizer) by gavage at dose levels equivalent to 0, 0.075, O.15, or 0.3 mg GB/kg (Bucci et al., 1991; Bucci and Parker, 1992). The doses were given once per day, 5 days per week for 13 weeks. All animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals, and on

those tissues of lower dose animals that were abnormal at necropsy. Hematological analyses and clinical chemistry (including RBC- and plasma-AChE) were evaluated in the same 6 male and 6 female rats in each dose group one week before the exposures began and also at weeks 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for neuropathy target esterase (NTE) activity. In both studies there were several statistically significant changes in clinical chemistry (i.e., aspartate aminotransferse in mid-dose males exposed to GB Type II) and hematology (decrease at week 7 in white blood cells in high-dose males exposed to GB Type II and increase at week 13 in erythrocytes in mid-dose females exposed to GB Type II); however, these effects were not sufficiently consistent to suggest organ dysfunction. Brain NTE was not altered significantly in any rats dosed with GB Type II; however, it was significantly decreased ($p < 0.05$) in female rats dosed with 0.3 mg/kg/day GB Type I. The latter, however, did not exhibit any histological signs of delayed neuropathy. GB Type II was not associated with any neoplastic or non-neoplastic lesions. Two high-dose females and one low-dose female dosed with GB Type I had brain lesions consisting of necrosis and vacuolization of individual hippocampal pyramidal cells. Bucci et al. (1991) reported this type of lesion to be consistent with hippocampal hypoxia resulting from the respiratory convulsant effects of GB; however, Bucci et al. (1991) also noted that post-mortem autolysis could have mimicked cerebral necrosis in two of the animals which were found dead. The third animal exhibited signs consistent with nerve agent toxicity (e.g., rapid breathing, salivation, lacrimation, hemorrhage in the urinary wall, and possible right forelimb paralysis), and the observed neural lesions were attributed to GB. This animal was in the test group receiving 0.075 mg/kg/day. As noted above, none of the rats dosed with up to 0.3 mg/kg/day of GB Type II

exhibited brain lesions. GB Type I contains the stabilizer tributylamine and GB Type II contains diisopropylcarbodiimide. Subchronic and chronic toxicity data for these stabilizers are lacking and in terms of acute toxicity, the stabilizers have only a fraction of the toxicity of the nerve agents (e.g., the oral LD_{50} for tributylamine is 114 mg/kg in rats; RTECS, 1995). Although there is one report indicating that tributylamine is a CNS stimulant (Windholz et al., 1983), there is no evidence to suggest that it contributed to the neurotoxic effect seen in the GB Type I study.

In the NCTR studies, RBC cholinesterase levels in the dosed animals were compared to control values for the same sampling times. In both studies there were significant decreases in plasma and RBC-ChE levels at certain time periods. The results for the GB Type II study were more internally consistent than those for GB Type I; i.e., the control values did not vary as greatly and the test groups in general more clearly exhibited dose-related changes in enzyme activity. For GB Type II, plasma ChE activity in high- and mid-dose males at week 1; in high- and mid- dose females at weeks 1 and 7; and in high dose females at week 3 was significantly lower than the corresponding control values (0 mg/kg) for the same time periods.

Inhibition of RBC-AChE was dose-related for females in the two highest dose groups and for males in all dose groups. Maximum RBC-ChE depression (48%) occurred in week 7 in both high-dose males and females. Male rats exposed to the lowest dose of GB Type II exhibited a 38% decrease in RBC-ChE activity in week 1; females exhibited a 12% decrease. By week 13, RBC-ChE activity levels in females returned to near pre-exposure levels (>90%); however, levels in males were still depressed 16-&24%. The AChE data were re-analyzed statistically by ANOVA and Dunnett's and Scheffe's Comparisons. This analysis indicated that RBC-AChE levels in males were significantly lower ($p < 0.05$) than baseline values in all dose groups at

weeks 1, 3, and 7, and for the two highest dose groups at week 13. The values for all dose groups were also significantly lower than controls at week 1, 3, and 7. Similar results were seen in females except that the RBC-AChE levels were not significantly different from controls or baseline values in the low-dose group. From this subchronic study, a LOAEL of 0.075 mg/kg/day is suggested, adjusting to a continuous dosing concentration (7 days-a-week, 24 hours-a-day) of 0.054 mg/kg/day).

In a subchronic inhalation study conducted on Fischer 344 rats, no signs of toxicity were observed in animals exposed to 0.0001 or 0.001 mg GB/m^3, 6 hr/day, 5 days/week, for up to 24 weeks (Weimer et al., 1979). Compared to the dose levels used in the subchronic oral studies described above, the exposures used in the Weimer et al. (1979) study were relatively low. For example, assuming an inhalation rate of 0.29 m^3/day and an average body weight of 0.35 kg for rats and 100% pulmonary absorption, the highest concentration in the Weimer et al. study would be equivalent to an internal dose of only 0.15 μg/kg/day.

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. There are no human data to suggest that GB is carcinogenic. As part of chronic inhalation studies conducted by Weimer et al. (1979), the tissues of animals exposed to GB for up to one year were examined for microscopic lesions including tumors. The test species included I.C.R. Swiss mice, strain A mice, Sprague-Dawley/Wistar rats, Fischer 344 rats, and purebred beagle dogs. The exposures were to 0.0001 or 0.001 mg GB/m^3, 6 hr/day, 5 days/week. Weimer et al. (1979) reported that agent-related tumors did not occur in any of the exposed species. Pulmonary tumors did occur in strain A mice; after 52 weeks of exposure, pulmonary adenomas were present in 3/19 animals exposed to 0.0001 mg GB/m^3 in 3/20 animals exposed to

0.001 mg GB/m^3, and in 0/20 controls; and for animals maintained for 6 months post-exposure, the incidence rates for pulmonary adenocarcinomas were 5/19, 6/18, and 9/29, respectively. However, these lesions were not considered to be agent-related. Strain A mice have a high natural propensity to form pulmonary tumors; the incidence of spontaneous pulmonary tumors being about 53% in animals 12 months of age and 90% in animals 18 months of age (Heston, 1942). Overall, the studies of Weimer et al. (1979) failed to provide positive evidence of GB's carcinogenicity.

1.3.2. Chronic Toxicity. There is limited information concerning the effects of GB following prolonged exposure to low concentrations. In a retrospective study of workers occupationally exposed to GB for one year or longer prior to the testing, increased brain beta activity, increased delta and theta slowing, decreased alpha activity, and increased amounts of rapid-eye-movement (REM) sleep were observed (Duffy et al., 1979; Burchfiel and Duffy, 1982). DHHS (1988) considered these changes to be of questionable importance due to the absence of clinically significant neuropsychological effects.

There are no animal studies involving chronic oral exposures to GB. In the chronic inhalation studies conducted by Weimer et al. (1979), I.C.R. Swiss and strain A mice, Sprague-Dawley/Wistar and Fischer 344 rats, and purebred beagle dogs were exposed to 0, 0.0001, or 0.001 mg GB/m^3, 6 hr/day, 5 days/week, for up to 52 weeks. Four male and 8 female beagles were exposed to each test concentration. In the rodent studies, 50 animals of each sex of each strain were exposed to each test concentration. The control groups were identical to the test groups except that an additional 100 F344 rats and A strain mice were used. RBC-AChE activity levels were monitored throughout the study for all the test species. No dose-related, statistically

significant changes in RBC-AChE occurred in any species at any sampling time. Using an inhalation rate of 0.29 m^3/day for rats, and assuming 100% pulmonary absorption, the 6 hr/day, 5 days/week exposure would correspond to an average daily dose of 0.00015 mg/kg. This dose is considerably below the gavage doses of 0.075 mg/kg/day that produced cholinesterase depression in the subchronic studies described in Section 1.2.2.2.

Five of 20 dogs exhibited abnormal EKGs at the time of sacrifice; elevated P waves were suggestive of right atrial hypertrophy; however, there was no evidence of enlargement or physical abnormalities of the heart. The absence of pre-exposure data precludes identifying this effect as due to GB exposure.

A higher incidence of tracheitis occurred in colony rats (a Sprague Dawley/Wistar population) and in Fischer rats exposed to GB in comparison to control animals, with the most severe cases occurred in the high-exposure group. The investigators could not determine whether the occurrence of tracheitis was agent-related. No other overt signs of GB-related toxicity were observed at either exposure level.

Atrophy of the seminiferous tubules, starting at 12 weeks of exposure, was also seen in the Fischer rats. The investigators noted that this inbred strain of rat is susceptible to numerous genetically-based defects which may appear under experimental conditions of stress. The tests were repeated using the same experimental protocol for 12 and 24 weeks, with none of the rats in this second assay exhibiting testicular atrophy.

1.4. REPRODUCTIVE STUDIES

Data pertaining to the reproductive toxicity of GB in human beings or laboratory animals are currently unavailable.

1.5. DEVELOPMENTAL TOXICITY

No data are available to evaluate the potential reproductive and developmental effects of GB in humans; however, studies in laboratory animals indicate that such effects are unlikely even at dose levels that are maternally toxic. LaBorde and Bates (1986, 1996) conducted developmental toxicity studies on agent GB Type I and GB Type II using CD rats and New Zealand rabbits. In the rat studies, the test animals were dosed with 0, 100, 240, or 380 μg/kg of GB orally on days 6-15 of gestation. Females were weighed on gestational days 0, 616 and prior to sacrifice on gestational day 20. The test animals were observed for clinical signs of toxicity. At sacrifice, gravid uteri were weighed and examined for number and status of implants (alive, resorbed or dead). Individual fetal body weight and internal or external malformations were recorded. Maternal toxicity (evidenced by excessive salivation, ataxia, lacrimation) and mortality (8/29 for GB Type I and 13/29 for GB Type II) occurred in the high-dose group. There were no significant differences among treatment groups in the incidence of resorptions or in the average body weight of live fetuses per litter. The only fetal morphological anomaly was fetal hydroureter which occurred at a rate of 5.2, 1.9, 5.3 and 2.1% with GB Type I and 4, 5, 3.2, and 0.5% with GB Type II in the 0, 100, 240, and 300 μg/kg dose groups, respectively. The observed effect was not dose-related and was, therefore, considered to be a spontaneous variant. Skeletal and cartilage variants occurred between dose group but these were not statistically significant. In similar studies conducted on New Zealand rabbits using the same experimental protocol, oral doses of 0, 5, 10, or 15 μg/kg/day on gestational days 6-19, resulted in no fetal toxicity or teratogenicity (LaBorde and Bates, 1986, 1996). The only observed fetal anomaly was retinal folding which occurred at a rate of 6.8, 3.9, 4.3, and 7.4% for GB Type I and 17, 18,

25, and 19% for GB Type II in the 0, 5, 10, and 15 Hg/kg dose groups, respectively. The frequency of the anomaly was not dose-related and the variant was, therefore, considered to be a spontaneously occurring malformation. Maternal toxicity, evidenced by excessive salivation, ataxia, and lacrimation, occurred at the highest dose.

The developmental toxicity of GB was also evaluated by Denk (1975). In this study Sprague Dawley rats were exposed to GB vapors (0.1 or 1 $\mu g/m^3$) for 6 hr/day, 5 days/week, for varying time periods. In one series of tests, male rats were exposed for 1 week to 1 yr, and then mated to unexposed females. Nineteen days after mating, the females were sacrificed and examined for number of corpora lutea, deciduomata, number of fetal deaths, and number of live fetuses. In a second series of tests mated pairs of rats were exposed to GB for 1, 2, or 3 weeks or until the pups were whelped. The incidence of intrauterine deaths was recorded and all fetuses were examined for abnormalities. In a third series of tests, males and females were exposed to GB for 10 months and then mated. The F1 generation was mated, as was the F2 generation. The number and sex of offspring, number of preweaning deaths, number weaned, and pup weights at various ages were recorded. Denk (1975) reported that GB, at the doses and by the route used, had no adverse effects with respect to dominant lethal mutations, reproductive performance, fetal toxicity, and teratogenesis.

1.5.1. Mutagenicity. No information is available regarding the genotoxicity of GB in humans. In bioassays using bacteria and mammalian cell cultures, GB was not genotoxic or mutagenic when tested with or without metabolic activation (Goldman et al., 1987). GB did not induce biologically significant increases in mutations when tested in the Ames Salmonella assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538) (Goldman et al., 1987). GB

Type I and GB Type II did not induce a significant increase in forward mutations when tested on mouse L5178Y lymphoma cells at concentrations of 50, 100, or 200 Hg/mL (Goldman et al., 1987). No increase in sister chromatid exchanges (SCE) was observed in Chinese hamster ovary cells exposed in vitro to 200 Hg/mL of GB (Goldman et al., 1987). Mice treated in vivo with a maximally tolerated intraperitoneal dose of 360 μg GB/kg did not exhibit a significant increase in SCE in splenic lymphocytes (Goldman et al., 1987). Exposure of rat hepatocytes to GB concentrations as high as 2.4×10^{-3} M resulted in a decrease in DNA repair synthesis, leading Goldman et al. (1987) to conclude that GB probably did not damage DNA directly but that it might inhibit DNA synthesis after non-agent-induced DNA damage had occurred.

1.6. TOXICOKINETIC STUDIES

1.6.1. Toxicokinetics. Shih, et al., (1994) used GC/MS to measure the appearance of putative metabolites of the nerve agents, GB, GD and GF, in the urine of male Crl:CD BR COBS or VAF/Plus rats. The alkyl phosphonic acids were considered to be the single major metabolites of these nerve agents. When 75 μg/kg GB was administered subcutaneously, its alkyl phosphonic metabolite appeared quickly in the blood after injection and was then rapidly eliminated in the urine, with 59% of the dose voided in the first four hours after injection.

1.6.2. Metabolism. The review by Munro, et al., (1994) summarizes the key information concerning potential mechanisms by which GB is metabolized, with emphasis on the important role played by certain plasma carboxylesterases. The presence of significant amounts of this enzyme in the plasma of rats and mice, with consequent ability of these species to form isopropyl phosphonic acid, as discussed in section 1.6.1., is considered to represent a possible explanation

for their relative resistance to GB attack. a feature in contrast to that existing in human beings which appear to lack this enzyme activity.

1.6.3. Percutaneous Absorption. Munro et al., (1994) pointed to the volatility of GB as a contributing factor to the agent's substantial lack of dermal absorption. Site-of entry effects were also considered to be possible mitigating factors. Procedures designed to impede GB evaporation from the skin surface, such as using a closed patch or inverted sample cup, dissolving the agent in a lipophilic base, and/or abrading the skin, markedly increased the dermal toxicity.

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

GB is one of a series of closely related organophosphate nerve agents, characterized by their ability to kill at low concentration through the inhibition of acetylcholinesterase (AchE). Though GB, along with its related compounds, GA, VX, and GD, may each display unique physico/chemical characteristics, their collective ability to irreversibly inhibit AChE represents their primary biological interaction and results in excessive accumulation of ACh at synapses, overstimulation of the portions of the nervous system that control smooth muscle, cardiac muscle, and exocrine glandular functioning. The suite of debilitating symptoms arising from these perturbations are compounded by those consequent on ACh overstimulation of the nervous system, and by respiratory failure arising from depression of the brain's respiratory center, neuromuscular block of the respiratory muscles, bronchial constriction, and increased lung secretions.

1.8. MECHANISTIC STUDIES

Nerve agents are inhibitors of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine at some neuronal synapses and myoneural junctions. Nerve agents interact with the enzyme by phosphorylation, with consequent inactivation, thereby preventing breakdown acetylcholine. The organophosphate-inhibited enzyme can be reactivated by dephosphorylation, but this occurs at a slower rate than the initial inactivation of acetylcholine. Consequently, there is an overall depletion of acetylcholinesterase and a buildup of acetylcholine. In addition, the nerve agent-enzyme complex can also undergo an "aging" process (thought to be due to a loss of an allyl or alkoxy group), whereby it becomes resistant to dephosphorylation (see review by Munro et al., 1994). In fact, differences in the rates of aging and reactivation may be important in evaluating toxicity data especially when extrapolating from animal studies to humans. In vitro tests conducted by Grob and Harvey (1958) indicate that both GA and GB combine with cholinesterase almost irreversibly during the first hour of their reaction. Sidell and Groff (1974) reported that the GB-ChE complex ages very rapidly in vivo, with 45-70% completion by 5 hours after infusion. By contrast, the complex formed between ChE and the nerve agent VX does not age significantly, and the rate of spontaneous reactivation can be as fast as 1%/hr in humans (Sidell and Groff, 1974).

The anticholinesterase effects of the organophosphate nerve agents can be characterized as being muscarinic, nicotinic, or central nervous system (CNS)-related. Muscarinic effects occur in the parasympathetic system (bronchi, heart, pupils of the eyes; and salivary, lacrimal and sweat glands) and result in signs of pulmonary edema, bradycardia, miosis, tearing, and sweating. Nicotinic effects occur in somatic (skeletal/motor) and sympathetic systems, and result

in muscle fasciculation, muscle weakness, tachycardia, and diarrhea. Effects on the CNS by organophosphates are manifested as giddiness, anxiety, emotional lability, ataxia, confusion, and depression (O'Brien, 1960).

Although the inhibition of cholinesterase within neuro-effector junctions or the effector itself is thought to be responsible for the major toxic effects of organophosphate agents, these compounds can apparently affect nerve-impulse transmission by more direct processes as well. Direct effects may occur on excitable tissues, receptors, and ionic channels. According to Somani et al. (1992), the direct action of nerve agents on nicotinic and muscarinic ACh receptors may occur when concentrations in the blood rise above micromolar levels, whereas at lower levels the action is mainly the result of inhibition of AChE. Albuquerque et al. (1985) have shown that agent GA, as well as agents GB and GD, are capable of changing receptor sites in a manner similar to that exhibited by acetylcholine, which promotes the conductance of electrophysiological signals associated with stimulation of neuromuscular function. VX "may directly affect a small population of muscarinic ACh receptors that have a high affinity for [^{3}H]cis-methyldioxalane binding" (Somani et al., 1992). VX may also counteract the effects of ACh by acting as an open channel blocker at the neuromuscular junction, thereby interrupting neuromuscular function (Rickett et al., 1987).

Exposure to some organophosphate cholinesterase inhibitors results in a delayed neuropathy characterized by degeneration of axons and myelin. This effect is not associated with the inhibition of acetylcholinesterase, but rather with the inhibition of an enzyme described as neuropathy target esterase (NTE); however, the exact mechanism of toxicity is not yet fully understood (Munro et al., 1994). For some organophosphate compounds, delayed neuropathy

can be induced in experimental animals at relatively low exposure levels, whereas for others the effect is only seen following exposure to supralethal doses when the animal is protected by antidotes from the acute toxic effects caused by cholinesterase inhibition.

Although there is the potential for nerve agents to have direct toxic effects on the nervous system, there is no evidence that such effects occur in humans at doses lower than those causing cholinesterase inhibition. For the purpose of evaluating potential health effects, inhibition of blood cholinesterase is generally considered the most useful biological endpoint.

Acetylcholinesterase is bound to the surface of red blood cells (termed RBC-ChE). RBC-ChE activity, as well as the activity of a second type of cholinesterase found in blood plasma (butyrylcholinesterase, or plasma cholinesterase) have been used as bioindicators of exposure to organophosphate compounds (pesticides and nerve agents). There is some evidence that RBC-AChE is as sensitive as brain ChE to the effects of nerve agents. Grob and Harvey (1958) reported that the *in vitro* concentrations producing 50% depression of brain-ChE and RBC-AChE activity were the same in the case of GA (1.5 x 10-8 mol/L), and only slightly different (3×10^{-9} mol/L and 3.3×10^{-9} mol/L) in the case of GB. However, *in vivo* animal studies indicate a poor correlation between brain and RBC-AChE in acute exposures (Jimmerson et al., 1989), and this is reflected in the fact that blood cholinesterase activity may not always be correlated with exposure or with signs and symptoms of toxicity. Acute exposures to high concentrations may cause immediate toxic effects before significant changes occur in blood ChE activity, and repeated exposures over a period of several days may result in a sudden appearance of signs and symptoms due to cumulative effects (Grob and Harvey, 1958). Conversely, blood ChE activity can become very low without overt signs or symptoms during chronic exposures to

low concentrations of organophosphates. This may be due to a slower rate of recovery of RBC-ChE compared to tissue ChE, or to a non-cholinesterase-dependent recovery pathway for neural tissue (Grob and Harvey, 1958). Sumerford et al. (1953) reported that orchard workers exposed to organophosphate insecticides had RBC-AChE values as low as 13% of average pre-exposure levels without any other signs or symptoms of toxicity. Animal studies have demonstrated that chronic exposures to low concentrations of organophosphate insecticides can also result in increased tolerance levels (Barnes, 1954; Rider et al., 1952; Dulaney et al., 1985). Similarly, Sumerford et al. (1953) reported increased levels of tolerance to organophosphate insecticides in people living near orchards subject to insecticide applications. Such adaptation may result from increased rates of formation of blood ChE, or from increased rates of detoxification.

The blood cholinesterases and other esterases may, to some degree, provide a protective effect by binding with some fraction of the anticholinesterase compound (Wills, 1972). However, not all nerve agents bind equally well with all cholinesterases. Agent GB inhibits both RBC-ChE (80-100%) as well as plasma-ChE (30-50%) (Grob and Harvey, 1958). By contrast, agent VX preferentially inhibits RBC- ChE (70% compared with about 20% inhibition of plasma ChE) (Sidell and Groff, 1974). Rodents (but not humans) have other enzymes in the blood, termed aliesterases, which can bind to organophosphates, thereby reducing the amount available for binding with acetylcholinesterase (Fonnum and Sterri, 1981). Agent GB binds with aliesterases; however, according to Fonnum and Sterri (1981), the quaternary ammonium group of VX prevents it from being a substrate for aliesterases. The strong specificity of agent VX for AChE may account, in part, for the fact that it is much more acutely toxic than agents GA and GB.

2. INTERPRETATION OF AVAILABLE INFORMATION

Agent GB is a colorless liquid with a molecular weight of 140.1 (DA, 1974, MacNaughton and Brewer, 1994); it has a vapor density of 4.8 (air = 1) and a liquid density of 1.09 g/mL at 25° C (DA, 1974). The vapor pressure of GB is 2.9 mm Hg at 25° C. It is miscible with water and readily soluble in organic solvents (DA, 1974). GB is very volatile with a vapor pressure of 2.9 mm Hg at 25° C (MacNaughton and Brewer, 1994). A vapor concentration of 22 g/m^3 has been reported for a temperature of 25° C (DA, 1974).

GB is completely miscible with water (DA, 1974), with a rate of hydrolysis dependent on temperature, pH, and other water quality parameters (Epstein, 1974; Morrill et al., 1985; Clark, 1989). At 20°C, the half-life ranges from 461 hr at pH 6.5 to 46 hr at pH 7.5. At 25°C, the half-life is 237 hr at pH 6.5 and 24 hr at pH 7.5. For comparison, GA is much more persistent at low temperature; at 0° C, with a half life of 8,300 hours at pH of 6.5. The rate of hydrolysis under natural conditions is accelerated by the presence of ions (dissolved solids) in solution. Metal cations such as copper and manganese in seawater increase the rate of hydrolysis (Epstein, 1974). Based on an estimated Henry's Law Constant of 5.4 x 10-7 atm m3/mol (MacNaughton and Brewer, 1994), evaporation of GB from water is expected to be slow.

According to Morrill et al. (1985), evaporation is the primary mechanism for the loss of GB from soil, and this is supported by the estimated volatility potential (slope of the vapor pressure vs. concentration in soil organics) of 4.9 x 104 mm Hg/mg/kg and by the air-soil partition coefficient of 135 x 10-5 mg/m^3 (for a soil density of 1.4 g/cm^3) as reported by MacNaughton and Brewer (1994). In a field test conducted in Finland detectable concentrations

of GB (> 1 pg/dm^3) were found in the air for up to 9 days following application of 10 mg of GB over a 10 x 10 meter area of moss (temperature 2.5-8°C, humidity 60-100%, wind speed 1-10 m/s) (Sanches et al., 1993). Studies conducted with soil samples from Dugway Proving Ground and Edgewood Arsenal showed that 90% of GB added to soil and maintained in closed containers at room temperature (20-25°C) was lost in the first 5 days (Small, 1984).

Binding of GB to soil organics is likely to be limited considering the relatively low log K_{OW} of 0.72 and low K_{OC} value of 59 (MacNaughton and Brewer, 1994); therefore, there is a potential for leaching and groundwater contamination. MacNaughton and Brewer (1994) calculated a leaching index of 3.7 for GB, (i.e., the number of leachings required to reduce the GB soil concentration to one-tenth of the original amount, assuming that for each leaching one kilogram of soil is in equilibrium with one liter of water). However, the amount reaching ground water is likely to be limited by hydrolysis.

The potency of the anticholinesterase activity of nerve agents and other organophosphates is expressed by the bimolecular rate constant (k_i) for the reaction of the phosphate compound with the enzyme and by the molar concentration causing 50% inhibition of the enzyme (I_{50}). The relationship between I_{50} and k_i as a function of time (t) is expressed by the following equation (Eto, 1974): $I_{50} = 0.693/t \times k_i$.

I_{50} data for several organophosphate nerve agents have been tabulated by Dacre (1984). The pI_{50} (negative log of the molar concentration causing 50% inhibition) for GB was reported to be 8.8 by Tammelin (1958) and 8.9 by Dacre (1984), and calculated as 8.5 from an I_{50} of 3.7 x 10-9 mol/L reported by Grob and Harvey (1958).

The potency of nerve agents can also be expressed in terms of the dose necessary to produce 50% inhibition of cholinesterase (ChE_{50}). In humans, RBC-ChE_{50} values for GB are 0.003 mg/kg and 0.01 mg/kg, respectively, for i.v. and oral doses (Grob and Harvey, 1958). The relative effectiveness of nerve agents in inhibiting cholinesterase is closely correlated with their acute toxicity.

3. DOSE-RESPONSE ASSESSMENT

3.1. INGESTION EXPOSURE

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Agent GB (methyl-phosphonofluoridate isopropyl ester, sarin)
CASRN -- 107-44-8
Date prepared -- September, 1996

ORAL RfD SUMMARY

Critical Dose -- 0.075 mg/kg-day (LOAEL)
UF -- 2700
MF -- 1
RfD — 2E-5 mg/kg-day

Critical Study

Critical Effect -- Inhibition of acetylcholinesterase in red blood cells (RBC).

Study Type -- Rat Subchronic Oral Study

Reference -- Bucci and Parker, 1992

NOAEL —
NOAEL(ADJ) --

LOAEL — 0.075 mg/kg-day
LOAEL(ADJ) — 0.054 mg/kg-day

Conversion Factors and Assumptions --

PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Bucci, T.J. and R.M. Parker. 1992. Toxicity Studies on Agents GB and GD (Phase II), 90 Day Subchronic Study of GB (Sarin Type II) in CD-Rats. Final Report. Prepared for U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, MD. DTIC AD-A248618.

In this study male and female CD rats (12 per sex per dose group) were administered GB Type II (GB containing diisopropylcarbodiimide as a stabilizer) by gavage at dose levels equivalent to 0 (saline vehicle control), 0.075, 0.15 and 0.3 mg GB/kg/day. The agent was administered once per day, 5 days per week for 13 weeks. All animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals, and on tissues of lower dose animals that appeared abnormal at necropsy. Hematological analyses and clinical chemistry (including RBC and plasma cholinesterase) were evaluated in the same 6 male and 6 female rats in each dose group one week before the exposures began and also at weeks 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for the enzyme neuropathy target esterase (NTE). Although there were several statistically significant changes in clinical chemistry (i.e., aspartate aminotransferse in mid-dose males) and hematology (decrease in white blood cells in high-dose males at week 7, and increase in erythrocytes in mid-dose females at week 13), these effects were not sufficiently consistent to suggest organ dysfunction. Administration of GB Type II at dose levels up to 0.3 mg/kg/day had no effect on brain NTE and was not associated with any neoplastic or non-neoplastic lesions.

Cholinesterase (ChE) levels in dosed animals (6 per sex/group) were compared to control values for the same sampling times. Plasma ChE was significantly lower than control values at the two highest dose levels at week 1 in males and at weeks 1 and 7 in females (and also at the highest dose at week 3). Inhibition of red blood cell acetylcholinesterase (RBC-AChE) was dose related for females in the two highest dose groups and for males in all dose groups. RBC-AChE levels in male rats were reported to be significantly lower than the corresponding controls for all dose levels at weeks 1 and 3, and for the two highest dose levels at week 7. In females, RBCAChE levels were significantly lower than controls for the two highest dose levels at weeks 1, 7 and 13, and for the highest dose level at week 3. For males in the low, mid- and high-dose groups, mean RBC-AChE levels were 62, 61, and 53% of the baseline (pre- exposure) value at week 1; 77, 71, and 66% of baseline at week 3; 63, 55, and 52% at week 7; and 84, 76, and 79% at week 13. For females in the low-, mid- and high-dose groups, RBC-AChE levels were 88, 63, and 59% of baseline at week 1; 96, 55, and 54% at week 3; 84, 59, and 52% at week 7; and >100, 93 and 91% at week 13, respectively. The data were re-analyzed statistically (using standard deviations) with ANOVA and Dunnett's and Scheffe's Comparisons. This analysis indicated that RBC-AChE levels in males were significantly lower ($p < 0.05$) than baseline values for all dose groups at weeks 1, 3 and 7. The RBC-AChE values for all dose groups were also significantly lower than controls at week 1, 3 and 7. Similar results were seen in females except that the RBCAChE levels were not significantly different from controls or baseline values in the low-dose group. Because of the significant decrease in RBC-AChE in male rats, the lowest dose of 0.075 mg/kg/day is considered a LOAEL.

Bucci et al. (1991) conducted a second rat subchronic toxicity study using GB Type I (GB containing tributylamine as a stabilizer). Male and female CD rats (12 per sex per dose group) were administered GB Type I by gavage at dose levels equivalent to 0.075, 0.15 and 0.3

mg GB/kg/day. The chemical was administered once per day, 5 days per week for 13 weeks. All animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high- dose and control animals, and on those tissues of lower dose animals that were abnormal at necropsy. Hematological analyses and clinical chemistry (including RBC and plasma cholinesterase) were evaluated in the same 6 male and 6 female rats in each dose group one week before the exposures began and also at weeks 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for NTE. Although there were several statistically significant changes in clinical chemistry and hematology, these effects were not sufficiently consistent to suggest organ dysfunction. Administration of GB Type I at dose levels up to 0.3 mg/kg/day had no effect on NTE except for a significant ($p < 0.05$) depression of NTE in high-dose females. No neoplastic or non-neoplastic lesions were seen except for the occurrence of brain lesions in two high-dose females and one low-dose female. These lesions consisted of necrosis and vacuolization of individual hippocampal pyramidal cells. It was reported by Bucci et al. (1991) that this type of lesion is consistent with hippocampal hypoxia resulting from the respiratory convulsant effects of GB. Bucci et al. (1991) noted that post-mortem autolysis could have mimicked cerebral necrosis in the two animals found dead. The third animal exhibited signs consistent with nerve agent toxicity (e.g., rapid breathing, salivation, lacrimation, hemorrhage in the urinary wall, and possible right forelimb paralysis). This animal was in the test group receiving 0.075 mg/kg/day. Information was not reported on the blood ChE levels of this animal; therefore, it cannot be determined whether this animal may have been accidentally overdosed. Cholinesterase levels in the dosed animals (6 per sex/group) were compared to control values for each sampling time. Plasma ChE in the high-dose females was significantly lower than control values ($p < 0.05$) at weeks 3, 7 and 13, and in the mid-dose females at week 7. In males, plasma ChE was significantly lower than the corresponding controls at weeks 1, 3 and 7 in the high-dose group, at weeks 1, 3, 7, and 13 in the mid-dose group, and at weeks 7 and 13 in the low-dose group. RBC-AChE was not significantly lower than control values except at week 7 for the low-, mid- and high-dose females.

UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

Ten to account for sensitive subpopulations, 10 for interspecies extrapolation, 3 for subchronic to chronic extrapolation, 3 for LOAEL-to-NOAEL extrapolation, and 3 for deficiencies in the database.

UF -- 2700

MF -- 1

JUSTIFICATION FOR UNCERTAINTY FACTOR COMPONENTS.

An uncertainty factor of 10 for sensitive subpopulations is considered necessary because some individuals have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whitaker, 1962). For homozygous individuals, the activity can be as low as 8-21% of the normal mean (Bonderman and Bonderman, 1971). These individuals may be unusually sensitive to organophosphate anticholinesterase compounds (Morgan, 1989).

An uncertainty factor of 10 is used for animal-to-human extrapolation because there is ample evidence that humans are more sensitive to GB than laboratory rodents. In humans, the single dose oral RBC-$AChE_{50}$ (dose required to lower red blood cell cholinesterase by 50%) is 0.01 mg/kg (Grob and Harvey, 1958), and an average daily dose of 0.034 mg/kg for three days resulted in moderate signs of toxicity. In comparison, rats receiving 0.3 mg GB Type II/kg/day for 90 days exhibited decreases in blood cholinesterase levels but no signs of toxicity (Bucci and Parker, 1992).

An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of oral RfDs for other organophosphate compounds, EPA has used NOAELs for cholinesterase inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data indicate that maximum ChE inhibition usually occurs 30-60 days or more after exposure begins, after which it levels off or even shows signs of recovery. In the Bucci and Parker (1992) study, plasma and RBC-ChE activity levels at week 13 were no longer significantly different from both baseline and control values, particularly for the lowest dose level; therefore, increased ChE inhibition is not expected to occur at longer exposure periods. However, an uncertainty factor of 3 is used here because studies are not available to verify that adverse effects would not occur following chronic exposures.

A LOAEL-to-NOAEL uncertainty factor of 3 is used instead of 10 because the endpoint, cholinesterase inhibition, was not associated with signs of toxicity.

The data base for GB consists of two well designed and well conducted subchronic toxicity studies in rats, developmental studies in two species (rats and rabbits), delayed neuropathy studies in chickens and rats, and chronic inhalation studies in mice, rats and dogs. In addition, there is substantial human data for acute and short-term exposures. These studies support the use of cholinesterase inhibition as the critical endpoint for deriving an oral RS. The data base for GB is, however, lacking a multi- generational reproductive toxicity study. Because studies on other organophosphate cholinesterase inhibitors, including a multi-generational study on agent VX, indicate that reproductive effects are unlikely, a full Uncertainty Factor of 10 is not considered necessary.

Although some animal data suggest that GB may produce delayed neuropathy in some species by a mechanism other than cholinesterase inhibition, such effects occur at dose levels

above those causing cholinesterase inhibition; therefore, an RFD based on cholinesterase inhibition should also be protective of delayed neurotoxicity.

ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

Acute studies: Grob and Harvey (1958): Single or multiple oral doses to human volunteers.

An evaluation of cholinesterase inhibition in humans following acute and short-term exposures can be used to support the use of cholinesterase inhibition as an endpoint for deriving an oral RfD for GB. In tests on humans, Grob and Harvey (1958) found that a single oral dose of 0.022 mg/kg produced mild toxic effects including anorexia, nausea, heartburn, tightness in the stomach and chest, increased fatigue, nervous tension, anxiety, and other central nervous system responses including insomnia and excessive dreaming. An additional dose of 0.008 mg/kg within 8 hr resulted in moderate toxic effects including stomach cramps, vomiting, diarrhea, increased salivation and lacrimation, slightly decreased heart rate, and abnormal breathing. According to Thienes and Haley (1972), a single dose of 0.002 mg GB/kg caused excessive dreaming and talking during sleep and a dose of 0.020 mg/kg caused insomnia, excessive dreaming, withdrawal, and depression. At high exposures, brain damage may occur as a result of oxygen deprivation in brain tissue during GB-induced seizures (Sidell, 1992).

Grob and Harvey (1958) reported that the first appearance of toxicity in humans occurred when RBC- ChE activity was depressed 88% (to 22% of the baseline value) following a single oral dose of GB. The single dose oral ChE_{50} value was reported to be 0.01 mg GB/kg, and the single oral dose causing lethality was estimated to be 0.14 mg/kg. In comparison, the single dose intra-arterial ChE_{50} was reported to be 0.003 mg/kg, and the single intramuscular dose causing lethality was estimated to be 0.03 mg/kg. Following i.v. administration, toxic effects occurred when RBC-ChE activity was depressed 40-50% (60-50% of baseline) indicating a more immediate effect on the nervous system than that caused by oral dosing (Grob and Harvey, 1958).

Grob and Harvey (1958) also administered to human volunteers multiple oral doses of GB over a period of 3 days (3-24 hr apart; average 7.4 hr). In two individuals, doses of 0.0005 or 0.005 mg/kg, totaling 0.007 mg/kg over the 3-day period, reduced RBC-ChE 33% and 27%, respectively, but neither produced toxic effects. Multiple doses of 0.008-0.016 mg/kg, totaling 0.064-0.096 mg/kg (average 0.088 mg/kg) over the 3-day period resulted in >90% reduction in RBC-AChE and produced mild symptoms of toxicity. Similar incremental doses, totaling 0.102 mg/kg over 3 days resulted in 99% reduction in RBC-ChE activity and caused moderate symptoms of toxicity. Grob and Harvey (1958) reported that exposure to GB had a cumulative effect that resulted in increased sensitivity to the chemical. For the three-day exposure period, the average daily dose producing no signs of toxicity was 2.3 μg/kg, about 115 times larger than the RfD. The average dose causing mild effects was 2.9 μg/kg, about 145 times larger than the RfD; and the average dose causing moderate toxicity was 3.4 μg/kg/day, about 170 times larger than the RfD.

There is limited information available concerning the chronic toxicity of GB in humans. In a retrospective study of workers occupationally exposed to GB for one year or longer, Duffy et al. (1979) and Burchfiel and Duff (1982) found increased brain beta activity, increased delta and theta slowing, decreased alpha activity, and increased amounts of rapid-eye-movement (REM) sleep were observed. DHHS (1988) did not consider these data to be clinically significant. In related studies, Rhesus monkeys were dosed with 5 μg GB/kg. i.v., or with 10 μg GB/kg, i.m. (one 1 μg/kg dose per week for 10 weeks) and their EKGs were evaluated after 1 year (Burchfiel and Duffy, 1982). The single dose was acutely toxic, but the animals exposed to smaller multiple doses showed no signs of toxicity. It was not reported whether blood cholinesterase activity levels were measured in the test animals. Both exposure groups exhibited significant and persistent increases in high frequency beta activity. Burchfiel and Duffy (1982), attributed the changes in EEG activity to the GB exposure; however, they also noted that a clear relationship has not been established between such alterations in EEG frequency spectrum and alterations in brain function. Neurobehavioral tests were not conducted on the exposed animals in the Burchfiel and Duffy (1982) study.

Information on the chronic toxicity of GB to animals is limited to inhalation studies (Weimer et al., 1979). No signs of toxicity were observed in Fischer 344 rats exposed to 0.0001 or 0.001 mg GB/m^3, 6 hr/day, 5 days/week, for up to 24 weeks (Weimer et al., 1979). In a continuation of these studies, I.C.R. Swiss and A strain mice, Sprague-Dawley/Wistar and Fischer 344 rats, and purebred beagle dogs were exposed to 0.0001 or 0.001 mg GB/m^3, 6 hr/day, 5 days/week, for up to 52 weeks. Four male and 8 female beagles were exposed to each test concentration. In the rodent tests, 50 animals of each sex of each strain were exposed to each test concentration. The control groups were identical to the test groups except that an additional 100 F344 rats and A strain mice were used. Five of 20 dogs exhibited abnormal EKGs at the time of sacrifice; elevated P waves were suggestive of right atrial hypertrophy; however, there was no evidence of enlargement or physical abnormalities of the heart. The absence of pre-exposure data precludes identifying this effect as due to GB exposure. A higher incidence of tracheitis occurred in colony rats (a Sprague Dawley/Wistar population) and in Fischer rats exposed to GB in comparison to control animals. The most severe cases of tracheitis occurred in the high exposure group. No other overt signs of GB-related toxicity were observed at either exposure level. RBC-AChE activity levels were monitored throughout the study for all the test species. No consistent statistically significant change in RBC-AChE occurred in any species at any sampling time.

Although AChE inhibition is considered to be the primary mechanism of toxicity of GB, particularly following acute exposures, there has been concern that nerve agents such as GB may have direct, non-cholinesterase mediated, toxic effects on the nervous system. Exposure to some organophosphate compounds results in a delayed neuropathy characterized by degeneration of axons and myelin. This toxic effect, termed organophosphate-induced delayed neuropathy (OPIDN), is not associated with AChE inhibition but rather with inhibition of the nervous system enzyme, neuropathy target esterase (NTE). OPIDN has not been observed in humans exposed to GB, but studies have shown that GB can cause elevated levels of NTE and signs of OPID in

some laboratory species (Munro et al., 1994). Signs suggestive of OPIDN have been reported in female Swiss albino mice exposed to 5 mg GB/m^3 for 20 min daily for 10 days (Husain et al., 1993). Muscular weakness of the limbs and slight ataxla occurred on the 14th day after the start of the exposures. These changes were accompanied by significant ($p < 0.001$) inhibition of NTE activity in the brain (59.2%), spinal cord (47.4%) and platelets (55.4%). Histological examination of the spinal cord revealed focal axonal degeneration which was reported to be moderate in 2 animals and light in four. The same exposure inhibited AChE in blood by 27.3% and in brain by 19.2% and was not associated with any anti-AChE symptoms. Significant ($p < 0.05$) reductions in brain NTE were also seen in female rats receiving 0.3 mg GB Type I/kg/day for 90 days; however, histopathological examination revealed no neural lesions indicative OPIDN (Bucci et al, 1991). Statistically significant decreases in brain NTE were not seen in a related study in which rats were dosed with the same amount of GB Type II (Bucci and Parker, 1992). OPIDN did not occur in cats receiving single supralethal doses or multiple low doses of GB for up to 10 days (Goldstein et al., 1987; Goldstein, 1989), but has been observed in chickens exposed to supralethal dose of GB. Davies et al. (1960), Davies and Holland (1972) and Gordon et al. (1983) reported that delayed neuropathy could be induced with doses 20 and 30 times the LDso if the animals were protected from acute toxic effects by the use of antidotes such as atropine and oxime compounds.

Studies in laboratory animals indicate that GB-induced reproductive or developmental effects are not likely to occur even at dose levels that are maternally toxic. LaBorde and Bates (1986,1996) conducted developmental toxicity studies on agent GB Type I and GB Type II using CD rats and New Zealand rabbits. In the rat studies, the test animals were dosed with 0, 100, 240, and 380 μg/kg of GB orally on days 6-15 of gestation. Females were weighed on gestational days 0, 6-16 and prior to sacrifice on gestational day 20. The test animals were observed for clinical signs of toxicity. At sacrifice, gravid uteri were weighed and examined for number and status of implants (alive, resorbed or dead). Individual fetal body weight and internal or external malformations were recorded. Maternal toxicity (evidenced by excessive salivation, ataxia, lacrimation) and mortality (8/29 for GB Type I and 13/29 for GB Type II) occurred in the high-dose group. There were no significant differences among treatment groups in the incidence of resorptions or in the average body weight of live fetuses per litter. The only fetal morphological anomaly was hydroureter which occurred at a rate of 5.2, 1.9, 5.3 and 2.1% with GB Type I and 4, 5, 3.2, and 0.5% with GB Type II in the 0, 100, 240, and 300 μg/kg dose groups, respectively. The observed effect was not dose-related and was therefore considered to be a spontaneous variant. Skeletal and cartilage variants occurred but these were not statistically significant. In studies conducted on New Zealand rabbits using the same experimental protocol, oral doses of 0, 5, 10, or 15 μg/kg/day on gestational days 6-19, resulted in no fetal toxicity or teratogenicity (LaBorde and Bates, 1986,1996). The only observed fetal anomaly was retinal folding which occurred at a rate of 6.8, 3.9, 4.3, and 7.4% for GB Type I and 17, 18, 25, and 19% for GB Type II in the 0, 5, 10 and 15 Hg/kg dose groups, respectively. The frequency of the anomaly was not dose-related and the variant was therefore considered to be a spontaneously occurring malformation. Maternal toxicity (evidenced by excessive salivation, ataxia, lacrimation) occurred at the highest dose.

The developmental toxicity of GB was also evaluated by Denk (1975). In this study Sprague-Dawley rats were exposed to GB vapors (0.1 and 1 $\mu g/m^3$) for 6 hr/day, 5 days/wk, for varying time periods. In one series of tests, male rats were exposed for 1 week to 1 yr, and then mated to unexposed females. Nineteen days after mating the females were sacrificed and examined for number of corpora lutea, deciduomata, number of fetal deaths, and number of live fetuses. In a second series of tests mated pairs of rats were exposed to GB for 1, 2, or 3 weeks or until the pups were whelped. The incidence of intrauterine deaths was recorded and all fetuses were examined for abnormalities. In a third series of tests males and females were exposed to GB for 10 months and then mated. The F1 generation was mated, as was the F2 generation. The number and sex of offspring, number of pre-weaning deaths, number weaned, and pup weights at various ages were recorded. Denk (1975) reported that GB, at the doses and by the route used, had no adverse effects with respect to dominant lethal mutations, reproductive performance, fetal toxicity, and teratogenesis.

CONFIDENCE IN THE ORAL RfD

Study: High
Data Base: Medium
RfD: Medium

The data base for GB consists of well designed and well conducted subchronic gavage studies in rats, chronic inhalation studies in rats, mice and dogs, developmental toxicity studies in rats and rabbits, and delayed neuropathy studies in rats, mice and chickens. In addition, data are also available evaluating the effects of GB in humans following acute and short-term exposures. The data base supports the use of cholinesterase inhibition as the appropriate endpoint for deriving an oral RfD. Confidence in the RfD based on this endpoint is medium to high.

EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document --

Other EPA Documentation --

Agency Work Group Review --

Verification Date --

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3.2. INHALATION EXPOSURE

Available data are insufficient to support development of a chronic inhalation exposure (RfC) estimate for agent GB.

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- Agent GB (methyl-phosphonofluoridate isopropyl ester, sarin)
CASRN -- 107-44-8
Last Revised -- No data

3.3. DERMAL EXPOSURE

Available data are insufficient to support development of a chronic dermal exposure (RfD_d) estimate for agent GB.

REFERENCE CONCENTRATION FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- Agent GB (methyl-phosphonofluoridate isopropyl ester, sarin)
CASRN -- 107-44-8
Last Revised -- No data

4. DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Data are insufficient to assess the carcinogenicity of GB in humans or animals for oral or inhalation exposures.

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

GB cannot be classified as to potential carcinogenicity because of the lack of adequate data.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Not classifiable

Basis -- Lack of adequate human or animal data

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Attachment 1

TOXICITY ASSESSMENT FOR THE NERVE AGENT SOMAN (GD)

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PREFACE

This report assesses the potential non-cancer and cancer effects of the nerve agent GD, (methylphosphonofluoridic acid, 1,2,2-trimethylpropyl ester, soman) (CAS Number 96-64-0). Information pertaining to non-cancer and cancer effects of GB has not been assessed previously by the United States Environmental Protection Agency (U.S. EPA).

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This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

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ABSTRACT

Data pertaining to the potential cancer/non-cancer effects of agent GD (methylphosphonofluoric acid 1,2,2-trimethylpropyl ester, soman) are reviewed. The compound is one of a number of organophosphorus nerve agents that are defined by their acute toxicity at very low doses, extremely rapid internalization, and the near-instantaneous onset of a suite of devastating symptoms that lead to death or incapacity within a short time. The group are characterized by their anti-acetylcholinesterase (AChE) activity, with consequent ACh overstimulation representing the trigger for profound clinical signs and physiological responses, preeminent among which are a general respiratory failure and perturbation of the nervous system. Longer term studies of GD administration at concentrations below the threshold for acute effects have failed to provide any evidence for the carcinogenicity of agent GD, an observation supported by the absence of evidence of mutagenicity or of any increased tumor incidence in occupationally-exposed workers. Similarly, GD was not associated with the onset of developmental toxicity in either rats or rabbits, even at concentrations that induced toxicological responses in the dams. However, a subchronic study exposing rats to agent GD has been used to derive a chronic oral RfD, with inhibition of plasma AChE activity representing the critical effect. An uncertainty factor of 2700 (consisting of 10 for inter-species extrapolation, 10 to protect sensitive subpopulations, 3 for subchronic to chronic extrapolation, 3 for LOAEL to NOAEL extrapolation, and 3 for deficiencies in the data base) was used to reduce an adjusted LOAEL of 0.0125 mg/kg-day to a chronic oral RfD of 4E-6 μg/kg-day.

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1. SUMMARY OF TOXICITY INFORMATION

1.1. EPIDEMIOLOGICAL STUDIES

Information is currently unavailable on the human health effects of GD as revealed through epidemiological studies.

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity. Nerve agents such as GD are acutely toxic by all routes of exposure. Initial symptoms of acute poisoning are fatigue, headache, mild vertigo, weakness, and loss of concentration. Moderate exposures result in miosis and excessive sweating, tearing, and salivation. Acidosis and hyperglycemia may also occur in addition to muscular weakness, muscular twitching, lacrimation, urination, and defecation. Acute poisoning can result in prostration, clonic convulsions (rapid repetitive movements) and tonic convulsions (limbs stretched and rigid) (Matsumura, 1976).

In general, acute exposures to nerve agents are known to cause EEG changes (Grob and Harvey, 1958; Sidell, 1992) which may persist for long periods of time after exposure (Metcalf and Holmes, 1969; Duffy et al.,1979; Duffy and Burchfiel, 1980); however, the reported changes have been considered to be clinically insignificant and not correlated with behavioral or physiological changes (DHHS, 1988). Acute exposures can also induce neuropsychological changes; however, there is no evidence of these effects persisting for months or years as has been reported for some organophosphate insecticides (Savage et al., 1988; Gershon and Shaw, 1961; Mick, 1974; Rodnitzky, 1974; Wagner, 1983; Tabershaw and Cooper, 1966).

1.2.2. Animal Toxicity.

1.2.2.1. Acute Toxicity —

1.2.2.1.1. Oral Toxicity. Bucci et al. (1992a) conducted range finding studies with GD, in which the test material was administered by gavage to male and female CD rats once per day, 5 days per week for 2 weeks. A maximum tolerated dose of 70 μg/kg/day was identified, with a dose of 300 μg/kg/day being lethal to 100% of the test animals.

1.2.2.1.2. Inhalation Toxicity. Anzueto et al., (1990) examined the acute inhalation toxicity of GD (and of Sarin) in anaesthetized baboons, by measuring blood pressure, electrocardiogram (ECG) output, arterial/mixed blood gases, lung volumes, lung pressures, and efferent phrenic nerve activities in animals receiving GD vapor via a self-sealing breathing tube with attached delivery syringe. Controls received isopropanol via a similar system. In addition to the above parameters, bronchoalveolar lavage (BAL) was also used to evaluate the changes in composition of pulmonary epithelial cells occurring in response to GD challenge. Measurements were carried out pre-treatment, then after 4 hours, 4 days and 28 days post-treatment. Animals receiving either nerve agent displayed decreased blood pressure, and bradyarrhythmias, conditions found to be reversible by atropine administration. Hematological, hemodynamic and clinical chemistry parameters were perturbed, with both plasma and red blood cell (RBC) acetylcholinesterases (AChE) inhibited at 4 hours and 4 days, but with partial recoveries of activity after 28 days. However, BAL data showed no significant differences or trends in total cell count or viability between treated and control groups, and changes in the numbers of macrophages and neutrophils appearing in the BAL recovery fluid showed no consistent treatment-related responses.

1.2.2.1.3. Dermal Toxicity. Somani et al., (1992) estimated an LD_{50} for dermal exposure to GD in human beings of 50-300 μg/kg.

1.2.2.1.4. Toxicity via Other Exposure Routes. In a study by Blick et al. (1994), rhesus monkeys were administered GD parenterally daily for 5 days. The effects of the treatment on performance of a well-learned, compensatory tracking task was assessed, and an ED_{50} of 0.97 μg/kg/day for decrement in this performance was obtained. This decrement in performance was concurrent with a 85-90% inhibition of serum ChE. The 0.97 ug/kg/day dose was about 40% of the single acute dose required to produce a similar performance decrement. It was also noted that the single acute dose (2.43,ug/kg) was associated with a 65-70% serum ChE inhibition.

Values for the acute lethality of GD in laboratory animals for varying routes of exposure range from 3.8 μg/kg/day in monkeys via intramuscular injection (Petras, 1984; Baze, 1993; Wall et al., 1990) to 165 μg/kg/day for subcutaneous injection in rats (Somani et al., 1986; Petrali, 1989; Maxwell et al., 1987a,b., Lennox et al., 1985).

As an example of the extensive library of studies that have addressed the potential for using chemical antidotes to protect against nerve agent attack, Koplovitz and Stewart, (1994) exposed pathogen- and atropinesterase-free New Zealand White rabbits to intramuscular injections of nerve agents such as GD at dose levels an order of magnitude higher than the 24-hour LD_{50}. The study sought to evaluate the combined ability of intravenous atropine (a cholinergic antagonist) and AChE-reactivating oximes such as pralidoxime chloride (2-PAM) or 1-2-hydroxy-iminomethyl-1-pyridino-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI6) to protect against a GD challenge, in the presence or absence of pyridostigmine pretreatment. Injections of atropine and HI6 combined to protect against the acute lethal effects of the super-

optimal dose levels of agent GD at much greater efficiency than combinations of pretreatments and treatments featuring the oxime 2-PAM. The most effective regimen for protection against agent GD, featured pretreatment with pyridostigmine, then treatment with injections of atropine, HI6, and diazepam.

1.2.2.2. Subchronic Toxicity — In a subchronic study conducted by the National Center for Toxicological Research (Bucci et al., 1992a), male and female CD rats (12/sex/group) were administered GD by gavage at dose levels equivalent to 15.5, 35.0 and 70 μg GD/kg. The doses were given once per day, 5 days per week for 13 weeks. All animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examination was performed on all animals. Terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals, and on those tissue of lower dose animals that were abnormal at necropsy. Hematological analyses and clinical chemistry (including RBC and plasma cholinesterase) were evaluated in the same 6 male and 6 female rats in each dose group one week before the exposures began and also at weeks 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for neuropathy target esterase (NTE).

Relative to untreated controls, the group mean body weight gain was significantly decreased ($p < 0.001$) in the high-dose (70 μg/kg) males (b.w. change 160.9 in controls vs. 83.7 g). A decrease in body weight gain also occurred in female rats (75 vs 59 g decrease for high-dose and controls, respectively) but the difference was not statistically significant. A definitive dose-response was not present in either males or females.

Although changes were observed in some clinical chemistry and hematologic parameters, the changes were not dose-related and therefore considered incidental to treatment. Brain NTE

was not altered in rats dosed with GD. Histopathological examinations revealed no gross or microscopic findings that could be attributed to treatment with GD. Special attention was given regarding intercostal and cardiac muscle lesions, and neurological lesions which have been previously reported in rats treated with GD (McLeod, 1985; Singer et al., 1987). However, none of these lesions was observed in GD-treated rats in the Bucci et al. (1991) study.

Considerable variability was noted in RBC-AChE and plasma-ChE activities among the control and treatment group baseline values, a contributing factor to the difficulty of interpreting the dose-response data. In fact, no significant changes in RBC-AChE activities between treatment and control groups were noted by the study authors. By contrast, a dose-related decrease in plasma-ChE activities in both male and female rats was observed for weeks 1 and 7. Relative to untreated rats, significant ($p<0.05$) depression of plasma cholinesterase levels was observed in both males and females of the high-dose (70 μg/kg) group during week 1 (25% and 33% for males and females, respectively) and week 7 (20% and 33% for males and females, respectively), and in males of the 35 μg/kg group during week 7 (28%). In females, the control plasma-AChE levels exceeded pre-treatment (baseline) values by week 13 but remained depressed (54 %, 66 %, and 50 % in the low, mid, and high dose groups, respectively) in males at week 13 although not significantly so.

The plasma- and RBC-AChE data from the Bucci et al. (1992a) study were re-analyzed statistically (using standard deviations) with ANOVA and Dunnett's and Scheffe's Comparisons. In the reevaluation, RBC and plasma cholinesterase levels were compared to respective controls for the same sampling times as well as to the baseline values within each group (Tables 5-8). This analysis also indicated an absence of definitive changes in RBC-AChE levels that could be

attributed to GD treatment. For plasma-AChE, a dose-related decrease ($p<0.05$) relative to controls was detected during weeks 1 and 3 for both male and female rats. With the exception of high-dose females at week 3, a comparison of values to baseline indicated that plasma-AChE levels of both the mid and high-dose groups were significantly ($p<0.05$) lower for weeks 1, 3, and 7 for females and throughout the 13-week period for males. Under the conditions of this study, GD treatment appeared to affect plasma-AChE levels at a dose as low as 17.5 ,μg/kg as exemplified by the significant ($p<0.05$) decrease relative to controls and baseline values in male (39% of baseline) and female (57% of baseline) rats at week 1. Accordingly, 17.5 μg/kg/day was taken as the LOAEL for subchronic oral toxicity for this study.

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. There are no data available regarding the potential carcinogenicity of agent GD in humans or laboratory animals.

1.3.2. Chronic Toxicity. There are no data regarding the chronic toxicity of agent GD following chronic exposure.

1.4. REPRODUCTIVE EFFECTS

There are no studies reporting the reproductive effects of agent GD in humans or laboratory animals.

1.5. DEVELOPMENTAL EFFECTS

There are no studies reporting the developmental effects of agent GD in humans or laboratory animals.

1.5.1. Mutagenicity. Goldman et al. (1987) reported on the results of genotoxicity studies of agent GD. In tests on bacteria and mammalian cell cultures, GD was not genotoxic or mutagenic

when tested with and without metabolic activation. There were no biologically significant increases in mutations when tested in the Ames Salmonella assay using five revertant strains (TA135, TA100, TA98, TA1537, and TA1538), either with and without metabolic activation. GD did not induce a significant increase in forward mutations when tested on mouse L5178Y lymphoma cells at concentrations of 50, 100, or 200 μg/mL, and no increase in sister chromatid exchanges (SCE) was observed when Chinese hamster ovary cells were exposed in vitro to 200 μg/mL of GD. Mice treated in vivo with a maximally tolerated intraperitoneal dose of 300 μg GD/kg did not exhibit significant increases in SCE in splenic lymphocytes. Exposure of rat hepatocytes to GD concentrations as high as 600 μl/3ml culture medium (2.5 x 10^6 hepatocytes) did not result in DNA damage or unscheduled DNA synthesis.

1.6. TOXICOKINETIC STUDIES

1.6.1. Toxicokinetics. Shih, et al., (1994) used GC/MS to measure the appearance of putative metabolites of the nerve agents, GD, GB and GF, in the urine of male Crl:CD BR COBS or VAF/Plus rats. The alkyl phosphonic acids were considered to be the single major metabolites of these nerve agents. When 75 μg/kg GD was administered subcutaneously, its alkyl phosphonic metabolite appeared in the urine at a much slower rate than did those of GB and GF. For example, 33% of the given dose was excreted after 6 hours, but, cumulatively only reached 62% after 7days. The authors discussed this incomplete elimination in relation to the potentially different catabolic mechanisms associated with the compound's stereoisomers.

1.6.2. Metabolism. The review by Munro, et al., (1994) summarizes the key information concerning potential mechanisms by which nerve agents GB, GA, and VX are metabolized, with emphasis on the important role played by certain plasma carboxylesterases in catalyzing the

formation of these compounds' alkyl phosphonic acid derivatives. Considering the work of Shih et al., (1994), who demonstrated the formation of an alkyl phosphonic acid derivative from agent GD. in combination with the chemical similarity among all the anti-AChE nerve agents, including GD, allows the conclusion that the plasma carboxylases may play an important role in the metabolism of this nerve agent also. The presence of significant amounts of this enzyme in the plasma of rats and mice, with consequent ability of these species to form isopropyl phosphonic acid, as discussed in section 1.6.1., is considered to represent a possible explanation for their relative resistance to GD attack, a feature in contrast to that existing in human beings which appear to lack this enzyme activity.

1.6.3. Percutaneous Absorption. There are no available data on the rate of absorption of agent GD via the dermal route.

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

GD is one of a series of closely related organophosphate nerve agents, characterized by their ability to kill at low concentration through the inhibition of acetylcholinesterase (AchE). Though GD, along with its related compounds, GA, VX, and GB, may each display unique physico/chemical characteristics, their collective ability to inhibit AChE represents their primary biological interaction and results in excessive accumulation of ACh at synapses, and overstimulation of the portions of the nervous system that control smooth muscle, cardiac muscle, and exocrine glandular functioning. The suite of debilitating symptoms arising from these perturbations are compounded by respiratory failure arising from depression of the brain's respiratory center, neuromuscular block of the respiratory muscles, bronchial constriction, and increased lung secretions.

1..8. MECHANISTIC STUDIES

Nerve agents are inhibitors of acetylcholinesterase (AChE), an enzyme responsible for deactivating the neurotransmitter acetylcholine (AChE) at some neuronal synapses and myoneural junctions. Nerve agents bind to the enzyme and phosphorylate it, causing structural changes and consequent loss of activity. The organophosphate-inhibited enzyme can be reactivated by dephosphorylation, but this occurs at a rate that is slower than agent-mediated inactivation, thus, there is a depletion of acetylcholinesterase and buildup of acetylcholine. In addition, the nerve agent-enzyme complex can also undergo an "aging"process (thought to be due to a loss of an alkyl or alkoxy group), whereby it becomes resistant to dephosphorylation (see review by Munro et al.,1994). Differences in rates of aging and reactivation may be important in evaluating toxicity data especially when extrapolating from animal studies to humans. In vitro tests conducted by Grob and Harvey (1958) indicate that both GA and GB combine with cholinesterase almost irreversibly during the first hour of their reaction. Sidell and Groff (1974) reported that the GB-ChE complex ages very rapidly in vivo, with 45-70% completion by 5 hours after infusion. In contrast, the complex formed between ChE and the nerve agent VX does not age significantly, and the rate of spontaneous reactivation can be as fast as 1 %/hr in humans (Sidell and Groff, 1974). These features may be assumed to apply to agent GD also.

The anticholinesterase effects of the organophosphate nerve agents can be characterized as being muscarinic, nicotinic, or central nervous system (CNS)-related. Muscarinic effects occur in the parasympathetic system (bronchi, heart, pupils of the eyes; and salivary, lacrimal and sweat glands) and result in signs of pulmonary edema, bradycardia, miosis, tearing, and

sweating. Nicotinic effects occur in somatic (skeletal/motor) and sympathetic systems, and result in muscle fasciculation, muscle weakness, tachycardia, and diarrhea. Effects on the CNS by organophosphates are manifested as giddiness, anxiety, emotional lability, ataxia, confusion, and depression (O'Brien, 1960).

Although the inhibition of cholinesterase within neuro-effector junctions or the effector itself is thought to be responsible for the major toxic effects of organophosphate agents, these compounds can apparently affect nerve-impulse transmission by more direct processes as well. Direct effects may occur on excitable tissues, receptors, and ionic channels. According to Somani et al. (1992), the direct action of nerve agents on nicotinic and muscarinic ACh receptors may occur when concentrations in the blood rise above micromolar levels, whereas at lower levels the action is mainly the result of inhibition of AChE. Albuquerque et al. (1985) have shown that agent GA, as well as agents GB and GD are capable of changing receptor sites in a manner similar to that exhibited by acetylcholine, which promotes the conductance of electrophysiological signals associated with stimulation of neuromuscular function. VX "may directly affect a small population of muscarinic ACh receptors that have a high affinity for [3H]-cis-methyldioxalane binding" (Somani et al., 1992). VX may also counteract the effects of ACh by acting as an open channel blocker at the neuromuscular junction, thereby interrupting neuromuscular function (Rickett et al.,1987).

Exposure to some organophosphate cholinesterase inhibitors results in a delayed neuropathy characterized by degeneration of axons and myelin. This effect is not associated with the inhibition of acetylcholinesterase, but rather with the inhibition of an enzyme described as neuropathy target esterase (NTE); however, the exact mechanism of toxicity is not yet fully

understood (Munro et al., 1994). For some organophosphate compounds, delayed neuropathy can be induced in experimental animals at relatively low exposure levels, whereas for others the effect is only seen following exposure to supralethal doses when the animal is protected from the acute toxic effects caused by cholinesterase inhibition.

Although there is the potential for nerve agents to have direct toxic effects on the nervous system or to cause delayed neuropathy, there is no evidence that such effects occur in humans at doses lower than those causing cholinesterase inhibition. However, it should be noted that there are very little animal or human data evaluating the potential effects of long-term exposure to low doses. Nevertheless, for the purpose of evaluating potential health effects, inhibition of cholinesterase is generally considered the most useful biological endpoint.

In addition to being found in the nervous system, acetylcholinesterase also occurs in the blood where it is bound to the surface of red blood cells (RBC-AChE). RBC-AChE activity, as well as the activity of a second type of cholinesterase found in blood plasma (butyrylcholinesterase, or plasma cholinesterase) have been used to monitor exposure to organophosphate compounds (pesticides and nerve agents). Changes in RBC-AChE activity are generally considered to be a convenient bioindicator of the potential effects of anti-AChE nerve agents (Morgan, 1989). There is also evidence that RBC-AChE is as sensitive as brain ChE to the effects of nerve agents. Grob and Harvey (1958) reported that the in vitro concentrations producing 50% depression of brain-ChE and RBC-AChE activity were the same in the case of GA (1.5×10^{-8} mol/L), and only slightly different (3×10^{-9} mol/L and 3.3×10^{-9} mol/L) in the case of GB. However, in vivo animal studies indicate a poor correlation between brain and RBC-AChE in cases of acute exposures (Jimmerson et al.,1989), and this is reflected in the fact

that blood cholinesterase activity may not always be correlated with exposure or with signs and symptoms of toxicity (Holmstedt, 1959). Acute exposures to high concentrations may cause immediate toxic effects before significant changes occur in blood ChE activity, and repeated exposures over a period of several days or more may result in a sudden appearance of symptoms due to cumulative effects (Grob and Harvey, 1958). Conversely, blood ChE activity can become very low without overt signs or symptoms during chronic exposures to low concentrations of organophosphates. This may be due to a slower rate of recovery of RBC-AChE compared to tissue ChE, or to non-cholinesterase-dependent recovery pathways for neural tissue (Grob and Harvey, 1958). Sumerford et al. (1953) reported that orchard workers exposed to organophosphate insecticides had RBC- and plasma-ChE values as low as 15% of normal values without any other signs or symptoms of exposure. Animal studies have demonstrated that chronic exposures to low concentrations of organophosphate insecticides and nerve agents can result in increased tolerance levels (Barnes, 1954; Rider et al.,1952; Dulaney et al., 1985). Similarly, Sumerford et al. (1953) reported increased levels of tolerance to organophosphate insecticides in people living near orchards treated with organophosphate insecticides. Such adaptation may result from increased rates of formation of blood ChE, or from increased rates of detoxification. Additional information on the development of tolerance to organophosphate cholinesterase inhibitors can be found in a review paper by Hoskins and Ho (1992).

The blood cholinesterases may, to some degree, provide a protective effect by binding with some fraction of the anticholinesterase compound (Wills, 1972). However, not all nerve agents bind equally well with all cholinesterases. In tests conducted on dogs, Holmstedt (1959) found that GA affected RBC and plasma cholinesterase to a nearly equal degree. By contrast,

agent VX preferentially inhibits RBC-AChE (70% compared with about 20% inhibition of plasma ChE) (Sidell and Groff, 1974). Rodents (but not humans) have other enzymes in the blood, termed aliesterases, which can bind organophosphates, thereby reducing the amount available for binding with acetylcholinesterase (Fonnum and Sterri, 1981). Agent GB binds with aliesterases; however, according to Fonnum and Sterri (1981), VX has a quaternary ammonium group which prevents it from being a substrate for aliesterases. The strong specificity of agent VX to AChE may account, in part, for the fact that it is much more acutely toxic than agents GA and GB (Munro et al.,1994).

2. INTERPRETATION OF AVAILABLE INFORMATION

Agent GD is a colorless liquid with a molecular weight of 182.2 (HSDB, 1996); it has a liquid density of 1.022 g/mL at 25°C,, with ready solubility in both polar and apolar solvents. A vapor concentration of 3.06 g/m^3 has been reported for a temperature of 25° C (HSDB, 1996).

Agent GD may hydrolyze to relatively nontoxic hydrofluoric and pinacolyl methylphosphonic acids (MacNaughton and Brewer, 1994; Rosenblatt et al.,1995). The hydrolysis rate is a function of temperature and pH; the rate is minimum between pH 4 and 6. The $t_{1/2}$ GD is approximately 100 hours with 20 x $t_{1/2}$ being required to attain a 1 x 10^6 reduction in GD concentration.

GD is likely to undergo hydrolysis in most soils. As noted above, the rate of hydrolysis will be dependent upon temperature and pH. According to Morrill et al. (1985), evaporation is the primary mechanism for the loss of the GA and GB nerve agents from soil. Although the G agents are liquids under ordinary environmental conditions, their relatively high volatility and vapor pressure permits them to be disseminated in vapor form. Because of this volatility, GD is not expected to persist in soils.

The potency of the anticholinesterase activity of nerve agents (such as GD) and other organophosphates is expressed by the bimolecular rate constant (k_i) for the reaction of the phosphate compound with the enzyme and by the molar concentration causing 50% inhibition of the enzyme when tested in vitro (I_{50}). I_{50} data for several organophosphate nerve agents have been tabulated by Dacre (1984). The relationship between I_{50} and k_i as a function of time (t) is expressed by the following equation (Eto, 1974): $I_{50} = 0.693/t \times k_i$.

The pI_{50} (negative log of the molar concentration causing 50% inhibition) for GD is 9.2 as reported by Dacre (1984).

Relative potency of nerve agents can also be expressed in terms of the in vivo dose necessary to produce the same level of cholinesterase inhibition by a specific exposure route. As would be expected, the effectiveness of the agents in inhibiting cholinesterase is closely correlated with their acute toxicity .

3. DOSE-RESPONSE ASSESSMENT

3.1. INGESTION EXPOSURE

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Agent GD (methylphosphonofluoridic acid 1,2,,2-trimethylpropyl ester, soman)
CASRN -- 96-64-0
Date prepared -- September, 1996

ORAL RfD SUMMARY

Critical Dose -- 0.0175 mg/kg-day (LOAEL)
UF — 2700
MF -- 1
RfD — 4E-6 mg/kg-day

Critical Study

Critical Effect -- Inhibition of acetylcholinesterase in plasma.

Study Type -- Rat Subchronic Oral Study

Reference -- Bucci et al., 1992a

NOAEL —
NOAEL(ADJ) —

LOAEL — 0.0175 mg/kg-day
LOAEL(ADJ) — 0.0125 mg/kg-day

Conversion Factors and Assumptions --

PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Bucci, T.J. R.M. Parker, and P.A. Gosnell. 1992a. Toxicity Studies on Agents GB and GD (Phase II), 90 Day Subchronic Study of GD (Soman) in CD-Rats. Final Report. Prepared for U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, MD. FDA 224-85-0007.

In the subchronic study conducted by the National Center for Toxicological Research (Bucci et al., 1992a), male and female CD rats (12/sex/group) were administered GD by gavage at dose levels equivalent to 15.5, 35.0 and 70 µg GD/kg. The doses were given once per day, 5 days per week for 13 weeks. All animals were observed daily for clinical signs of toxicity and weighed weekly. Necropsy examinations were performed on all animals and terminal body and organ weights were recorded. Microscopic evaluation was performed on all high-dose and control animals, and on those tissue of lower dose animals that were abnormal at necropsy. Hematological analyses and clinical chemistry (including RBC and plasma cholinesterase) were evaluated in the same 6 male and 6 female rats in each dose group one week before the exposures began and also at weeks 1, 3, 7, and 13. In addition, at necropsy a hemisection of each brain was prepared and tested for the enzyme neuropathy target esterase (NTE).

Relative to untreated controls, the group mean body weight gain was significantly decreased ($p < 0.001$) in the high-dose (70 Hg/kg) males (b.w. change 160.9 vs. 83.7 g). A decrease in body weight gain also occurred in female rats (75 vs 59 for high-dose and controls, respectively) but the difference was not statistically significant. A definitive dose-response was not present in either males or females.

Although changes were observed in some clinical chemistry and hematologic parameters, the changes were not dose-related and therefore considered to be incidental to treatment. Brain NTE was not altered in rats dosed with GD. Histopathological examinations revealed no gross or microscopic findings that could be attributed to treatment with GD. Special attention was given regarding intercostal and cardiac muscle lesions, and neurological lesions which have been previously reported (McLeod, 1985; Singer et al., 1987). However, none of these lesions were observed in GD-treated rats.

Considerable variability was noted in plasma- and RBC-AChE levels for control and treatment group baseline values. For example, a dose-related decrease in plasma-AChE levels in both male and female rats was observed for weeks 1 and 7. Relative to untreated rats, significant ($p<0.05$) depression of plasma cholinesterase levels were observed in both males and females of the high-dose (70 µg/kg) group during week 1 (25% and 33% for males and females, respectively) and week 7 (20% and 33% for males and females, respectively), and in males of the 35 µg/kg group during week 7 (28%). In females, the plasma-AChE levels exceeded pretreatment (baseline) values by week 13 but remained depressed (54%, 66%, and 50% in the low, mid, and high dose groups, respectively) in males at week 13 although not significantly so. No significant changes in RBC- AChE levels were noted by the study authors, or during a subsequent statistical re-evaluation of the data using ANOVA and Dunnett's and Scheffe's Comparisons. However, for plasma-AChE, a dose-related decrease ($p<0.05$) relative to controls

was detected during weeks 1 and 3 for both male and female rats. With the exception of high-dose females at week 3, a comparison of values to baseline indicated that plasma-AChE levels of both the mid and high-dose groups were significantly ($p<0.05$) lower for weeks 1, 3, and 7 for females and throughout the 13-week period for males.

Under the conditions of this study, GD treatment appeared to affect plasma-AChE levels at a dose as low as 17.5 μg/kg as exemplified by the significant ($p < 0.05$) decrease relative to controls and baseline values in male (39% of baseline) and female (57% of baseline) rats at week 1. For plasma- AChE, decreases in activity levels appeared to be dose-related. The LOAEL of 17.5 μg/kg/day was adjusted for continuous exposure (5 days/7 days) to obtain an adjusted LOAEL of 12.5 μg/kg/day (0.0125 mg/kg/day).

UNCERTAINTY AND MODIFYING FACTORS (ORAL (RfD)

Ten to account for sensitive subpopulations, 10 for interspecies extrapolation, 3 for subchronic to chronic extrapolation, 3 for LOAEL-to-NOAEL extrapolation, and 3 for deficiencies in the database.

UF -- 2700

MF -- 1

JUSTIFICATION FOR UNCERTAINTY FACTOR COMPONENTS.

An uncertainty factor of 10 for sensitive subpopulations is considered necessary because some individuals have a genetic defect causing their blood cholinesterase activity to be abnormally low (Evans et al., 1952; Harris and Whitaker, 1962). These individuals, therefore, may be unusually sensitive to organophosphate anticholinesterase compounds.

An uncertainty factor of 10 is used for animal-to-human extrapolation because there is no evidence suggesting that humans less sensitive to GD than are laboratory animals.

An uncertainty factor of 3 is used to extrapolate from a subchronic to chronic exposure. In the derivation of the oral RfDs for other organophosphate compounds, the EPA has used NOAELs for cholinesterase inhibition following short-term exposures without adjustment for a more prolonged exposure period because of the unlikelihood that the endpoint would change over time (i.e., a subchronic-to-chronic UF of 1 was used). In addition, animal data indicate that maxlmum ChE inhibition may occur 30-60 days or more after exposure begins, after which it levels off or even shows recovery. In the Bucci et al. (1992a) study, both plasma and RBC-AChE levels exhibited signs of recovery at week 13, especially for the lower doses (Tables 5-8). Therefore, increased ChE inhibition is not expected to occur at longer exposure periods.

However, an uncertainty factor of 3 is used because studies are not available to verify that adverse effects would not occur following chronic exposures.

A LOAEL-to-NOAEL uncertainty factor of 3 is used instead of 10 because the endpoint, cholinesterase inhibition, was not associated with signs of toxicity.

The database for GD lacks chronic oral studies in two species, and studies assessing reproductive/developmental effects. Because studies on other organophosphate cholinesterase inhibitors, including a multigeneration study on agent VX, indicate that reproductive/developmental effects are unlikely, a full uncertainty factor of 10 is not warranted.

ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

Evidence of delayed neurotoxicity was not observed in the previously described rat study by Bucci et al. (1992a). A delayed neurotoxicity study in SPF white leghorn chickens was also negative (Bucci et al., 1992b).

The endpoint for identifying a NOAEL for nerve agents such as GD is the level at which there is no significant depression in cholinesterase activity. In humans, 15% ChE inhibition is generally considered to be the minimal change that can be observed with any statistical reliability (Callaway et al., 1951). Existing response data for other organophosphates indicate that RBC-ChE inhibition of as much as 20% is not associated with adverse clinical signs or symptoms in humans and should be considered only as evidence of exposure (Marquis, 1988). Although nerve agents may induce toxic effects through pathways other than cholinesterase inhibition, there is at present insufficient evidence to evaluate dose-response functions. Additionally, the U.S. EPA (1995) reports scientific agreement that statistically significant inhibition of cholinesterase in multiple organs and tissues accompanied by clinical effects constitutes a hazard; however, in the absence of clinical effects, such inhibition may not be of biological significance. It is generally agreed that inhibition of RBC and/or plasma cholinesterase contributes to the overall hazard identification of cholinesterase inhibiting agents by serving as biomarkers (U.S. EPA, 1995).

Blood ChE activity levels have been used by EPA as the critical endpoint in the establishment of an oral RfD for the organophosphate insecticide malathion (U.S. EPA, 1995). In this case, a NOEL was identified as the highest dose level at which no change in RBC-ChE or plasma ChE was recorded in 5 human volunteers who received the compound orally for up to 56 days. The next highest dose was associated with a depression of about 25 % in both RBC- and plasma-ChE, but no clinical signs of toxicity. Although the NOAEL was adjusted by a factor of 10 to account for sensitive subpopulations, no adjustment was made for length of exposure (i.e., even though the experimental exposure period was not more than 56 days, a subchronic-to-chronic uncertainty factor was not applied to the data).

In the derivation of an oral RfD, human oral exposure data are preferred (as in the case of malathion); however, the only available human data for GD pertain to acute exposures. Although such data can be used to establish short-term exposure limits; acute toxicity endpoints are generally not used for developing subchronic or chronic reference values.

The only subchronic or chronic exposure studies for GD that were found in the available literature consist of a 90-day study in which rats were given GD by gavage (Bucci et al.,1992a).

The use of the subchronic rat study for developing an oral RfD for GD is complicated by the fact that rodents have a much lower RBC-AChE activity level compared to humans (Ellin, 1981). By itself, this could cause rats to be relatively more sensitive than humans to anticholinesterase compounds; however, the lower RBC-ChE activity may be offset by the presence of aliesterase in rat blood. Aliesterase, which is not present in humans (Cohen et al.,1971), is known to bind to and thereby reduce the toxicity of GD (Fonnum and Sterri, 1981). Other species differences, such as the rates of aging of the nerve agent-ChE complex, the rates of synthesis of plasma cholinesterase in the liver, and the levels of AChE in various parts of the nervous system (Ivanov et al.,1993) may also result in differences in species' sensitivities. There is insufficient data to determine the relative susceptibilities of humans and rodents to GD; therefore, for the purpose of this assessment, the EPA method will be followed which assumes that humans may be as much as ten times more sensitive to a chemical than laboratory rodents.

CONFIDENCE IN THE ORAL RfD
Study: Low
Data Base: Low
RfD: Low

EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document --

Other EPA Documentation --

Agency Work Group Review --

Verification Date —

EPA CONTACTS (ORAL RfD)

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3.2. INHALATION EXPOSURE

Available data are insufficient to support development of a chronic inhalation exposure (RfC) estimate for agent GD.

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- Agent GD (methylphosphonofluoridic acid 1,2,2-trimethylpropyl ester, soman)
CASRN — 96-64-0
Last Revised -- No data

3.3. DERMAL EXPOSURE

Available data are insufficient to support development of a chronic dermal exposure (RfD_d) estimate for agent GD.

REFERENCE CONCENTRATION FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- Agent GB (methylphosphonofluoridic acid 1,2,2-trimethylpropyl ester, soman)
CASRN — 96-64-0
Last Revised -- No data

4. DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Data are insufficient to assess the carcinogenicity of GD in humans or animals for oral or inhalation exposures.

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

GD cannot be classified as to potential carcinogenicity because of the lack of adequate data.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Not classifiable

Basis -- Lack of adequate human or animal data

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Attachment 1

TOXICITY ASSESSMENT FOR SULFUR MUSTARD (HD)

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PREFACE

This report assesses the potential non-cancer and cancer effects of sulfur mustard (HD, bis(2-chloroethyl)sulfide) (CAS Number 505-60-2). Information pertaining to the toxic (primarily cancer) effects of HD was previously assessed by the United States Environmental Protection Agency (U.S. EPA) in the 1991 issue paper *Upper-bound quantitative cancer risk estimate for populations adjacent to sulfur mustard incineration facilities*, EPA/600/8-91/053. The quantitative non-carcinogenic effects of HD have not been subject to official agency review.

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This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

PREAMBLE NOTICE

This document was produced under the auspices of the Environmental Risk Assessment Program (ERAP), which has its genesis in the DOD/DOE Strategic Environmental Research and Development Program (SERDP) that was established through Public Law 101-510 (10 United States Code 2901-2904). ERAP was established as a cooperative effort of DOD, DOE, and EPA to improve health and ecological risk assessments and to foster consistence in risk assessments across federal agencies. The program has three working groups chartered under its mission which are the Materials/Chemicals Risk Assessment (MCRA) Working Group, Human Risk Assessment Methodology (HRAM) Working Group, and the Ecological Risk Assessment Methodology (ERAM) Working Group. The program also has an Advisory and Coordinating Committee (ACC) that oversees the program and the working group's activities.

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ABSTRACT

Data pertaining to the potential cancer/non-cancer effects of Agent HD (sulfur mustard, mustard gas, 2,2'-dichlorodiethyl sulfide) are reviewed. As one of the chemical warfare agents used in World War I, agent HD has been and continues to be the subject of many reports of long- and short-term toxicity in human beings and in laboratory animals. Characterized by rapid and near-complete absorption through the skin and lungs, acute responses center on vesication of the skin, irritation and injury to the respiratory system, and damage to the eyes. Longer term exposures to human beings and laboratory animals have established agent HD as a potent carcinogen. The U.S. EPA used the incidence of skin tumors in rats and mice exposed to HD vapor to develop estimates for an inhalation unit risk. Similarly, the agency used a relative potency method to derive an oral slope factor for the compound. Supporting these determinations are a considerable body of *in vivo* and *in vitro* evidence of HD's mutagenicity that, with the extensive library of occupational exposure studies in human beings, forge the link between long-term exposure and an elevated risk of respiratory tract and skin tumors. Data on subchronic oral exposure to rats have been used to develop a reference dose (RfD) for agent HD, with epithelial acanthosis and hyperplasia of the forestomach as the critical effect. A LOAEL of 0.022 mg/kg-day was identified, that, under the influence of a combined uncertainty factor of 3000 (10 to protect sensitive subpopulations, 10 for animal to human extrapolation, 10 for subchronic to chronic extrapolation, and 3 for estimating the NOAEL from the LOAEL), reduced to an RfD of 0.007 μg/kg-day.

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1. SUMMARY OF TOXICITY INFORMATION

1.1. EPIDEMIOLOGICAL STUDIES

IARC (1975), Waters et al., (1983), Watson et al (1989), and the Institute of Medicine (1993) have reviewed the epidemiological evidence for the carcinogenicity of HD in humans, the majority of the information coming from studies of soldiers exposed to HD during World War I, or from studies of workers exposed at chemical agent manufacturing facilities. For example, Morganstern et al. (1947) reported that many workers in a munitions plant handling sulfur mustard developed chronic bronchitis which in some cases developed into bronchiectasis. Wada et al. (1962a,b) reported that a large proportion of workers at a Japanese plant manufacturing mustard, as well as Lewisite and several other agents, exhibited productive cough, irregular fever, chronic bronchitis, emphysematous changes, and pleural adhesions. It is likely that in this case the reported effects were due to concentrations of HD sufficiently high to cause acute toxic effects; exposure levels were estimated to reach as high as 50-70 mg/m^3 at times (Inada et al., 1978).

Acute exposures to HD can also result in long-term respiratory damage manifested as asthma-like conditions, emphysematous bronchitis, and increases in the incidence of secondary respiratory infections (bronchopneumonia and tuberculosis), as set forth in a review by Watson and Griffin, (1992). Beebe, (1960) evaluated the occurrence of respiratory tract disease among a group of World War I soldiers. Those who had been exposed to HD exhibited greater mortality from tuberculosis and pneumonia than either of two reference groups. Manning et al. (1981) reported a significantly increased incidence of mortality from pneumonia among 428 former

workers at an HD manufacturing facility. The ratio of observed to expected cases was 2 ($p \leq 0.05$). Some individuals exposed to HD concentrations that were damaging to the eyes were susceptible to relapsing keratitis (delayed keratopathy) (Watson and Griffin. 1992). Dahl et al., (1985) described the condition's potential to reappear at time points from 8-40 years from the initial exposure.

Case and Lea (1955) reported 29 deaths from cancer of the lungs and pleura among a sample of 1267 World War I veterans who had been exposed to sulfur mustard, 80% of whom also suffered from chronic bronchitis. For comparison, 14 cases would have been expected in a population of that size based on the mortality rates for the male population of England and Wales. The apparently elevated incidence of risk of respiratory tract neoplasms (mortality ratio 207, $p \leq 0.01$) was similar to that of veterans unexposed to HD but who had had bronchitis, leading these workers to conclude that the evidence did not support the designation of HD as a direct carcinogen. However IARC (1975) noted the difference in smoking habits between veterans exposed to HD versus non-exposed veterans, a factor considered likely to obscure any carcinogenic effect of exposure to HD.

Beebe (1960) evaluated the occurrence of respiratory tract cancers among a group of 2718 American soldiers exposed to sulfur mustard during World War I and found that the ratio of observed to expected cases was 1.47 (based on U.S. mortality rates) compared with 1.15 for wounded soldiers not exposed to sulfur mustard, and 0.81 for soldiers who had pneumonia, but who had not been exposed to mustard. Norman (1975) evaluated the same group of soldiers after a 10-year follow-up period (study completed in 1965) and found that the exposed men had a 40% excess of lung cancer mortality, with an estimated relative risk of 1.3 (95% confidence limits of

0.9-1.9) compared to a control group consisting of wounded soldiers without exposure to mustard. The latency period was estimated to be 22-37 years. Norman (1975) also reported that in a limited subgroup of veterans, the relative risk of lung cancer mortality among cigarette smokers who were exposed to mustard agents was approximately equal to that of non-smoking veterans exposed to mustard (4.3 vs 4.4). Norman (1975) concluded that there was no evidence in this limited data set that mustard exposure and cigarette smoking had a synergistic effect on lung cancer mortality.

Retrospective studies of Japanese workers who had been employed at a chemical agent manufacturing plant from 1929 to 1945 have revealed that these individuals have an increased risk of developing respiratory tract cancers. Although sulfur mustard was the main product of the facility, Lewisite, diphenylarsine, hydrocyanic acid, phosgene, and chloracetophenone were also produced there (Inada et al., 1978), and it is not known to what degree these other chemicals contributed to the observed effects. The concentration of mustard in the work place was estimated to be as high as 50-70 mg/m^3 (Nakamura, 1956), and reportedly, the workers frequently exhibited signs of mustard toxicity including acute conjunctivitis, acute rhinitis, acute bronchitis, and acute dermatitis with blister formation. Studies completed in the 1950's documented individual cases of bronchial and laryngeal carcinoma in this population of workers (Yamada et al., 1953, 1957). Yamada (1963) reported that 16.3% of 172 deaths of former workers were due to cancers of the respiratory tract and oropharynx. The incidence rate among 5030 non-exposed inhabitants from the same geographic area was reported to be 0.4% (Yamada, 1963). Mortality rates among the former factory workers during the years 1952-1967 were studied by Wada et al. (1968) who found that the incidence of mortality due to respiratory tract

cancer was 33/495 (30 confirmed by histological evaluation) compared to an expected 0.9, based on national mortality rates for males with the same age distribution as the mustard workers. Of 930 former factory workers not directly involved in the mustard production process, three had died of respiratory tract cancer compared to 1.8 expected. Neoplasms occurred in the tongue, pharynx, sphenoidal sinus, larynx, trachea, and bronchi, with only one occurring peripherally in the lung. The median length of employment was 7.4 years, and the median interval between first employment and death from cancer of the respiratory tract was 24.4 years (Wada et al., 1968).

Additional studies of this population of workers were conducted by Nishimoto et al. (1983, 1988) who incorporated histopathological and mortality data gathered between 1952 and 1986. For 1632 of these workers, the standardized mortality ratio (SMR) for respiratory tract tumors was 3.9 (70 observed vs. 17.8 expected, $p<0.001$, based on data for the Japanese male population) and the SMR for all malignant tumors was 1.2 (173 observed vs. 142 expected, $p<0.01$). These individuals were divided into three groups; (A) those directly involved in the manufacture of sulfur mustard or Lewisite; (B) those not involved in mustard or Lewisite manufacture, but who experienced some exposure; and (C) those engaged in the manufacture of other gases and those who were never exposed. The SMR for groups A and B (1.6 and 1.9) were also significantly elevated ($p<0.001$) whereas that for group C was not. Nishimoto et al (1988) also showed that the SMR was about 2.7 for individuals who had worked at the factory 0.5 to 5 years, but 7.17 for individuals who had been employed for more than 5 years. The SMR was not significantly elevated for individuals who had worked at the factory for 7 months or less. SMRs were also calculated for each of six age groups. For individuals between 30 and 39 years old the SMRs for respiratory tract cancer were not significantly elevated; however, the SMRs for the 40-

49, 50-59, 60-69, and 70-79 yr olds were 10.3, 3.9, 4.4, and 2.5, respectively; all statistically significant at $p<0.01$ or $p<0.001$.

There is some evidence that these former factory workers may have also suffered elevated rates of digestive tract and skin tumors. Histopathological studies conducted by Yamada (1974, as reported by Inada et al., 1978) on 94 autopsy cases and 8 surgical cases revealed 17 cases of digestive tract cancers among these workers (no comparisons with control groups were reported). Of 488 former workers examined dermatologically, 115 had abnormal pigmentation and 22 had skin tumors of which 8 were cases of Bowen's disease (Inada et al., 1978). Pigmentation disorders were present in 57 cases out of 109 engaged only in the production of mustard and in only 1 of 16 cases engaged only in the production of Lewisite. Hyperkeratotic skin lesions such as Bowen's disease, basal cell carcinomas, and hyperkeratotic papular eruptions, were present in 14 cases out of 109 engaged only in mustard production and in 1 case out of 16 engaged only in Lewisite production. No abnormalities were observed in 77 former factory workers who had no exposure to chemical agents (Inada et al., 1978). It was also observed that the longer an individual had been exposed to mustard, the more marked the skin lesions tended to become (Inada et al., 1978).

The studies of Nishimoto et al. (1988), Yamada (1974) and Inada et al. (1978) provide strong evidence for a causal link between chemical agent exposure and cancer; however, because the workers were exposed to multiple chemicals, it is not possible to state conclusively that the cancers were due solely to sulfur mustard. Furthermore, it should be noted that several possible confounding factors, such as tobacco smoking habits, pre-existing health conditions, and post-exposure occupational histories of the workers, were not evaluated. In addition, SMRs

themselves may not provide an accurate estimate of relative cancer risk if they do not correlate with tumor incidence rates in exposed and control groups (i.e., if social/economic or other differences between control and exposed groups result in differences in health care which affect survival rates).

Weiss and Weiss (1975) conducted studies evaluating the health of 271 German workers employed for varying lengths of time between 1935-1945 at a munitions depot where the production, testing and destruction of sulfur and nitrogen mustard (as well as bromoacetone, phosgene, chloropicrin and organic arsenicals) had occurred. Ninety percent of the group had chronic health problems and 114 had died by the end of 1974. Thirty-five percent died from cancer of which 38% were bronchial cancers. The total number of deaths from cancer was significant ($p<0.01$) and the number of bronchial cancers was also significant (11 observed vs. 5 expected for the population of the geographic region where the facility was located). The number of cancers of the gastrointestinal tract was 35% greater than expected. The average tumor induction time was 21.6 years. IARC (1975) notes that the study was limited to workers with available medical records, which "raises the possibility that the proportion with cancer may have been inflated, since medical records or autopsy records would more likely have been preserved for workers with cancer". Furthermore, IARC (1975) does not indicate whether smoking habits and other confounding factors were accounted for in the study of Weiss and Weiss (1975).

According to Klehr (1984), German workers involved in the dismantling of an HD facility developed multiple skin lesions including basal cell carcinoma, Bowen's disease,

Bowen's carcinomas, and carcinoma spinocellulare. The incidence rate for all tumors (including skin tumors) was 34% in 53 workers evaluated.

Manning et al. (1981) evaluated the incidence of cancer among former workers of a British mustard manufacturing facility (1939-1945). As of 1974, the number of deaths from all neoplasms combined (45) was slightly greater than that expected from national death rates, but the increase was not statistically significant. Two deaths were attributed to cancer of the larynx and one to carcinoma of the trachea compared to an expected number of 0.40 ($p<0.02$; relative risk 7.5). Seven individuals were known to have developed cancer of the larynx, compared with 0.75 expected ($p<0.001$, relative risk 9.3). Lung cancer deaths were also elevated (21 observed vs. 13.43 expected) but not to significant levels (relative risk 1.6). In follow-up investigations of this group of workers, Easton et al. (1988) evaluated the mortality records of 3354 individuals and found greater numbers of cancer deaths when compared to national mortality rates. Significant increases were observed in deaths from cancer of the larynx (11 observed, 4.04 expected, $p = 0.003$), pharynx (15 observed, 2.73 expected, $p<0.001$), and all other buccal cavity and upper respiratory sites combined (12 observed, 4.29 expected, $p = 0.002$). There were also 200 deaths from lung cancer compared with 138.39 expected ($p<0.001$). It was also reported that the risks of developing cancer of the lung and pharynx were significantly related to the duration of employment. Significant excess mortality was also observed for cancers of the esophagus (20 observed vs. 10.72 expected) and stomach (70 observed vs. 49.57 expected) but there was no correlation with time since first exposure or duration of exposure.

Manning et al. (1981) concluded that it was very likely that the observed cancers of the pharynx, larynx and other upper respiratory sites were due to exposure to sulfur mustard because

the excesses were too large to be accounted for by confounding factors (the effects of smoking, however, were not evaluated), increased with increasing duration of employment, and were limited to the period more than 10 years after first employment. Evidence for a causal relationship between HD exposure and other cancers, was not considered to be as strong.

Although a large number of American military personnel were exposed to sulfur mustard in chamber and field tests conducted during World War II, the morbidity and mortality records of this cohort have not been adequately evaluated to document long-term health risks (Institute of Medicine, 1993).

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity. Sulfur mustard vesicants are acutely toxic by direct contact. Edema, ulceration, and necrosis of the skin and respiratory tract epithelium can occur, as well as conjunctivitis and blindness. General symptoms of systemic toxicity include nausea, vomiting, fever, and malaise (ITII, 1975). Delayed effects which may occur following acute exposures include: eye lesions, chronic bronchitis, and cancers of the respiratory tract and skin. However, information on adverse effects following long-term exposures to less than acutely toxic concentrations is very limited. Health effects of sulfur mustard agents have recently been reviewed by ATSDR (1992), Somani (1992), Sidell and Hurst (1992), Watson and Griffin (1992), and the Institute of Medicine (1993). The following is a brief summary of the most important toxicological data for sulfur mustard.

Acute exposures to sulfur mustard can result in skin and eye damage, gastrointestinal irritation, and depressed myelopoiesis (resulting in leukopenia and anemia) (Vogt et al., 1984). Damage to the respiratory tract, which is the principal cause of mortality in the first few days to

weeks after exposure to sulfur mustard, involves acute edema, inflammation, and destruction of the airway epithelial lining (Institute of Medicine, 1993). Infection of the respiratory tract resulting in bronchopneumonia is a common complication of exposure to sulfur mustard.

The skin and eyes are especially sensitive to the toxic effects of HD. When applied to human skin, about 80% of the dose evaporates and 20% is absorbed (Vogt et al., 1984). About 12% of the amount absorbed remains at the site and the remainder is distributed systemically (Renshaw, 1946). Doses up to 50 $\mu g/cm^2$ cause erythema, edema, and sometimes small vesicles. Doses of 50-150 $\mu g/cm^2$ cause bulbous-type vesicles and larger doses cause necrosis and ulceration with peripheral vesication Droplets of liquid HD containing as little as 0.0025 mg may cause erythema (Ward et al., 1966). Eczematous sensitization reactions were reported in several early studies and may occur at concentrations below those causing direct primary irritation (Rosenblatt et al., 1975). In humans, the LCt_{50} (estimated concentration x exposure period lethal to 50% of exposed individuals) for skin exposures is 10,000 mg-min/m^3 (DA, 1974) (for masked personnel; however, the amount of body surface area exposed was not reported). The ICt_{50} (estimated concentration x exposure period incapacitating to 50% of exposed individuals) for skin exposures is 2000 mg-min/m^3 at 70-80°F in a humid environment and 1000 mg-min/m^3 at 90°F in a dry environment (DA, 1974, 1992). The LCt_{50} for contact with the eyes is 200 mg-min/m^3 (DA, 1974, 1992). The LD_{Lo} for skin exposure is 64 mg/kg and the LD_{50} is estimated to be about 100 mg/kg (DA, 1974, 1992).

By contrast to some eye irritation data in laboratory animals which were negative for adverse effects at repeated dosing levels of 1.4 mg-min/m^3 (Rosenblatt et al., 1975), a Ct of <12 mg-min/m^3 is considered a no-effects dose for eye irritation in humans, at ambient temperatures

(McNamara et al., 1975). At higher temperatures, other biological effects occur at lower concentrations. Cts of 40-90 can cause eye irritation and conjunctivitis after a latency period of 2 to 48 hours; and Cts of 90-100 mg-min/m^3 produce moderately severe burns, ulcers, opacity, and perforation, after a latency period of 2 to 10 hrs (Doull et al., 1980). In some cases there may be a recurrent vascularization and ulceration many years after the initial exposure.

The LCt_{50} for inhalation exposures in humans has been estimated to be 1500 mg-min/m^3. From comparatively limited data, DA (1992) developed an acute oral LD_{50} of 0.7 mg/kg.

1.2.2. Animal Toxicity.

1.2.2.1. Acute Toxicity —

1.2.2.1.1. Oral Toxicity. The oral LD_{50} for rats has been estimated at 17 mg/kg (DA, 1974). Rats treated with 2.5 mg/kg/day for 14 days developed inflammation, petechial hemorrhage, thickening, and sloughing of the gastric mucosa (Hackett et al., 1987).

1.2.2.1.2. Inhalation Toxicity. In animals, median lethal Ct values for HD have been estimated to range from 600 to 1900 mg-min/m^3 for 10 min exposures (see Rosenblatt et al., 1975 for review). An LC_{Lo} (lowest lethal concentration) of 189 mg/m^3/10 min has been reported for mice (Lewis and Sweet, 1984), and a 5-min LC_{Lo} of 77 ppm has been reported for dogs (ITII, 1975).

1.2.2.1.3. Percutaneous Toxicity. A review by Dacre and Goldman (1996) has collated much of the available toxicological and pharmacological information on HD, including a summary of the rapid responses that are evident when HD is applied to the skin of laboratory animals in acute tests. The reviewers describe the experiments of Vogt (1984), who used histochemistry, light and electron microscopy to follow the response of rabbit or guinea pig skin

to the application of between 25-250 $\mu g/cm^2$ of HD. An initial erythema was evident within 30-60 minutes of challenge, followed by further progression and the development of edema and necrosis. Initial damage to the basal epithelial cells, superficial small capillaries, with resulting vascular leakage, was followed by a massive infiltration of granulocytes, then by death of the epidermal cells, increased lysosomal enzyme activity, and muscular leakage. The inflammatory response achieved its maximal activity between 27-72 hours, to be followed by scab formation and ultimate healing.

These essentially local effects of topical application of HD to animal skin are compounded by the consequences of the substances' ability to readily penetrate the skin. Though the initial step in the development of injury appears to be the fixation of mustard molecules at the site of contact, a large proportion of the load is none-the-less carried away by the circulation with resulting systemic injury. Application of isotopically-labeled HD to the skin of rats has been followed by the appearance of counts throughout the body within 30 minutes of challenge.

1.2.2.2. Subchronic Toxicity — In a subchronic study conducted by Sasser et al. (1989a), Sprague-Dawley rats (12/sex/group) were dosed by gavage with 0, 0.003, 0.01, 0.03, 0.1 or 0.3 mg HD (in sesame oil)/kg body weight, 5 days/week, for 13 weeks. No mustard-related mortality occurred at any dose level. Body weights were significantly decreased in animals in the high-dose group. Epithelial hyperplasia of the forestomach occurred in 5/12 males and 5/12 females of the high-dose group and in 1/12 males receiving 0.1 mg/kg/day, but not in any other treatment group or controls. No other treatment-related pathological lesions, clinical chemistry changes, or hematological abnormalities were reported.

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. McNamara et al., (1975) exposed SDW rats, ICR Swiss albino and A/J/ mice, rabbits, guinea pigs, and dogs to HD vapors for varying exposure periods up to one year. The test animals were exposed to 0.001 mg HD/m^3 continuously or to 0.1 mg HD/m^3 for 6.5 hr followed by 0.0025 mg HD/m^3 for 17.5 hr per day, 5 days/week. No tumors were observed in rabbits, guinea pigs, dogs, or mice, but skin tumors occurred in the exposed rats. Rats were tested in two separate studies; (1) a "toxicity study" in which 70 males and 70 females were exposed to each concentration for up to 52 weeks and then observed for 6 months at which time they were sacrificed, and (2) a "carcinogenicity study" in which the same number of males and females were exposed to each concentration of sulfur mustard for time periods ranging from 1 to 52 weeks and then observed for 2 to 21 months before being sacrificed. Fifty animals of each sex were maintained as controls. Animals dying naturally during either of the tests were not included in the results. In both tests, skin tumors occurred primarily in animals exposed to the highest concentration of sulfur mustard. Of the tumors observed, only basal cell and squamous cell carcinomas, trichoepitheliomas, and keratoacanthomas of the skin were considered to be related to the sulfur mustard exposure (McNamara et al., 1975). In the 52-week toxicity test, these skin tumors were observed only in test animals maintained for at least 70 days after the exposure period ended. Of the 29 animals still on test after 180 days post-exposure, 9 (4 males and 5 females) were found to have skin tumors. In the carcinogenicity test, only 1 of 49 rats exposed to 0.001 mg HD/m^3 developed a skin tumor, but 21 of 56 rats exposed to 0.1/0.0025 mg HD/m^3 developed skin tumors.

Heston (1950) reported an increase in the occurrence of pulmonary tumors in strain A mice injected intravenously with 0.25 mL of a 1:10 dilution of a saturated solution of HD in water (0.06-0.07%) at 2-day intervals for a total of 4 doses. The tumor incidence was 93.3% (2.6 tumors/mouse) compared with 61% in controls (0.9 tumors/mouse). In a second test in which a slightly lower dose was used, pulmonary tumors were found in 68% of the surviving treated animals (1.09 tumors/mouse) compared with 13% in the controls (0.13 tumors/mouse) ($p \leq 0.001$). A significant increase in the incidence of pulmonary tumors in strain A mice was also seen in an inhalation study in which the test animals were exposed for 15 minutes to vapors released from 0.01 mL of HD applied to filter paper (Heston and Levillain, 1953; exposure levels were not otherwise quantified). Eleven months after exposure, lung tumor incidence was 49% (33/67) in the exposed animals and 27% (21/77) in the controls ($p \leq 0.01$).

In another study, Heston (1953) found that subcutaneous injections of HD (0.05 cc of a 0.05% solution at weekly intervals for 6 weeks, or 0.1 cc of a 0.1% solution in olive oil at 2-day intervals for a total of 6 doses) into the mid-dorsal region of mice (strains A, C3H, and C3Hf) resulted in injection-site tumors, whereas injections of vehicle alone did not induce tumor formation. Tumors found at the injection site included 11 sarcomas, 2 sarcomas neurogenic in origin, a rhabdomyosarcoma, 3 papillomas, a squamous cell carcinoma, a hemangioendothelioma, and a mammary carcinoma.

1.3.2. Chronic Toxicity. The McNamara et al. (1975) study exposed male and female SDW rats (140), A/J mice (140), rabbits 212), guinea pigs (30), and dogs (6 initially) to a sulfur mustard vapor concentration of 0.001 mg HD/m^3 for 24 hr/day, 5 days/wk, for varying exposure durations up to one year (Note: the investigators reported that the experimental protocol involved

a continuous exposure, implying that it was for 7 days/wk; however, in several places in the report it is specifically mentioned that the exposures were for only 5 days/wk). The same number of animals of each species were exposed to 0.1 mg HD/m^3 for 6.5 hr followed by 0.0025 mg HD/m^3 for 17.5 hr per day, 5 days/week for up to one year. The latter exposure is equivalent to a 5 day/wk time-weighted average concentration of 0.029 mg/m . Unexposed controls consisted of 10 dogs, 7 rabbits, 20 guinea pigs, 100 rats and 120 mice. Exposed animals were sacrificed periodically during the study and were replaced with new animals. One hundred ICR mice were added to the test chambers about 6 months after the tests began, and 50 A/J mice were added to the chambers about 3 months later.

Signs of toxicity that could be attributed to the sulfur mustard exposure occurred only in rats and dogs. Of 39 rats exposed to 0.001 mg HD/m^3 for 12 months, 5 exhibited chronic keratitis, a condition that McNamara et al. (1975) reported could possibly have been agent-related; however, this effect was not observed in any rats exposed to 0.1 mg HD/m^3. No signs of toxicity were seen in any of the dogs exposed to 0.001 mg HD/m^3; however, it should be noted that only 2 animals were exposed for the full 52-week period and only 4 animals were exposed for 32 weeks. The major signs of toxicity seen in the dogs exposed to 0.1 mg HD/m^3 were ocular changes consisting of corneal opacity, pannus, vascularization, pigmentation, keratitis, and granulation. McNamara et al. (1975, p. 12) state that chronic keratitis and conjunctivitis occurred in 3 of 10 dogs exposed for 7.5 or 12 months. The data presented by McNamara et al. indicate that chronic keratitis was also seen in some animals as early as 16 weeks after exposure began, and may have occurred in as many as 5 of 10 animals exposed for 32, 40 or 52 weeks. McNamara et al. (1975) concluded that it was "possible" that these effects were agent related.

Pneumonitis occurred in several of the dogs exposed to 0.1 mg HD/m^3, but this condition was also seen in the control animals, and because no other respiratory tract lesions were found, McNamara et al. (1975) indicated that the observed pneumonitis was not agent-related. There were no changes in blood chemistry of the exposed dogs except for a possible increase in serum glutamic oxaloacetic transaminase after 12-28 weeks of exposure to 0.1 mg/m^3. Two dogs exposed to 0.1 mg HD/m^3 for 12 months also exhibited anaphylactic syndrome, gastroenteritis, and petechia.

Although these effects were considered by McNamara et al. (1975) to be unrelated to the exposure to sulfur mustard, they are consistent with the known vesicant and sensitization actions of the agent. It is possible that the HD condensed on the fur of the animals and was subsequently ingested as a result of grooming behavior. Gastroenteritis could then have resulted from direct contact of the vesicant with the gastrointestinal epithelium.

1.4. REPRODUCTIVE EFFECTS

Azizi et al. (1995) investigated changes in serum concentrations of reproductive hormones and sperm counts in men who had been exposed to HD during wartime. In 16 individuals, serum free and total testosterone and dehydroepiandrosterone were markedly decreased in the first five weeks after exposure; but levels returned to normal by 12 weeks. In 28 of 42 men evaluated one to three years after exposure, sperm counts were less than 30 million cells/mL and follicle-stimulating hormone was increased compared to controls having sperm counts above 60 million cells/mL. Testicular biopsy of the test subjects revealed partial or complete arrest of spermatogenesis.

1.5. DEVELOPMENTAL EFFECTS

In a study conducted by Hackett et al. (1987), HD (dissolved in sesame oil) was administered by intragastric intubation to rats and rabbits on gestation days 6-15 (rats) or 6-19 (rabbits). Female rats were dosed with 0, 0.2, 0.4, 0.8, 1.6, 2.0 or 2.5 mg/kg/day in a range-finding study (3-9 animals per dose group of which 2-7 per dose group were pregnant) and with 0, 0.5, 1 .0, or 2.0 mg/kg/day in a teratology study (25-27 animals per dose group of which 20-26 per dose group were pregnant). Maternal and fetal toxicity was observed at all dose levels. In the range-finding study significant ($p < 0.05$) maternal effects included mortality (1/3) at the highest dose; severe gastric lesions (petechial hemorrhage and sloughing of gastric mucosa) at 2.0 and 2.5 mg/kg/day; and inflamed mesenteric lymph nodes at doses of 0.4 mg/kg/day and higher. Significant decreases in body weight and decreased extragestational weight occurred at 1.6 mg/kg/day and decreased hematocrit at 0.8 mg/kg/day. There were no adverse effects on fetal weight and no evidence of morphological abnormalities in the fetuses. In the rat teratology study, maternal toxicity was evidenced by gastric inflammation at 2.0 mg/kg/day, and inflamed mesenteric lymph nodes at doses of 0.5 mg/kg/day and higher. Decreased body weight and decreased extragestational weight occurred at 0.5 mg/kg/day; decreased hematocrit at 1.0 mg/kg/day; and decreased weight of the placenta and gravid uteri at 2.0 mg/kg/day. Fetal effects included decreased weight in females and hydroureter at 0.5 mg/kg/day; decreased weight of males at 1.0 mg/kg/day; increased incidences of supernumerary ribs, misaligned sternebrae, and reduced ossification of sternebrae at 2.0 mg/kg/day. The investigators reported that the study did not reveal any evidence for an HD-induced teratogenic effect in rats because all of the observed

fetal changes occurred at dose levels that also produced maternal toxicity. The NOAEL for maternal and fetal toxicity was reported to be <0.5 mg/kg/day.

In the second part of the Hackett et al., (1987) study, rabbits were dosed with 0, 0.5, 1.0, 2.0, and 2.5 mg/kg/day in a range-finding study (7-8 per dose group), and with 0, 0.4, 0.6, or 0.8 mg/kg/day in the teratology study (7-8 per dose group). Dose levels of 0.8 mg/kg/day or higher were lethal to the dams. Damage to the gastric mucosa and enlarged Peyer's patches were observed in animals that received the lowest dose (0.4 mg/kg/day). Depressed body weight, depressed extragestational weight gain, and depressed hematocrit values occurred at 0.8 mg/kg/day. In the range-finding study a significant depression in fetal body weights occurred at a dose level of 2.0 mg/kg/day; however, in the teratology study no significant effects were observed on intrauterine survival, placental and fetal body weights, or incidence of fetal abnormalities. The investigators concluded that the study provided no evidence that sulfur mustard induced a teratogenic effect in rabbits. The NOAELs for maternal and fetal toxicity were reported to be <0.4 mg/kg/day and >0.8 mg/kg/day, respectively.

In a two-generation reproductive toxicity study conducted by Sasser et al. (1989b), groups of Sprague-Dawley rats (27 females and 20 males/group/generation) were gavaged with 0, 0.03, 0.1 or 0.4 mg/kg/day. The animals were treated according to the following exposure protocol: male and female rats were dosed 5 times/week for 13 weeks prior to mating and during a 2-week mating period; female rats were dosed daily throughout the 21-day gestation and parturition period; and females were dosed 4-5 times/week during the 21-day lactation period. Males who had mated with females were sacrificed at the birth of their pups; dams who had given birth were sacrificed when the pups were weaned. Male and female F1 pups received

sulfur mustard until they were mated, the females became pregnant, and gave birth. At this point, F1 males were sacrificed and F1 dams continued on the dosage schedule until weaning at which point the study was terminated. Thus, two generations of rats received subchronic exposure to sulfur mustard, with each generation going through a mating cycle. Similarly, two generations of pups were born to parents who had received sulfur mustard. Body weight gain was significantly ($p < 0.05$) lower than control values in the F1 rats of both sexes born to parents who had received the highest dose of sulfur mustard. There were no significant adverse effects on reproductive parameters at any dose level. However, dose-related lesions of the squamous epithelium of the forestomach (acanthosis and hyperplasia) occurred in both sexes of each treatment group. The lesions were described as mild at the lowest dose level, 0.03 mg/kg, compared with the higher dose groups. The incidence and severity of acanthosis was 0/94 in the controls, 71/94 in the low-dose group, 89/94 in the mid-dose group, and 94/94 in the high-dose group. Benign neoplasms of the forestomach occurred in 8/94 animals in the 0.1 mg/kg group and in 10/94 animals of the 0.4 mg/kg group. The results of this study indicate that lowest dose tested (0.03 mg/kg/day) is a LOAEL for maternal toxicity.

1.5.1. Mutagenicity. IARC (1975), Fox and Scott (1980) and ATSDR (1992) have summarized the available evidence concerning the genotoxicity of HD. Because the compound is a strong DNA alkylating agent, genotoxic effects occur through cross-link formation, inhibition of DNA synthesis and repair, point mutations, and chromosome and chromatid aberrations (ATSDR, 1992). Some of these conditions have been observed in humans following exposure to HD, others in various test systems including bacteria, yeast, insects, and mammalian cell cultures.

In studies conducted on a group of 28 former employees of a chemical agent manufacturing plant, Yanagida et al. (1988) found that the frequency of mutations to hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) deficiency was significantly elevated when compared to two control groups matched for age and smoking status. One control group consisted of healthy men and the other of individuals with bronchitis. The data also showed that the mutations were significantly more frequent in those workers who had longer exposures to HD. A chromosome study of 16 former workers of this same factory indicated a significantly higher incidence of sister chromatid exchanges (SCE) in peripheral lymphocytes when compared to a control group ($p<0.03$) (Shakil et al., 1993). Two individuals with chronic myelocytic leukemia had an almost three-fold higher SCE rate than controls and also a high (12.1%) incidence of chromosome abnormalities (Shakil et al., 1993). In an evaluation of the p53 mutations found in lung tumors of HD workers, Takeshima et al. (1994) found that the mutations were similar to those in lung tumors of tobacco smokers (the factory workers were also tobacco smokers), however, the prominence of G:C to A:T transitions and the occurrence of double mutations in two of twelve cases suggested that the exposure to HD did contribute to the development of the lung cancers.

Wulf et al. (1985) reported significant ($p<0.001$) increases in sister chromatid exchanges in lymphocytes of eleven fisherman who had accidently been exposed to sulfur mustard in sufficiently high concentrations to cause signs of acute toxicity.

HD was found to be genotoxic and mutagenic in several microbial assays. The agent caused alkylation of DNA in the yeast Saccharomyces cerevisiae (Kircher and Brendel, 1983), and interstrand DNA cross-links (Venitt, 1968) and inhibition of DNA synthesis (Lawley and

Brookes, 1965) in *Escherichia coli*. Using the histidine reversion assay, Stewart et al. (1989) found that sulfur mustard induced point mutations in Salmonella typhimurium strain TA102 and frameshift mutations in TA 97, but neither type of mutation in strains TA98 and TA100.

HD inhibited DNA synthesis in mouse lymphoma cells (Crathorn and Roberts, 1965), HeLa cells (Crathorn and Roberts, 1966), and L-strain mouse fibroblasts (Walker and Thatcher, 1968). It also induced chromosomal aberrations in cultured rat lymphosarcoma and mouse lymphoma cells (Scott et al., 1974) and chromosomal aberrations and reverse mutations in male BDF_1 mice in a host-mediated assay using the murine leukemia L5178Y/Asn$^-$ as an indicator (Capizzi et al., 1973).

Several studies have also demonstrated that sulfur mustard causes dominant lethal mutations. Rozmiarek et al. (1973) reported a dominant lethal mutation rate of 9.4% (+1.9%) in rats after adult males had been exposed to 0.1 mg HD/m^3 for 12 weeks. Sasser et al. (1990) reported that a dominant lethal effect occurred after male Sprague-Dawley rats were dosed orally with 0.5 mg HD/kg/day 5 days/week for 10 weeks. The observed effects included increases in early fetal absorptions, preimplantation losses, and decreases in total live embryo implants. A significant increase in the percentage of abnormal sperm was also reported. Dominant lethal mutations, as well as chromosome rearrangements, have also been observed in Drosophila melanogaster exposed to sulfur mustard (Auerbach and Robson, 1946).

The cytotoxic, clastogenic and mutagenic effects of HD in Chinese hamster ovary cells have also been evaluated by Jostes et al. (1989). Chromosomal aberration frequency increased in a dose-dependent manner over the dose range of 0.0625 to 0.25μM. Mutation induction at the

HGPRT locus was sporadic, but the majority of the exposures resulted in mutation frequencies that were 1.2 to 4.0 fold higher than the spontaneous frequencies.

1.6. TOXICOKINETIC STUDIES

1.6.1. Toxicokinetics. Information on the toxicokinetics of agent HD has been provided by Black, et al., (1992), who tracked the appearance of some putative HD metabolites in urine following cutaneous exposure to male albino Porton strain rats. Gas chromatography/Mass Spectrometry (GC/MS) was used to measure thiodiglycol, thiodiglycol sulfoxide, 1-1'-sulfonylbis[2-(methylthio)ethane] (SBMTE), 1-methylsulfinyl-2-[2-(methylthio)ethyl sulfonyl] ethane (MSMTESE), and 1,1'-sulfonylbis[2-(methylsulfinyl-ethane] (SBMSE) in urine samples collected for up to 8 days after exposure. Free and esterified thiodiglycol peaked in the 24 hour samples, but cumulatively comprised less than 2% of the applied dose, whereas thiodiglycol sulfoxide peaked at 6 hours and comprised about 4% of the dose. In a novel approach, the collective amounts of SBMSE and MSMTESE, presumably derived from the action of β-lyase on cysteine conjugates following S-methylation and oxidation, were determined as a common analyte (SBMTE), after treatment of the urine specimens with titanium dioxide. Urinary excretion of hydrolysis or β-lyase products accounted for up to 19% of the initial cutaneous dose.

That urine is a major route for HD elimination was demonstrated by experiments in which ^{35}S-labeled HD was administered to male albino Porton strain rats by either intravenous or intraperitoneal injection or subcutaneous application (Hambrook et al., 1992). Urine and feces were collected at 6 and 24 hours after exposure, daily for 8 days, then intermittently until the counts returned to background. In the cutaneous application experiments, the administered doses were 0.2, 1 or 5 $\mu mol/cm^2$, although the estimate for amount absorbed depended on the residual

HD remaining in the applicator after exposure. After intraperitoneal or intravenous injection, most of the counts were eliminated in the urine during the first three days, with lower amounts appearing up to 105 days from the point of injection. By contrast, after subcutaneous application counts appeared in the feces as well as urine. Overall recovery was incomplete, suggesting that HD or its sulfur-containing metabolites may have been retained in the skin.

The tissue distribution of radioactivity after intravenous injection of ^{14}C-labeled HD was followed in male Wistar rats (Maisonneuve et al., 1994). Doses of either 500 μg/kg or 10 mg/kg were injected by either the femoral or jugular veins, then groups of animals were sacrificed at time points ranging from 2 minutes to 96 hours after exposure. Animals were necropsied, and organs were excised, weighed, then measured for radioactivity. Radioactivity peaked after 2 to 3 hours, with counts predominating in the kidney or liver. In some experiments, counts were evident in muscle, a phenomenon considered to be a site-of-entry effect, since injection via the jugular vein resulted in a different pattern of distribution. Overall, the specific activity in organs of animals receiving HD via the jugular vein appeared to be much higher than in those receiving HD via the femoral vein.

1.6.2. Metabolism. The acute toxic effects of mustard vesicants are usually attributed to the consequences of alkylation reactions with organic compounds including nucleoproteins such as DNA. Alkylation reactions can result in physiological and metabolic disturbances as well as mutagenic effects such as those discussed in section 1.5.1. Several hypotheses have been advanced concerning the primary cause of cell death following acute exposures. As reviewed by Papirmeister et al. (1991), the three major hypotheses are:

1. Poly(ADP-ribose) polymerase (PADPRP) hypothesis. - In this theory DNA is the initial target of the mustard agent. Alkylated DNA purines undergo spontaneous and enzymatic depurination, leading to the production of apurinic sites which are cleaved by apurinic endonucleases to yield DNA breaks. Accumulation of DNA breaks leads to activation of the chromosomal enzyme PADPRP, which utilizes nicotinamide adenine dinucleotide (NAD^+) as a substrate to ADP-ribosylate and a variety of nuclear proteins, causing severe lowering of cellular NAD^+. Depletion of NAD^+ results in the inhibition of glycolysis, and stimulation of the nicotinamide adenine dinucleotide phosphate (NADP+)-dependent hexose monophosphate shunt (HMS) pathway follows as a result of the accumulation of glucose-6-phosphate, a common precursor for both glycolysis and the HMS. Induction and secretion of proteases is stimulated as a result of enhanced HMS activity, and this leads to pathological changes in the cell.

2. Thiol-Ca^{++} peroxidation hypothesis. The first step in this process is thought to be the alkylation of glutathione (GSH) by the mustard agent. Depletion of GSH subjects protein sulfhydryl groups to damage from the agent or from reactive cellular oxidants. Proteins most susceptible to damage include Ca^{++} translocases (Ca^{++}-stimulated, Mg^{++}-dependent ATPase) which are dependent on thiol groups to maintain cellular Ca^{++} homeostasis, and microfilamentous proteins, where loss of sulfhydryl groups could result in disruptions of the cytoskeletal and structural integrity of the plasma membrane.

3. Lipid peroxidation hypothesis. According to this hypothesis the mustard agent causes depletion of GSH which, in turn leads to the buildup of highly toxic oxidants, usually through H_2O_2-dependent reaction sequences. The oxidizing agents react with membrane phospholipids to form lipid peroxides, initiating a chain reaction of lipid peroxidation which can lead to alterations in membrane fluidity, loss of membrane protein function, and loss of membrane integrity.

1.6.3. Percutaneous Absorption. The discussion of the acute dermal toxicity of HD in laboratory animals (Section 1.2.2.1.3.) and the summary of the extensive epidemiological data on human exposure (Section 1.1) combine to present a picture of a substance whose profound site-of-entry effects are compounded by its ability to quickly penetrate the skin, thereby gaining access to remote bodily sites and other major organ systems via the general circulation.

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

Data describing any structure-activity relationships between HD and other compounds are presently unavailable.

1.8. MECHANISTIC STUDIES

In experiments to assess the role of DNA damage and repair on the survival of primary cultures of rat cutaneous keratinocytes to agent HD, Ribeiro et al. (1991) employed a "nucleoid sedimentation" assay procedure to assess the potential for exposed cells to recover the structural integrity of their genome, post-challenge. In other experiments, keratinocytes grown on a nylon net at an air-water interface were exposed to HD for 1hr at 35°C in a 5% CO_2/air atmosphere, followed by extensive washing. Lysed cells were then sedimented on a continuous density gradient, and the stratified bands visualized under UV light. In some cultures exposed to HD, ^{3}H-Thymidine incorporation was measured after precipitation with trichloroacetic acid (TCA). HD was found to affect the rate of sedimentation of keratinocyte DNA at concentrations down to 0.1 μM, suggesting the loss of structural integrity of the nucleic acid, most likely due to the formation of strand breaks, and/or the loss of some supercoiling. However, in those cells pulsed with up to 5 μM then removed from exposure, some recovery of DNA structural integrity was evident, an effect not apparent with cells originally challenged with higher concentrations of HD. However, the ability of cells to incorporate ^{3}H-Thymidine was not recovered under these experimental conditions, allowing the speculation that the recovery of the DNA's tertiary structure may have resulted in a functionally inadequate genome due to mismatching that might occur on insertion of non-complementary bases into the nucleic acid.

2. INTERPRETATION OF AVAILABLE INFORMATION

Pure sulfur mustard (HD) is a colorless, odorless, oily liquid with a molecular weight of 159.08 (MacNaughton and Brewer, 1994). Commercial products, however, have a yellow-brown color and sweet odor due to contaminants (MacNaughton and Brewer, 1994). HD has a vapor density of 5.5 (air = 1), a liquid density of 1.27 g/mL at 25°C, a vapor pressure of 0.11 mm Hg at 25°C, and a water solubility of 0.092 g per 100 g at 22°C (DA, 1974). The vapor pressure of HD is 0.11 mm Hg at 25°C, indicating moderate volatility. A vapor concentration of 920 mg/m^3 has been reported for a temperature of 25°C (DA, 1974) (although not adequately described in the reference, this presumably is the saturation concentration above a pure liquid). Information on the half-life of HD in air under various environmental conditions was not found in the available literature.

The water solubility of HD has been reported as 0.092 g per 100 g water at 22°C (DA, 1974), and 5 x 10^{-3} M at room temperature (MacNaughton and Brewer, 1994). In dilute aqueous solutions HD hydrolyzes almost completely to thiodiglycol and hydrochloric acid (Papirmeister et al., 1991). For dissolved HD, the hydrolysis half-life ranges from about 4 to 15 min for temperatures of 20-25°C; however, bulk HD may persist in water for up to several years (Small, 1984). Small (1984) reported that it would take 15 days for the mass of a 1 cm droplet of HD in quiescent water to decrease by one half.

The Henry's Law Constant for HD has been estimated to be 2.1 x 10^{-5} atm m^3/mol (MacNaughton and Brewer, 1994), indicating a moderate potential for evaporation from water.

HD can be very persistent in soil (Rosenblatt et al., 1995), the extent depending on the soil type, pH, moisture content, and whether the agent is at the soil surface or buried. Small (1984) reported that when HD was applied to the soil surface, volatilization would be the main route of HD loss (half-life about 30 min), but if the soil was wet, hydrolysis would be the main loss pathway. When sprayed onto the soil surface, the vesicant action of HD persisted for about 2 weeks, however, when the agent leaked into the soil, such activity was still present after 3 years (DA, 1974). Rosenblatt et al. (1995) considered the persistence of sulfur mustard in soil to be due to the formation of oligomeric degradation products that coat the surface of the mustard agent and that are resistant to hydrolysis.

3. DOSE-RESPONSE ASSESSMENT

3.1. INGESTION EXPOSURE

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name --Sulfur Mustard (bis(2-chloroethyl)-sulfide; Agent HD)
CASRN -- 505-60-2
Preparation Date -- September 1996

ORAL RfD SUMMARY

Critical Dose --
UF -- 3000
MF -- 1
RfD -- 7E-6 mg/kg-day
Confidence -- Medium/High

Critical Study

Critical Effect -- Forestomach hyperplasia, acanthosis

Study Type -- Rat gavage study (two generations)

Reference -- Sasser et al., 1989b

NOAEL --
NOAEL(ADJ) --

LOAEL -- 0.03 mg/kg-day
LOAEL(ADJ) -- 0.022 mg/kg-day

Conversion Factors and Assumptions --

PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Sasser, L.B., R.A. Miller, D.R. Kalkwarf, et al. (1989) Toxicology studies on Lewisite and sulfur mustard agents: Two-generation reproduction study of sulfur mustard (HD) in rats. Final

report from Pacific Northwest Laboratories (PNL-6944) to U.S. Army Medial Research and Development Command, Fort Detrick, MD, AD A216423.

Male and female Sprague-Dawley rats were gavaged with 0, 0.03, 0.1, or 0.4 mg/kg dissolved in sesame oil, with a resulting significantly ($p < 0.05$) decreased body weight gain in the F1 rats of both sexes born to parents who had received the highest dose of HD. In addition, dose-related lesions of the squamous epithelium of the forestomach (acanthosis and hyperplasia) occurred in both sexes of each treatment group. The lesions were described as mild at the lowest dose level, 0.03 mg/kg, compared with the higher dose groups. The incidence and severity of acanthosis was 0/94 in the controls, 71/94 in the low-dose group, 89/94 in the mid-dose group, and 94/94 in the high-dose group. Benign neoplasms of the forestomach occurred in 8/94 animals in the 0.1 mg/kg group and in 10/94 animals of the 0.4 mg/kg group. The investigators reported that the NOAEL for toxicity was <0.03 mg/kg and the NOAEL for reproductive effects >0.4 mg/kg.

The lowest dose tested, 0.03 mg/kg, can be considered a LOAEL for rats orally exposed to HD, with epithelial acanthosis and hyperplasia of the forestomach as the critical effect. Using this LOAEL, a human chronic RfD can be derived by adjusting the dose to a 7 day/week exposure protocol and then applying the result to the RfD methodology. Dose adjustments for discontinuous exposure can be made as follows: female rats were gavaged 5 times/week for 15 weeks (75 days), total dose= 2.25 mg/kg; daily for 3 weeks (21 days), total dose = 0.63 mg/kg; and 4 times/week for 3 weeks (12 days), total dose - 0.36 mg/kg. The combined total dose over the 21-week exposure period, therefore, is 3.24 mg/kg; dividing the combined total dose of 3.24 mg/kg by 147 days (21 weeks) results in an adjusted LOAEL of 0.022 mg/kg/day. The adjusted LOAEL can then be applied to the equation for deriving an RfD.

UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

Ten for protection of sensitive subpopulations, 10 for interspecies extrapolation, 10 for subchronic to chronic exposure, and 3 for LOAEL to NOAEL extrapolation.

UF -- 3000

MF -- 1

ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

Reproductive/Developmental Study: Hackett et al,. 1987:

HD was administered by intragastric intubation to CD rats on gestation days 6-15. Test animals received 0, 0.2, 0.4, 0.8, 1.6, 2.0 or 2.5 mg/kg/day (in sesame oil vehicle) in a range-finding study (3-9 animals per dose group) and 0, 0.5, 1.0, or 2.0 mg/kg/day in a teratology study

(25-27 animals per dose group). In the range-finding study, maternal toxicity was evidenced by mortality (1/3) at the highest dose (2.5 mg/kg/day); severe gastric lesions (petechial hemorrhage and sloughing of gastric mucosa) at 2.0 and 2.5 mg/kg/day, and inflamed mesenteric lymph nodes at doses of 0.4 mg/kg/day and higher. Decreased body weight and decreased extragestational weight occurred at 1.6 mg/kg/day and decreased hematocrit at 0.8 mg/kg/day. In the teratology study maternal toxicity was evidenced at 0.5 mg/kg/day by decreased body weight and decreased extragestational weight; at 1.0 mg/kg/day by decreased hematocrit; and at 2.0 mg/kg/day by depressed weight of the gravid uteri. Developmental effects included decreased fetal weight (in females) and hydroureter at 0.5 mg/kg/day; decreased weight of males at 1.0 mg/kg/day; and increased incidences of supernumerary ribs, misaligned sternebrae, and reduced ossification of sternebrae at 2.0 mg/kg/day. The investigators reported that the NOAEL for fetal toxicity was <0.5 mg/kg/day.

Hackett et al. (1987) also administered sulfur mustard by intragastric intubation to pregnant New Zealand White rabbits on gestation days 6-19. The test animals were dosed with 0, 0.5, 1.0, 2.0, and 2.5 mg/kg/day in a range-finding study (7-8 animals per dose group) and with 0, 0.4, 0.6, or 0.8 mg/kg/day in a teratology study (18-19 per dose group). Maternal effects included gastric lesions at 1.0 mg/kg/day in the range-finding study and at 0.4 mg/kg/day in the teratology study; enlarged Peyer's patches at 0.5 and 0.4 mg/kg/day; and depressed body weight at 2.0 and 0.8 mg/kg/day. The only reported fetal effect was depressed fetal body weight at 2.0 mg/kg/day. The investigators reported that the NOAEL for fetal toxicity was therefore >0.8 mg/kg/day.

Vesicants such as sulfur mustard are acutely toxic by direct contact; consequently, much of the available human toxicity data pertain to skin, eyes and respiratory tract exposures. Edema, ulceration, and necrosis of human skin and respiratory tract epithelium can occur, as well as conjunctivitis and blindness. General symptoms of systemic toxicity include nausea, vomiting, fever, and malaise (ITII, 1975). Delayed effects which may occur following acute exposures include: eye lesions, chronic bronchitis, and cancers of the respiratory tract and skin. Acute exposures to sulfur mustard may also result in gastrointestinal irritation, leukopenia, and anemia (Vogt et al., 1984).

Acute exposures to sulfur mustard can also result in long-term respiratory damage manifested as asthma-like conditions, emphysematous bronchitis, and increases in incidence of secondary respiratory infections (bronchopneumonia and tuberculosis) (see review by Watson and Griffin, 1992). Beebe (1960) evaluated the occurrence of respiratory tract disease among a group of World War I soldiers. Soldiers who had been exposed to mustard gas exhibited greater mortality from tuberculosis and pneumonia than either of two reference groups. Manning et al., (1981) reported a significantly increased incidence of mortality from pneumonia among 428 former workers of a sulfur mustard manufacturing facility. The ratio of observed to expected cases was 2 ($p \leq 0.05$). Some individuals exposed to HD concentrations that are damaging to the eyes are susceptible to relapsing keratitis (Watson and Griffin, 1992). The condition may reappear 8 to 40 years after recovery from the initial exposure (Dahl et al., 1985).

Limited information on the toxicity of HD following long-term exposures comes from studies of workers at chemical agent manufacturing plants. Morganstern et al. (1947) reported that many workers in a munitions plant handling HD developed severe bronchitis which, in some cases, developed into bronchiectasis. Wada et al. (1962a,b) reported that a large proportion of workers at a Japanese plant manufacturing a number of chemical agents including HD exhibited productive cough, irregular fever, chronic bronchitis, emphysematous changes, and pleural adhesions. Exposure levels were estimated to be 50-70 mg/m^3 (Inada et al., 1978) and there is evidence that the workers were exposed to concentrations higher enough to cause acute effects (Tanaka, 1988).

CONFIDENCE IN THE ORAL (RfD)

Study -- High
Data Base -- Medium
RfD --Medium

The data base for HD consists of one subchronic study in rats, developmental studies in rats and rabbits, and a reproductive study in rats. In addition, there are considerable data available on the toxicity of HD by the inhalation pathway. Taken together these studies support the conclusion that the primary target organs in sulfur mustard exposures are the points of contact with the body, i.e., skin, eyes, and respiratory and gastrointestinal. tracts. Therefore, the endpoint observed in the principal study, gastric lesions, is a reasonable effect following chronic oral exposures. However, the principal study did not identify a NOAEL for this effect, and the route of administration (gavage) may have led to an enhanced response due to the bolus type of dosing. Consequently, the overall confidence in the RfD must be considered medium.

EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document --

Other EPA Documentation --

Agency Work Group Review --

Verification Date --

EPA CONTACTS (ORAL RfD)

Harlal Choudhury / NCEA-Cin -- (513)569-7536

U.S. ARMY CONTACTS

3.2. INHALATION EXPOSURE

Available data are insufficient to support development of a chronic inhalation exposure (RfC) estimate for agent HD.

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- -Sulfur Mustard (bis(2-chloroethyl)-sulfide; Agent HD)
CASRN -- 505-60-2
Last Revised -- No data

3.3. DERMAL EXPOSURE

Available data are insufficient to support development of a chronic dermal exposure (RfD_d) estimate for agent HD.

REFERENCE CONCENTRATION FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- -Sulfur Mustard (bis(2-chloroethyl)-sulfide; Agent HD)
CASRN -- 505-60-2
Last Revised -- No data

4. DOSE-RESPONSE FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name -- Sulfur mustard [bis(2-chloroethyl)-sulfide, Agent HD]
CASRN -- 505-60-2
Date prepared -- September 1996

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

HD is considered to be a known human carcinogen based on occupational exposure studies indicating an elevated risk of respiratory tract tumors in workers at a chemical agent manufacturing plant; an animal study identifying skin tumors in rats exposed to sulfur mustard vapors for up to one year; positive results in mutagenicity and genotoxicity studies, and on biochemical studies showing that HD is a potent DNA alkylating agent.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- HD is considered to be a known human carcinogen following inhalation exposures. It should also be treated as if it were carcinogenic by any other route of exposure.

Basis -- Several studies on workers occupationally exposed to HD indicate an elevated risk of respiratory tract and skin tumors after long-term exposure. In addition, animal studies, mutagenicity studies, genotoxicity data, and the fact that HD is a potent DNA alkylating agent all provide supporting evidence for the compound's carcinogenicity.

NOTE: The International Agency for Research on Cancer (IARC) has classified "mustard gas" as a Group 1 carcinogen (IARC, 1987). The National Toxicological Program (NTP) includes "mustard gas" in the category of "Substances or groups of substances, occupational exposures associated with a technological process, and medical treatments that are known to be carcinogenic" (NTP Annual Report on Carcinogens, 1994). The State of Maryland also considers "mustard gas" as a "known human carcinogen" (a Class I.A Toxic Air Pollutant as defined by the Code of Maryland Regulations, CMR Title 26 Subtitle 11, as amended).

HUMAN CARCINOGENICITY DATA

The extensive library on the carcinogenic responses to HD in humans has been summarized in section 1.1.

ANIMAL CARCINOGENICITY DATA

Information reporting on the carcinogenicity of HD in animals has been summarized in section 1.3.1.

SUPPORTING DATA FOR CARCINOGENICITY

Studies detailing the mutagenic and genotoxic effects of HD in *in vivo* and *in vitro* systems are summarized in section 1.5.1.

QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

SUMMARY OF RISK ESTIMATES

Oral Slope factor -- 0.073-0.095 per (μg/kg)/day
Drinking Water Unit Risk -- 0.002-0.0033 per (μg/L)
Estimation Method -- Relative potency method

Risk Level	Concentration
E-4 (1 in 10,000)	0.03-0.05 μg/L
E-5 (1 in 100,000)	0.003-0.005 μg/L
E-6 (1 in 1,000,000)	0.0003-0.0005 μg/L

DOSE-RESPONSE DATA

Long-term carcinogenicity studies have not been conducted on HD; consequently, the standard EPA approach for estimating an oral slope factor cannot be applied. However, U.S. EPA (1991) evaluated the carcinogenicity of HD using a relative potency method, as follows: From the results of the study conducted by Heston (1950) in which A strain mice were injected intravenously with HD, and the results of a study by Shimkin and McClelland (1949) in which A strain mice were injected intravenously with 20-methylcholanthrene (MC), U.S. EPA (1991) determined that the potency of these two compounds to induce pulmonary tumors in this strain of mice was about the same. The results of a study conducted by Stoner et al. (1984), in which A strain mice were dosed with MC and benzo(a)pyrene (BaP) by gavage and by intraperitoneal injection were then used to determine that MC was 10 to 13 times more potent than B(a)P in inducing lung tumors. Because the potency of HD was considered to be the same as that of MC, it was concluded that the cancer potency for HD would be 10-13 times that of B(a)P. Accordingly, since the oral slope factor of B(a)P is currently taken to be 7.3 per mg/kg-day (U.S. EPA, 1996), the oral slope factor for HD can be estimated to be 73-95.5 per mg/kg-day or 0.073-0.0955 per μg/kg-day. The drinking water unit risk, and concentrations at specific risk levels are based on a human body weight of 70 kg and a drinking water intake of 2 L/day.

ADDITIONAL COMMENTS

Watson et al. (1989) have estimated the carcinogenic potency of HD by the rapid screening of hazard (RASH) method developed by Jones et al. (1988). This approach compares exposures that produce documented toxic effects of a chemical of interest to exposures of a reference chemical producing a similar effect. The RASH procedure was applied to Heston's intravenous (1950) and subcutaneous injection studies (1953). Comparing the carcinogenicity of HD and B(a)P), Watson et al. (1989) showed that the two compounds are of approximate equivalent carcinogenic potency in experimental animals, with a best estimate relative potency of 1.3 for HD relative to B(a)P and an interquartile range (middle 50% of distribution) of 0.6-2.9. Multiplying the relative potency of 1.3 by the currently accepted oral slope factor of 7.3 per (mg/kg)/day for B(a)P results in an oral slope factor of 9.5 per (mg/kg)/day [0.0095 per (μg/kg)/day] for HD. This estimated slope factor is approximately one order of magnitude less than that arising from the relative potency method (U.S. EPA, 1991)

DISCUSSION OF CONFIDENCE

Although human and animal data are inadequate, there is indirect evidence suggesting that HD may be carcinogenic by the oral exposure route. The mechanism of action of HD as a direct DNA alkylating agent, its known genotoxicity in exposed humans and in various animal bioassays, its induction of skin tumors in animals, and its induction of forestomach hyperplasia in rats following subchronic gavage dosing, all support the hypothesis that this compound may function as a point-of-contact carcinogen on epithelial tissues. Furthermore, the mechanism of action of HD would be expected to be similar to that of other known or suspected mustard carcinogens such as nitrogen mustard (sulfur mustard tumorigenicity was determined to be comparable to that of the nitrogen mustard agents HN2 and HN2-HCI and the therapeutic nitrogen mustard compounds melphalan and BCME; (see Institute of Medicine, 1993, Appendix I), as well as bis (chloro) ethyl ether (BCEE).

In the absence of human or animal dose-response data, the relative potency approaches developed by Watson et al. (1989) and U.S. EPA (1991) are considered to be appropriate methods for estimating the tumorigenic potency of HD for the oral route of exposure. Although there are dose-response data from an animal inhalation exposure study (McNamara et al., 1975), route-to-route extrapolation (from inhalation to oral) is not considered appropriate because the exposure protocol of McNamara et al. (1975) resulted in rat skin tumors which might have occurred, not a result of systemic uptake, but as a result of dermal contact with sulfur mustard vapor (perhaps trapped by the rat pelt). Therefore, there is no good method for estimating the dermal dose of sulfur mustard, or for converting this to an oral dose.

QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FORM INHALATION EXPOSURE

SUMMARY OF RISK ESTIMATES

Inhalation Unit Risk -- 8.5E-2 per ($\mu g/m^3$)
Estimation method -- Weibull time-to-tumor method
Air Concentrations at Specified Risk Levels:

Risk Level	Concentration
E-4 (1 in 10,000)	0.001 $\mu g/m^3$
E-5 (1 in 100,000)	0.0001 $\mu g/m^3$
E-6 (1 in 1,000,000)	0.00001 $\mu g/m3$

EPA (1991) subjected the data shown above from the McNamara et al. (1975) toxicity study to a time-to-tumor analysis using the WEIBULL 82 computer program. Empirically, the latency time estimated from the program was about 2 months indicating that the tumors appearing at the end of the experiment were induced when the exposure was still occurring; therefore, the nominal concentrations of 0.001 and 0.029 mg/m^3 were used in the analysis (U.S. EPA, 1991). An upper bound on the unit risk was determined by calculating the linearized upper bound on estimated risk at a single unit of dose and at an appropriate age. At 18 months, the upper bound risk from continuous exposure at 1 $\mu g/m^3$ was 2.0×10^{-2} when estimated from male and female data combined. The lifetime upper-bound unit risk was estimated to be 8.5×10^{-2} per $\mu g/m^3$. For calculations of the air concentrations for specified risk levels, the human respiratory rate was assumed to be 20 m^3/day.

ADDITIONAL COMMENTS

EPA (1991) also analyzed the tumor incidence data derived from the McNamara et al. (1975) toxicity study using the GLOBAL 86 computer program for estimating a multistage model dose-response curve. Because the rats were observed for 6 months after the exposures ended, the daily average exposure was estimated to be equal to 2/3 of the nominal concentration for 52 weeks; i.e., 0.00067 mg/m^3 for the high-exposure group and 0.000193 mg/m^3 for the low-exposure group. In this analysis only those animals tested and observed long enough to exceed the demonstrated minimum tumor latency period (70 days post-exposure) were included. The resulting incidence rate was 4/11 in males and 5/18 in females exposed to the high concentration, 0/29 in males and females exposed to the low concentration, and 0/19 in the control group. The linearized upper bound on the low-dose slope of the estimated curve (ql^*) was 2.9×10^{-2} per $\mu g/m^3$. This value was then adjusted for a lifetime exposure of 24 months by multiplying by the ratio of the lifetime to experimental duration to the third power [i.e., $(24\ mo/18\ mo)^3$]. This adjustment resulted in an estimated inhalation unit risk of 6.8×10^{-2} per $\mu g/m^3$.

Incidences of Skin Tumors in Rats Exposed to Sulfur Mustard

Exposure Duration (months)	Post-Exposure (days)	Control M	Control F	0.001 (mg/m^3) M	0.001 (mg/m^3) F	0.1/0.0025 (mg/m^3) M	0.1/0.0025 (mg/m^3 F
2			0/5	0/5	0/5	0/5	
3		0/5	0/5	0/5	0/5	0/5	0/5
4					0/5	0/5	
6		0/5	0/5	0/5	0/5		
8		0/5	0/5	0/5	0/5	0/5	0/5
12		0/5	0/5	0/5	0/5[a]	0/5	0/5
12	70					4/4[4b]	
12	90	0/4	0/4[c]	0/4	0/5	0/1	0/5
12	180	0/7	0/4[a,d]	0/6	0/14[e]	0/6	5/13[4b,f,g]

SOURCE: McNamara et al., 1975; adapted by U.S. EPA, 1991

[1] Superscripts indicate the number and types of tumors:
- a. subcutaneous fibroma
- b. skin. squamous cell carcinoma
- c. squamous cell carcinoma of uterus
- d. pulmonary adenoma
- e. papilloma of the skin
- f. basal cell carcinoma of the skin
- g. thyroid adenoma.

[2] Only types b and f were considered by the authors to be related to the HD exposure and only these types are counted in the numerators of this table.

[3] Exposures were 0.001 mg/m^3 continuously or 0.1 mg HD/m^3 for 6.5 hr followed by 0.0025 mg HD/m^3 for 17.5 hr per day (0.0029 mg/m^3 average), 5 days/week.

U.S. EPA (1991) analyzed the data generated in the McNamara et al. (1975) carcinogenicity study using the GLOBAL 86 program. The data were grouped into 17 lifetime incidences (two dose levels times eight exposure durations plus a control group), and the exposures were converted to lifetime average concentrations, assuming a lifespan of 2 years. The lifetime average concentrations ranged from 0.0096 $\mu g/m^3$ to 14.5 $\mu g/m^3$. Skin tumors occurred only in animals exposed to lifetime average HD concentrations of 3.35 $\mu g/m^3$ or higher (i.e., 4/5 at 3.35, 4/5 at 7.25, 4/4 at 10.9, and 10/23 at 14.5 $\mu g/m^3$). It was assumed that the cancer incidence would be linearly related to the product of HD concentration and exposure duration even at very low concentrations, and that the dose-response relationship in humans would be the same as that in animals when doses are expressed as lifetime average air concentrations. The linearized upper bound on the low-dose slope ($q1^*$) estimated from these data by GLOBAL 86 was 9.4×10^{-2} per $\mu g/m^3$.

U.S. EPA (1991) also estimated the inhalation unit risk for HD from the relative potency data, and, by analogy with the toxicity of 20-methylcholanthrene, determined that the potency of HD would be 10-13 times the inhalation cancer unit risk for BaP. The latter was derived from the oral slope factor of 11.55 per mg/kg-day (a value recognized at the time of EPA's study) using the standard defaults of 20 m^3/day for ventilation rate and 70 kg for body weight. The resulting inhalation unit risk for HD was 0.033-0.043 per $\mu g/m^3$.

Considering all the above data, the U.S. EPA (1991) chose the unit risk of 8.5×10^{-2} per $\mu g/m^3$, derived from the Weibull time-to-tumor model, as the most appropriate estimate of the carcinogenic potency of HD. The exposures used in this model were long-term, the effect of killing the test animals before a full lifetime was adjusted for, and the sample size was the largest obtainable from the McNamara et al. (1975) study.

DISCUSSION OF CONFIDENCE

EPA (1991) notes that the dose-response estimates derived from the McNamara et al. (1975) study are highly uncertain due to the fact that the study was not of a standard design and too few animals were exposed and followed for a lifetime to give adequate sensitivity for detecting long-term effects. In addition, the uncertainty concerning the experimental conditions was too great to allow confidence about the absolute potency value.

Since, in the McNamara et al. (1975) study, malignant tumors appeared only at the highest mustard concentration and only late in life, EPA (1991) observed that "perhaps it may exert its carcinogenic activity secondarily through lifelong exposure to its cytotoxic or irritating effects. Under such circumstances, human exposures at low concentrations for limited times may entail much less risk than implied by the unit risk factor estimated for lifetime effects at higher doses. On the other hand, the lack of low-dose responses and early-appearing tumors in the McNamara data may be due simply to the inherent difficulty of detecting low-risk levels in experiments of reasonable size." Because HD is known to be a strong and direct DNA alkylating agent, the likelihood is very high that it functions as a non-threshold carcinogen. Consequently,

the risks associated with exposures to low concentrations require evaluation, and the McNamara et al. (1975) study provides the only inhalation data set which allows for some quantification of carcinogenic potency.

DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

DOCUMENTATION (CARCINOGENICITY ASSESSMENT)

U.S. EPA. (1991) Upper-bound quantitative cancer risk estimate for populations adjacent to sulfur mustard incineration facilities. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC. EPA/600/8-91/053.

REVIEW (CARCINOGENICITY ASSESSMENT)

CONTACTS (CARCINOGENICITY ASSESSMENT)

5. REFERENCES

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Attachment 1

TOXICITY ASSESSMENT FOR THIODIGLYCOL (TDG)

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PREFACE

This report assesses the potential non-cancer and cancer effects of TDG (thiodiglycol; 2,2'-thiodiethanol, CAS Number 111-48-8). Information pertaining to non-cancer and cancer effects was previously assessed by the United States Environmental Protection Agency (U.S. EPA) in a draft Drinking Water Toxicological Profile (1984) for this compound.

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This document supports the activities of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. Toxicity values will be submitted for consideration by the EPA's IRIS Consensus Process for inclusion on IRIS (EPA's Integrated Risk Information System).

PREAMBLE NOTICE

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ABSTRACT

Data pertaining to the potential cancer/non-cancer effects of TDG (thiodiglycol: 2,2'-thiodiethanol) are reviewed. Acute oral, subcutaneous and intravenous LD_{50}s are reported for rats, guinea pigs, mice and rabbits. In an extensive database search, no subchronic or chronic studies were found for any route of exposure. In occupational settings, TDG is considered to be a skin and eye irritant, though no epidemiological studies or occupational exposure data were identified. Insufficient data exist to derive oral or dermal RfDs, an inhalation RfC, or a carcinogenic slope factor for TDG.

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1. SUMMARY OF TOXICITY INFORMATION

1.1. EPIDEMIOLOGICAL STUDIES

Pertinent epidemiological studies are currently unavailable.

1.2. SHORT-TERM STUDIES

1.2.1. Human Toxicity. Information on the toxicity of TDG in humans was unavailable except for a report by Von Bramer and Davis (1981) that TDG exhibited moderate oral toxicity compared to isopropanol. However, specific data were not reported.

1.2.2. Animal Toxicity.

1.2.2.1. Acute Toxicity —

1.2.2.1.1. Oral Toxicity. Oral LD_{50}s of 6610 and 3960 mg/kg have been reported for rats and guinea pigs, respectively (Smyth et al., 1941). Smyth et al. (1941) noted that the toxic effects of TDG were similar to those of other glycols; for example, fatal or near-fatal doses produced "varying degrees of sluggish depressed functioning" but no narcosis; some digestive tract irritation, damage to the kidneys, and to a lesser degree, damage to the liver.

1.2.2.1.2. Non-Oral Toxicity. Information is unavailable on the toxicity of TDG via inhalation. A subcutaneous LD_{50} of 4-5 cc/kg (4742-5926 mg/kg) was reported for mice and one of 4 cc./kg (4742 mg/kg) was reported for rats (Anslow et al., 1948). Intravenous LD_{50}s of 2, 4, and 3 cc/kg (2370, 4742, and 3556 mg/kg) were reported for mice, rats, and rabbits, respectively (Anslow et al., 1948). TDG was mildly irritating when applied to the skin of rabbits at a dose level of 500 mg (Union Carbide, 1971), and moderately irritating to the eye of rabbits when applied at a dose level of 500 mg (Carpenter and Smyth, 1946). The 500 mg exposure to rabbit

eyes caused pain and conjunctival hyperemia without corneal damage (Carpenter and Smyth 1946; Hughes 1948).

1.2.2.2. Subacute Toxicity — Information on the subacute toxicity of TDG is currently unavailable.

1.2.2.3. Subchronic Toxicity — Information on the subchronic toxicity of TDG is currently unavailable.

1.3. LONG-TERM STUDIES

1.3.1. Carcinogenicity. Pertinent studies on the carcinogenic activity of TDG are currently unavailable.

1.3.2. Chronic Toxicity. Information on chronic toxicity of TDG is currently unavailable.

1.4. REPRODUCTIVE STUDIES

Information on the reproductive effects of TDG is currently unavailable.

1.5. DEVELOPMENTAL EFFECTS

Information on the developmental effects of TDG is currently unavailable.

1.5.1 Mutagenicity. Information on any mutagenic effects of TDG is currently unavailable.

1.6. TOXICOKINETIC STUDIES

1.6.1. Toxicokinetics. When male Porton strain rats were injected intraperitoneally with isotopically labelled TDG, approximately 90% of the administered dose was excreted in the urine within 24 hours. The primary metabolite was thiodiglycol sulfoxide (≥90% excreted activity). Minor metabolites included thiodiglycol sulfone, S-(2-hydroxy-ethylthio)acetic acid, and S-(2-hydroxyethylsulfinyl) acetic acid, with approximately 0.5-1% of the original dose excreted as free TDG (Black et al., 1993).

A number of studies have identified TDG as a metabolite of the chemical warfare agent sulfur mustard (bis(2-chloroethyl)sulfide; $C_4H_8Cl_2S$; CAS No. 505-60-2) in agent-exposed humans and laboratory rodents. Thus, following intraperitoneal administration of sulfur mustard agent to male Porton strain rats, approximately 60% of the initial dose was excreted in the urine within 24 hours. Primary metabolites were thiodiglycolsulfoxide, 1,1'-sulfonylbis[2-S(N-acetylcysteinyl)ethane], 1,1'-sulfonylbis [2-methylsulfinyl ethane], and/or 1-methylsulfinyl-2-[2-(methylthioethyl sulfonyl] ethane, with TDG as a minor metabolite. Similar results were also obtained in an earlier study by Black and Read (1988).

That TDG is a minor metabolite of sulfur mustard metabolism is also documented in earlier studies featuring male Wistar rats, where the compound was excreted in both free and conjugated form within 12-24 hours of intravenous or intraperitoneal administration of sulfur mustard (Davidson et al. 1961; Robert and Warwick. 1963).

In the presence of aqueous sulfur mustard or TDG solutions, the hydrolysis of p-nitrophenol phosphate (p-NPP) by mouse liver cytosol was inhibited by TDG concentrations of 30-300μM (Brimfield 1995). The author considered the effect to be an indication of serine/threonine phosphatase inhibition, and that any inhibition of p-NPP hydrolysis by sulfur mustard was likely to be a consequence of exposure to TDG rather than to sulfur mustard itself.

TDG has also been found as a urinary metabolite in humans either accidentally exposed to sulfur mustard (Black and Read, 1995a,b) or during wartime (Wils et al., 1985, 1988; Vyvudilik, 1985). In these cases also, TDG was a minor metabolite compared to the concentrations of thiodiglycolsulfoxide excreted (Black and Read, 1995a,b).

TDG is considered to be a more appropriate biomarker of sulfur mustard exposure because of the background levels of the sulfoxide in the urine of unexposed subjects (Black and Read 1995a,b). However, TDG from unexplained sources has been found (at up to 10 ng/ml) in blood of some subjects unexposed to sulfur agent (Black and Read, 1988).

1.6.2. Metabolism. Based on the appearance of TDG in the urine of rats exposed to sulfur mustard, Black et al. (1992) have proposed that potential metabolic mechanisms may include either, hydrolysis, and/or conjugation with glutathione (majority), followed by metabolism of glutathione conjugates to N-acetylcysteine conjugates, or to methylthio/methyl sufinyl derivatives under the action of β-lyase.

1.6.3. Percutaneous Absorption. Information on the percutaneous absorption of TDG is currently unavailable, although the compound is considered to be an irritant to human skin and mucous membranes.

1.7. STRUCTURE-ACTIVITY RELATIONSHIPS

Available data on TDG are insufficient to evaluate the structure-activity relationships of this compound.

1.8. MECHANISTIC STUDIES

At present there are no studies of the mechanism by which TDG causes toxicity.

2. INTERPRETATION OF AVAILABLE INFORMATION

Thiodiglycol (TDG; 2-2'thiodiethanol, thiodiethylene glycol, bis (β-hydroxy ethyl) sulfide, Kromfax solvent, etc., CAS No. 111-48-8: $C_4H_8SO_2$) is prepared from ethylene oxide and hydrogen sulfide, is hygroscopic, and possesses anti-oxidant properties (Von Bramer and Davis, 1981; Budaveri, et al., 1989). It is a water-soluble liquid of low vapor pressure, and has industrial uses not only as a solvent for vat, acid, and basic dye stuffs, (Von Bramer and Davis, 1981), but as a co-stabilizer in the production of polyvinyl chloride (Nass, 1981). As a hydrolysis product of the vesicant chemical warfare agent sulfur mustard as well as a precursor in its production (Small, 1984; Ember, 1996), TDG is listed as a "Schedule 2" compound ("compounds with low to moderate commercial use") within the terms of the Chemical Weapons Convention Treaty. It is thus subject to the reporting and inspection requirements of the convention (Ember 1993).

TDG is considered to be an irritant of the eye, skin, and mucous membranes in occupational settings, although there are no specific inhalation or dermal exposure criteria established by OSHA, ACGIH, or NIOSH. There are no human toxicity data and only sparse animal toxicity data, primarily consisting of single dose acute LD_{50}s for laboratory rodents and rabbits via oral, subcutaneous, or intravenous exposure. Some single dose data (for irritation, pain and temporary tissue injury) are also available for the skin and eyes or rabbits.

No data addressing chronic or subchronic toxicity, carcinogenicity, reproductive or developmental toxicity, mutagenicity, structure-activity relationships, or mechanisms of toxicity were found in the biomedical or pharmacotoxicological literature.

Available data are insufficient to support the development of RfD, RfC, or slope factor for this compound.

3. DOSE-RESPONSE ASSESSMENT FOR NON-CANCER EFFECTS

3.1 INGESTION EXPOSURE

Available data are insufficient to support development of a chronic oral RfD estimate for TDG In addition, there are no ambient water quality criteria or drinking water standards for TDG.

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Thiodiglycol
CASRN -- 111-48-8
Last Revised -- No Data

3.2. INHALATION EXPOSURE

Available data are insufficient to support development of a chronic RfC estimate for TDG. In addition, there are no OSHA, ACGIH, or NIOSH inhalation standards and criteria for TDG.

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name -- Thiodiglycol
CASRN -- 111-48-8
Last Revised -- No data

3.3. DERMAL EXPOSURE

Available data are insufficient to support development of a chronic dermal exposure (RfD_d) for TDG.

REFERENCE DOSE FOR CHRONIC DERMAL EXPOSURE (RfD_d)

Substance Name -- Thiodiglycol
CASRN -- 111-48-8
Last Revised -- No data

4. DOSE-RESPONSE ASSESSMENT FOR CARCINOGENICITY

CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Data are insufficient to assess the carcinogenicity of TDG in humans or animals for oral or inhalation exposures.

EVIDENCE FOR CLASSIFICATION AS TO HUMAN CARCINOGENICITY

TDG cannot be classified as to potential human carcinogenicity because of the lack of adequate data.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Not classifiable

Basis -- Lack of adequate human or animal data

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Attachment 1

SERDP/ACC Draft
October 1997

CANCER ASSESSMENT OF

TNT (2,4,6-TRINITROTOLUENE)

Material/Chemical Risk Assessment Working Group
Environmental Risk Assessment Program

United States Department of Defense
United States Department of Energy
United States Environmental Protection Agency

NOTICE

This document is a preliminary draft. It has not been formally released by the Department of Defense, Department of Energy, or Environmental Protection Agency and should not at this stage be construed to represent government policy.

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PREFACE

This report updates the cancer assessment of TNT (2,4,6-trinitrotoluene, Chemical Abstracts Service registry number 118-96-7). The noncancer reference dose (RfD) is not discussed in this report, as no new studies on noncancer toxicity have become available since the RfD was developed in 1988. Cancer information was assessed by the United States Environmental Protection Agency (U.S. EPA) in a 1989 health advisory and by the Agency for Toxic Substances and Disease Registry (ATSDR) in a 1995 toxicological profile. EPA had classified TNT as a possible human carcinogen, group C under its 1986 cancer guidelines. These earlier assessments should be considered along with this update; to not duplicate these efforts, detailed information summarized in the earlier assessments is not repeated here.

Although there are no new cancer studies since EPA's 1989 cancer assessment, this report updates the 1989 assessment in two ways. First, the 1989 health advisory discusses oral exposure only, providing no slope factor or unit risk estimate for inhalation or dermal exposure. This update uses information on absorption by different routes of environmental exposure to recommend slope factors for inhalation and dermal exposure, as well as oral exposure. Second, revisions to EPA's cancer guidelines were proposed in 1996. This update uses the new proposed guidelines in developing a narrative statement to describe the weight of evidence, in using the two-step approach to dose-response assessment, in using the new cross-species scaling factor, and in considering mode of action in the choice of an approach to low-dose extrapolation.

This assessment is a product of the Material/Chemical Risk Assessment Working Group of the Environmental Risk Assessment Program, a cooperative endeavor of the Department of Defense, Department of Energy, and Environmental Protection Agency. This working group is developing toxicity values for selected chemicals of concern at federal facilities. These toxicity values will be considered by EPA for inclusion on IRIS (EPA's Integrated Risk Information System).

The Working Group gratefully acknowledges the helpful and insightful comments from several scientists in EPA's National Center for Environmental Assessment: Robert Beliles, Harlal Choudhury, David Reese, John Schaum, Dharm Singh, and Lawrence Valcovic.

External peer review of this report was provided in July 1996 by Drs. Irwin Baumel, Michael Dourson, William Hartley, and Life Systems, Inc. The final report addresses their comments and recommendations.

Additional comments were received in February 1997 from several IRIS consensus reviewers: David Bennett, Ann-Marie Burke, Dorothy Canter, Gerald Carney, Vicki Dellarco, John Helvig, Richard Hill, Edward Ohanian, Marian Olsen, and Joseph Reinert.

PREAMBLE NOTICE

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ABSTRACT

Information pertaining to the potential carcinogenicity of TNT (2,4,6-trinitrotoluene) is reviewed. Female rats fed TNT developed rare urinary bladder carcinomas and papillomas; similar results were not observed in male rats or in male or female mice. Mutagenic activity has been found in the urine of exposed workers, and positive results *in vitro* without metabolic activation suggest that TNT is direct acting. Pharmacokinetic studies show that TNT is absorbed by ingestion, inhalation, and dermal exposure. As TNT causes cancer and other toxic effects internally following absorption into the circulation, it is concluded that TNT is likely to cause cancer by any route of environmental exposure.

For human ingestion exposure, an ED10 (estimated dose associated with a 10% increased cancer incidence) of 6.8 mg/kg-day is derived; its lower bound, 4.7 mg/kg-day. Using a linear model to extrapolate to lower doses, a central estimate of the slope of the dose-response curve at low doses is 1.5×10^{-2} per mg/kg-day average lifetime ingestion exposure to TNT; its upper bound, 2.1×10^{-2} per mg/kg-day.

Pharmacokinetic studies show that absorption by inhalation is comparable to absorption by ingestion. In the absence of inhalation cancer studies, it is recommended that the low-dose slope for ingestion be used for inhalation exposure.

Similarly, pharmacokinetic studies show that absorption through skin is comparable to, albeit considerably lower than, absorption by ingestion. In the absence of dermal cancer studies, it is recommended that dermal exposures be reduced by a factor of 2 before applying the ingestion slope factor.

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1. SUMMARY OF CANCER INFORMATION

TNT (2,4,6-trinitrotoluene, Chemical Abstracts Service registry number 118-96-7) is a yellow-to-white, odorless solid used as an explosive in military and industrial applications. It is manufactured only at military arsenals. Given the restricted production and use of TNT, it is likely to be a chemical of concern primarily at military facilities. The primary exposure routes for the general public are ingestion of contaminated drinking water, dermal contact with contaminated surface water, and inhalation of air contaminated through incineration or detonation of TNT at military facilities. Children can be exposed by eating contaminated soil.

1.1. EPIDEMIOLOGIC STUDIES

There are no epidemiologic studies investigating an association between TNT exposure and cancer. ATSDR (1995) reports on a preliminary study finding increased leukemia in adults living in proximity to former munitions plants in Germany. The study, however, is limited by few cases, no reporting of the proximity of the cases to TNT manufacturing or disposal sites, no reporting of environmental concentrations, and no investigation of confounding exposures.

Occupational studies showing mutagenic activity in the urine of workers exposed to TNT are discussed in Section 1.3. Occupational studies showing a potential for TNT to be absorbed are discussed in Section 1.4.

1.2. LABORATORY ANIMAL EXPERIMENTS

Carcinogenicity experiments have been conducted in rats and mice for TNT administered through the diet. There are no experiments of TNT administered by inhalation or dermal exposure.

Furedi et al. (1984a). Fischer 344 rats were fed TNT in the diet for 24 months at doses of 0, 0.4, 2, 10, and 50 mg/kg-day. These doses did not alter survival rates. Reduced food consumption was observed at 10 and 50 mg/kg-day; correspondingly, body weight was decreased by 5 and 22% in males and by 8 and 20% in females. Other than ocular discharge at 50 mg/kg-day, no other clinical signs of toxicity were apparent.

Anemia with secondary splenic lesions was found in both sexes at 10 and 50 mg/kg-day. Further, Howell-Jolley and Heinz bodies and methemoglobinemia were observed, suggesting the oxidizing nature of TNT or its metabolites. Also at 10 and 50 mg/kg-day, renal damage was observed in both sexes and liver hyperplasia was observed in males.

At 10 and 50 mg/kg-day, hepatocellular hyperplasia was observed in male rats (see Table 1-1), and renal and urinary bladder hyperplasia was observed in female rats (see Table 1-2). Urinary bladder carcinomas and papillomas, considered rare, were observed in the mucosal epithelium in female rats. Of these, one papilloma and one carcinoma were observed in the seven high-dose animals dying before the end of the experiment; hyperplasia was not reported for animals dying early.

TABLE 1-1

Liver and Urinary Tract Findings in Male Fischer 344 Rats Fed TNT

Administered dose (mg/kg-day)	0	0.4	2	10	50
Liver hepatocellular hyperplasia	9/54	10/54	7/54	23/54	34/55
Kidney increased pigmentation	22/54	20/54	15/54	47/54	50/55
Kidney lymphocytic inflammation	45/54	46/54	66/54	50/54	55/55

Source: Furedi et al., 1984a

TABLE 1-2 Urinary Tract Findings in Female Fischer 344 Rats Fed TNT					
Administered dose (mg/kg-day)	0	0.4	2	10	50
Kidney increased pigmentation Kidney lymphocytic inflammation Kidney mineralization, hyperplasia	35/54 14/37 0/37	41/54 16/40 0/40	53/55 16/40 0/40	51/55 33/46 0/46	55/55 43/47 7/47
Urinary bladder hyperplasia Urinary bladder papillomas Urinary bladder carcinomas	0/37 0/54 0/54	0/40 0/54 0/54	0/40 0/55 0/55	2/44 1/54 0/55	12/35 5/55 12/55
Urinary bladder carcinomas & papillomas	0/54	0/54	0/55	1/55	17/55

Source: Furedi et al., 1984a

The latter trend is statistically significant ($p<0.001$ by the Cochran-Armitage trend test, Snedecor and Cochran, 1967); the trend cannot be distinguished from a linear one ($p>0.10$ by the Cochran-Armitage test for deviations from linearity, Snedecor and Cochran, 1967).

Furedi et al. (1984b). In a parallel study, B6C3F1 mice were fed TNT in the diet for up to 24 months at doses of 0, 1.5, 10, and 70 mg/kg-day. These doses did not alter survival rates. At 70 mg/kg-day, body weight gain was decreased by about 30% in males and 25% in females; increased food consumption, however, was observed at this dose for most of the study. No clinical signs of toxicity were apparent. Anemia and hepatotoxicity were observed at 70 mg/kg-day. An increase in [lymphocytic and granulocytic] leukemia and malignant lymphoma of the spleen was reported in females (see Table 1-3).

TABLE 1-3 Lymphocytic and Granulocytic Leukemia and Malignant Lymphoma of the Spleen in Female B6C3F1 Mice Fed TNT				
Administered dose (mg/kg-day)	0	1.5	10	70
Incidence	9/54	15/54	17/54	21/54

Source: Furedi et al., 1984b

This increase, for the spleen alone, is statistically significant ($p<0.01$ at the highest dose). NTP guidance for combining tumor sites and types (McConnell et al., 1986), however, recommends combining malignant lymphoma and lymphocytic leukemia in all organs. Following NTP guidance, U.S. EPA (1989) (discussed by Ross and Hartley, 1990) retabulated the incidence of malignant lymphoma and lymphocytic leukemia (omitting granulocytic leukemia) in all organs (rather than in the spleen only). By this analysis, the increase is not statistically significant ($p>0.05$ by the Cochran-Armitage trend test). Retabulated incidences to support this later analysis were not, however, reported by U.S. EPA (1989) or by Ross and Hartley (1990), and individual animal results were not reported by Furedi et al. (1984b).

1.3. SHORT-TERM TESTS

Information on genetic toxicity has been reviewed by ATSDR (1995) and U.S. EPA (1989). Ahlborg et al. (1985) found mutagenic activity in urine samples from workers exposed to TNT. Urine samples from 80 males and 17 females were assayed for mutagenic activity using *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation; baseline data for each worker was assayed from samples taken after a 4-week vacation. Mutagenic

activity was found using *S. typhimurium* without metabolic activation and, to a lesser extent, with metabolic activation. These results were not related to smoking or other chemical exposures.

In a followup study, Ahlborg et al. (1988) studied urine samples from 50 workers exposed to TNT using *S. typhimurium* without metabolic activation; baseline data for each worker was assayed from samples taken after a free weekend. Again, mutagenic activity was higher in the exposed samples. The dominating urinary metabolite was 4-aminodinitrotoluene (4-ADNT), but 2-ADNT and much lesser quantities of TNT were also found in the urine. The investigators thought that 6-ADNT could be an equally important metabolite. Mutagenic activity was not correlated with urinary concentration of TNT. As ADNT was not shown to be mutagenic, the investigators suggested that the lack of correlation with urinary TNT could be attributable to the low levels of TNT compared with its practical detection limit, dermal exposure in workers classified by workplace air concentration as not exposed, and toxicokinetic variability between individuals.

The occupational studies are consistent with an *in vitro* study using a human cell line, which produced some evidence of unscheduled DNA synthesis without, but not with, metabolic activation (ATSDR, 1995). Several *in vitro* studies show that TNT is mutagenic in several *S. typhimurium* strains, causing frameshift mutations and base-pair substitution mutations with and without metabolic activation (ATSDR, 1995).

The few *in vivo* studies include negative results for clastogenesis in the mouse micronucleus assay and for unscheduled DNA synthesis in the rat liver assay. These results are consistent with the *in vitro* finding of unscheduled DNA synthesis in human cells without, but not with, metabolic activation.

1.4. PHARMACOKINETIC STUDIES

Animal cancer studies have been by ingestion only. Pharmacokinetic studies provide information about the potential for absorption and risk of cancer by other exposure routes.

1.4.1. Ingestion Exposure. Studies in four species, as described by ATSDR (1995) and U.S. EPA (1989), show that ingested TNT is absorbed into the systemic circulation.

Lee et al. (1975) administered a single gavage dose of 82 mg/kg radiolabeled TNT suspended in peanut oil to female Charles River CD rats. Twenty-four hours later, 54% of the administered dose had been recovered in the urine; 26%, in the gastrointestinal tract and feces.

In a later study, Hodgson et al. (1977) administered a single gavage dose of 100 mg/kg radiolabeled TNT suspended in peanut oil to male and female Charles River CD or Sprague-Dawley rats. Twenty-four hours later, 53-64% of the administered radioactivity had been recovered in the urine; 36-38%, in the gastrointestinal tract and feces.

In a further study, El-hawari et al. (1981) administered a single oral dose of 50 mg/kg radiolabeled TNT to rats, mice, dogs, and rabbits. Twenty-four hours later, 60, 59, 61, and 74%, respectively, of the administered radioactivity was recovered in the urine of these species; 92, 94, 94, and 104%, respectively, was recovered in the combined urine, feces, and gastrointestinal tract.

In the latter two studies, it was observed that the urine of the rats and mice was pigmented red, although a similar result was not observed in dogs and rabbits. The Army report cited a 1972 report by Hassman that red pigment occurs in humans poisoned by TNT.

1.4.2. Inhalation Exposure. Studies of humans and rats, as described by ATSDR (1995) and U.S. EPA (1989), show a potential for inhaled TNT to be rapidly absorbed into the systemic circulation.

Hassman and Hassmanova (1976) measured TNT's primary metabolite, 4-ADNT, in the urine of 88 factory workers exposed to TNT. There was a good correlation between air TNT concentrations (0.045–0.93 mg/m^3) and urinary 4-ADNT, which increased rapidly during the workday and returned to background levels within 24 hours. This suggests that TNT is rapidly absorbed and eliminated following inhalation exposure.

In Sprague-Dawley rats, El-hawari et al. (1981) administered 50 mg/kg TNT by either intratracheal instillation or orally. Four hours later, urinary excretion was 19% of the intratracheal dose and 15% of the oral dose. In view of the shorter, 4-hour interval used, this study suggests that absorption by inhalation and ingestion are comparable. Further, the high percentage excreted after 4 hours suggests that absorption by inhalation is rapid.

1.4.3. Dermal Exposure. Studies of humans and animals, as described by ATSDR (1995) and U.S. EPA (1989), show a potential for TNT to be absorbed through the skin.

Woollen et al. (1986) measured urinary 4-ADNT and 6-ADNT of 25 exposed workers; the dermal dose was not specified. There were wide variations in the rate of clearance. Excretion was characterized as slow; 8 of 9 workers had detectable ADNT after being away from the workplace for 17 days. Five workers monitored closely during two workshifts demonstrated rapid absorption.

Ahlborg et al. (1988) found that concentrations of TNT and metabolites in the urine of workers exposed to TNT were greater than what would be expected from uptake based on air

concentration. They suggested that dermal absorption is an important route of TNT uptake in humans, and may be more important than uptake through inhalation.

In rats, mice, dogs, and rabbits, El-hawari et al. (1981) compared dermal and oral absorption. Doses were 50 mg/kg in rats and mice; 5 or 50 mg/kg in dogs and rabbits. Twenty-four hours later, 24, 42, 16, and 57% of a 50 mg/kg dermal dose was recovered in rats, mice, dogs, and rabbits, respectively, in the combined urine, feces, gastrointestinal tract, and other tissues; for a 5 mg/kg dermal dose the corresponding percentages were 17 and 68% in dogs and rabbits, respectively. These percentages are lower than those recovered after oral exposure. This suggests that dermal absorption may be considerably less than oral absorption (cited earlier as over 90%), with relative efficiency depending on species.

1.5. STRUCTURE-ACTIVITY RELATIONSHIPS

Two chemicals structurally similar to TNT (2,4,6-trinitrotoluene) are 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT). Both have been assessed for potential carcinogenicity (U.S. EPA, 1988a,b).

Evidence reviewed in the assessment of 2,4-DNT includes feeding studies reporting skin and subcutaneous tissue fibromas in male rats, mammary gland fibroadenomas in female rats, hepatocellular tumors in male and female rats, and renal tumors in male mice; another feeding study found no tumors in male or female mice. Based on these studies, U.S. EPA (1988a) classified 2,4-DNT as probably carcinogenic to humans, group B2 under its guidelines for carcinogen risk assessment (U.S. EPA, 1986).

Evidence reviewed in the assessment of 2,6-DNT includes tests showing an ability to both initiate and promote tumors; 2,6-DNT was the only dinitrotoluene showing initiating

activity. In addition, a feeding study using a mixture of 20% 2,6-DNT, 75% 2,4-DNT, and 4% other dinitrotoluenes induced hepatocellular carcinomas in male and female rats. Based on these studies, U.S. EPA (1988b) classified 2,6-DNT as possibly carcinogenic to humans, group C under its guidelines for carcinogen risk assessment (U.S. EPA, 1986).

Dinitrotoluene mixtures are classified as probably carcinogenic to humans, group B2, by U.S. EPA (1988c).

In view of the difference in tumor sites and in tumor initiating activity with TNT and the structural analogues 2,4-DNT and 2,6-DNT, the pertinence of these results to TNT is uncertain.

1.6. MECHANISTIC INFORMATION

Although there are no mechanistic studies designed to ascertain the mode of action by which TNT may cause cancer, the noncancer toxicity in the experimental studies suggests several modes of action. The observation of renal and urinary bladder hyperplasia in female rats (Furedi et al., 1984a) suggests increased cell replication leading to urinary bladder carcinomas. The needle crystalline form of TNT also suggests bladder irritation. The observation of hematologic and bone marrow effects in rats and mice (Furedi et al., 1984a,b) suggests suppression of the immune system leading to nonsignificant increases in leukemia and lymphoma in female mice. It should be noted that anemia and other hematologic effects are among the major signs of TNT toxicity in humans.

The finding of mutagenic activity in the urine of workers exposed to TNT indicates a role for mutagenesis at low doses. Positive results *in vitro* without metabolic activation suggest that TNT itself is direct acting.

2. CANCER HAZARD ASSESSMENT

2.1. INTEGRATIVE SUMMARY OF CANCER HAZARD INFORMATION

Well-designed and well-conducted experiments have been completed in Fischer 344 rats and B6C3F1 mice for lifetime dietary exposure to TNT. There are no experiments of TNT administered by inhalation or dermal exposure. Female rats had a statistically significantly increased incidence of carcinomas and papillomas of the urinary bladder, considered rare in this strain. Female mice had an increase in lymphocytic and granulocytic leukemia and malignant lymphoma of the spleen. No increase in any tumor type was observed in male rats at doses up to 50 mg/kg-day or in male mice at doses up to 70 mg/kg-day.

Although there are no epidemiologic studies investigating an association between cancer and TNT exposure, mutagenic activity has been found in the urine of workers exposed to TNT. This suggests a role for mutagenesis as a mode of action at low doses. These occupational studies are consistent with *in vitro* studies using a human cell line or several *S. typhimurium* strains, in which TNT caused frameshift mutations and base-pair substitution mutations with and without metabolic activation. *In vivo* study results are negative for clastogenesis in the mouse micronucleus assay and for unscheduled DNA synthesis in the rat liver assay. These results are consistent with the *in vitro* finding of unscheduled DNA synthesis in human cells without, but not with, metabolic activation. This suggests that TNT itself is direct acting.

Occupational studies and experiments in rats, mice, dogs, and rabbits show that TNT is absorbed through ingestion, inhalation, and dermal exposure. TNT then enters the systemic

circulation, after which its metabolites, 4-ADNT, 6-ADNT, and, to a lesser extent, 2-ADNT, can be measured in urine.

Two chemicals structurally similar to TNT, 2,4-DNT and 2,6-DNT, cause cancer in animal studies, albeit at different sites than TNT.

2.2. WEIGHT OF EVIDENCE

TNT is considered likely to cause cancer by all routes of environmental exposure. The main lines of evidence supporting this classification are: (1) TNT induces urinary bladder papillomas and carcinomas, a rare tumor, when fed to female rats; (2) mutagenic activity is present in the urine of workers exposed to TNT; (3) studies in rats, mice, dogs, and rabbits show that TNT is rapidly and efficiently absorbed by ingestion, inhalation, and dermal exposure; and (4) occupational studies indicate that TNT is absorbed by inhalation and, presumably, dermal exposure.

2.3. IMPLICATIONS FOR DOSE-RESPONSE ASSESSMENT

To support the choice of an approach for dose-response assessment, information on mode of action includes: (1) renal and urinary bladder hyperplasia in female rats that suggests a role for increased cell replication at high doses (presumably nonlinear); (2) the needle crystalline form of TNT, suggesting a potential at high doses for bladder irritation and compensatory cell replication (again, presumably nonlinear); (3) observation of leukemia and lymphoma of the spleen in female mice, suggesting suppression of the immune system as a mode of action; and (4) evidence that TNT is a direct-acting mutagen (presumably linear at low doses). The currently available information is not, however, sufficient to establish that cell replication is the only mode of action relevant to human environmental exposure, especially in view of the evidence of

mutagenicity. Hence, the dose-response assessment will be based on linear extrapolation below the observed range.

2.4. ADDITIONAL CHARACTERIZATION

Experiments in rats have shown that multiple agents can act together to cause bladder cancer (Cohen, 1985). Thus, it is important to identify and quantify exposures to other bladder carcinogens in order to fully characterize the overall level of exposure and risk.

We do not know how children would respond. Their immature metabolic system, which would allow TNT to remain longer in the body unchanged, suggests a higher risk, because TNT can act directly without metabolic activation. Additionally, the rapid growth of the bladder early in life suggests a higher risk if humans and rats respond similarly. For example, in the case of saccharin, which appears to cause cancer through cytotoxicity and consequent hyperplasia, exposure during the relatively short neonatal period appears to be of disproportionate importance in producing bladder tumors in rats, because the number of bladder cells grows by more than an order of magnitude between birth and weaning (Ellwein and Cohen, 1988).

3. CANCER DOSE-RESPONSE ASSESSMENT

3.1. SELECTION OF APPROACH TO DOSE-RESPONSE ASSESSMENT

Dose-response assessment begins by considering development of a biologically based model (U.S. EPA, 1996), that is, a model whose mathematical structure reflects the ascertained mode of action and whose parameters are measured in experimental studies. No experiments, however, have measured the rate parameters that would be used in a biologically based model. Consequently, the tumor information available at this time is more suited to empirical modeling.

The only statistically significant tumor response is an increased incidence of urinary bladder carcinomas and papillomas in female Fischer 344 rats fed TNT (Furedi et al., 1984a). Although this response is not statistically different from a linear dose-response relationship, it does suggest a nonlinear curve in the experimental range. Thus, a flexible default model—allowing either linearity or nonlinearity—is fitted to describe tumor incidence as a function of dose in the experimental range. For extrapolation to lower doses, a linear approach is used to reflect the mutagenic potential of TNT and the presumption that this indicates a dose-response curve with a linear component at low doses.

Cancer potency is described by an ED10 (estimated dose associated with a 10% increased incidence) and its lower bound, LED10, expressed as equivalent human doses. For extrapolation to lower doses, an ED10 can be converted to a slope by computing 0.10/ED10[1]; similarly, an upper-bound slope can be obtained by computing 0.10/LED10. (Note that slopes are inversely

[1]The slope is the change in response divided by the change in dose. Extrapolating from a response of 0.10 at dose ED10 to a response of 0 at dose 0, the slope is (0.10–0)/(ED10–0), or 0.10/ED10.

proportional to ED10s; high potency is indicated by high slopes, but low ED10s.) Formerly, upper-bound slopes (known as "q_1*"s) were calculated by the linearized multistage procedure (U.S. EPA, 1980, 1986). The LED10 method and the linearized multistage procedure give similar upper-bound slopes.

3.2. DOSE-RESPONSE ASSESSMENT FOR INGESTION EXPOSURE

Furedi et al. (1984a) provide tumor information for empirically estimating the cancer risk from ingesting TNT. The dose-response trend is statistically significant ($p<0.001$ by the Cochran-Armitage trend test), and the trend cannot be distinguished from a linear one ($p>0.10$ by the Cochran-Armitage test for deviations from linearity). In the absence of cross-species pharmacokinetic studies on TNT, doses are scaled from rats to humans using a factor based on the 3/4 power of body weight (U.S. EPA, 1992).[2] Using this factor, doses of 0, 0.4, 2, 10, and 50 mg/kg-day in rats are considered to be equivalent to doses of 0, 0.1, 0.5, 2.6, and 12.8 mg/kg-day in humans.

Using this method, an ED10 is estimated to be 6.8 mg/kg-day average lifetime exposure; its lower bound, 4.7 mg/kg-day. From these values, a central estimate slope is 1.5×10^{-2} per mg/kg-day average lifetime exposure; its upper bound, 2.1×10^{-2} per mg/kg-day. These calculations are summarized in Table 3-1.

---

[2] For a 70-kg human, $(\text{mg/kg-day})_{\text{human}} = (\text{mg/kg-day})_{\text{animal}} \times (\text{animal weight} / 70)^{1/4}$

TABLE 3-1 **Dose-Response Assessment for Bladder Tumors**	
Tumor Animal Route Reference	Urinary bladder carcinomas and papillomas Female Fischer 344 rats Diet Furedi et al. (1984a)
Exposure duration Study duration Animal lifespan Animal weight	24 months 24 months 24 months (study duration) 0.3 kg
Administered dose Equivalent human dose Tumor incidence	0 0.4 2 10 50 mg/kg-day 0 0.1 0.5 2.6 12.8 mg/kg-day 0/54 0/54 0/55 1/55 17/55
Model Potency, slope estimates	Risk(d) = 1 -exp(-2.3 x $10^{-3}d^2$) in experimental range ED10=6.8 mg/kg-day, LED10=4.7, ED01=2.1, LED01=0.55; q_1*=1.8x10^{-2} per mg/kg-day

When drinking water concentrations are measured in units of μg/L, this upper-bound slope is equivalent to 6×10^{-7} per μg/L for a representative 70-kg person drinking 2 L/day.[3] This slope can be used to estimate concentrations associated with lifetime increased cancer risks of 1 in 10,000, 1 in 100,000, and 1 in 1,000,000.[4] These are 200, 20, and 2 μg/L, respectively.

---

[3] If a 70-kg person drinks water at 2 L/day, then a substance present in drinking water at a concentration of 1 μg/L would result in an intake of

(1 μg/L) × (0.001 mg/μg) × (2 L/day) / (70 kg) = 0.000029 mg/kg-day

Thus, the risk associated with a drinking water concentration of 1 μg/L is 0.000029 times the risk associated with an intake of 1 mg/kg-day.

[4] This follows from the risk equation, risk = slope × dose, equivalently, dose = risk / slope.

If, for comparison, a dose-response curve were fitted to the leukemia and lymphoma response observed in mice, the ED10 and LED10 would be 4.5 and 2.3 mg/kg-day, respectively. These doses are lower than the ED10 and LED10 derived from the bladder tumors. In view of the lack of statistical significance of the leukemia/lymphoma response when all organs are combined (U.S. EPA, 1989; Ross and Hartley, 1990), this shows that animal carcinogenicity experiments are not sensitive enough to rule out the possibility of other risks of this magnitude.

3.3. DOSE-RESPONSE ASSESSMENT FOR INHALATION EXPOSURE

Although there are no cancer studies of inhalation exposure to TNT, it is reasonable to conclude that inhaling TNT would increase the risk of cancer. Support for this comes from occupational studies and animal experiments showing that TNT is rapidly absorbed by inhalation (El-hawari et al., 1981). Thus TNT would enter the systemic circulation, after which TNT can be distributed to distant sites and cause tumors.

We do not know what would be the relative carcinogenic response from inhalation exposure. In the absence of inhalation cancer studies, the best available information comes from pharmacokinetic studies showing that TNT absorption by inhalation is comparable to absorption by ingestion (El-hawari et al., 1981). Thus it is recommended that the low-dose slope for ingestion exposure also be used for inhalation exposure.

When ambient air concentrations are measured in units of $\mu g/m^3$, this slope is equivalent to 6×10^{-6} per $\mu g/m^3$ for a representative 70-kg person breathing 20 m^3/day.[5] This slope can be

---

[5] If a 70-kg person breathes air at 20 m^3/day, then a substance present in ambient air at a concentration of 1 $\mu g/m^3$ would result in an intake of

$$(1\ \mu g/m^3) \times (0.001\ mg/\mu g) \times (20\ m^3/day) / (70\ kg) = 0.00029\ mg/kg\text{-}day$$

Thus, the risk associated with an ambient air concentration of 1 $\mu g/m^3$ is 0.00029 times the risk associated with an intake of 1mg/kg-day.

used to estimate concentrations associated with lifetime increased cancer risks of 1 in 10,000, 1 in 100,000, and 1 in 1,000,000; these are 20, 2, and 0.2 $\mu g/m^3$, respectively.

3.4. DOSE-RESPONSE ASSESSMENT FOR DERMAL EXPOSURE

Although there are no cancer studies of dermal exposure to TNT, it is reasonable to conclude that dermal exposure to TNT would increase the risk of cancer. Support for this comes from studies showing that TNT is rapidly absorbed through the skin (El-hawari et al., 1981). Thus TNT would enter the systemic circulation, after which TNT can be distributed to distant sites and cause tumors.

In the absence of cancer studies of dermal exposure to TNT, the most pertinent information comes from pharmacokinetic studies comparing dermal and ingestion exposure. Animal studies suggest that dermal absorption may be lower by a factor of 2 to 5; in rats, mice, dogs, and rabbits, absorption of a 50 mg/kg dermal dose was 24, 42, 16, and 57%, respectively, compared with over 90% by ingestion (El-hawari et al., 1981). A factor of 2, taken from the lower end of this range, is, thus, a plausible adjustment factor that is unlikely to underestimate risks for this important exposure pathway. Thus it is recommended that dermal exposures be reduced by a factor of 2 before applying the low-dose slope for ingestion exposure.

Uncertainty would arise in applying this slope to soil exposure. The animal experiments measured absorption after direct contact with TNT mixed in peanut oil; absorption of TNT from soil may be different. Quantitative information to adjust for this uncertainty is not now available; without it, no further adjustment in the absorption factor is recommended.

3.5. COMPARISON TO PREVIOUS DOSE-RESPONSE ASSESSMENTS

The new slope factor for ingestion exposure, 2.1×10^{-2} per mg/kg-day, is 30% less than that from EPA's 1989 Health Advisory. This difference is attributable primarily to use of the new cross-species scaling factor based on the 3/4 power of body weight (U.S. EPA, 1992, 1996). Much less significant is starting linear extrapolation from an LED10 (U.S. EPA, 1996) instead of using the linearized multistage procedure (U.S. EPA, 1986).

The new slope factor applies to dermal and inhalation exposure, after adjusting exposure estimates for absorption. Previously, no slope factors had been explicitly recommended for these exposure routes. This assessment's recommendation that the slope factor apply to dermal and inhalation exposure is supported by (1) clear evidence that TNT is absorbed by these exposure routes, and (2) evidence of cancer and other toxic effects internally following absorption of TNT into the systemic circulation. Further, dermal exposure is likely to be a significant route of environmental exposure.

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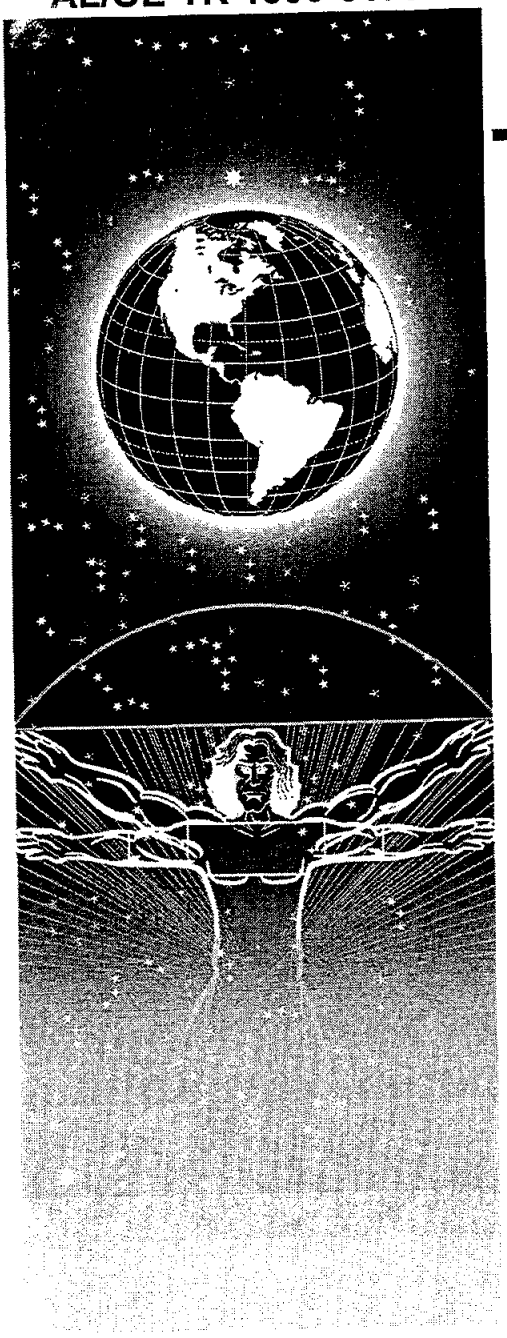

UNITED STATES AIR FORCE ARMSTRONG LABORATORY

Summary of Human Health Risk Assessment Guidelines and Methodologies

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September 1996

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13. ABSTRACT (Maximum 200 words)

This report presents an overview of risk assessment guidelines and methodologies for evaluating cancer and noncancer hazards due to exposure to environmental substances. An overview of the National Research Council's 1994 report *Science and Judgment in Risk Assessment* has been included. Although other federal agencies have established risk assessment guidelines, this report focuses on guidelines and methodologies currently in use or currently proposed by the U. S. Environmental Protection Agency. The primary purpose of this report is to identify key issues pertinent to cancer and noncancer risk assessments that impact the risk assessment process and result in more plausible risk estimates.

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PREFACE

This document was produced under the auspices of the Environmental Risk Assessment Program (ERAP), which has its genesis in the DOD/DOE Strategic Environmental Research and Developmental Program (SERDP) that was established through Public Law 101-510 (10 United States Code 2901-2904). ERAP was established as a cooperative effort of DOD, DOE, and EPA to improve health and ecological risk assessments and to foster consistency in risk assessments across federal agencies. The program has three working groups chartered under its mission which are the Materials/Chemicals Risk Assessment (MCRA) Working Group, Human Risk Assessment Methodology (HRAM) Working Group, and the Ecological Risk Assessment Methodology (ERAM) Working Group. The program also has an Advisory and Coordinating Committee (ACC) that oversees the program and the working group's activities.

This report is a product of the HRAM Working Group and presents issues concerning risk assessment guidelines and methodologies established for evaluating cancer and noncancer hazards due to exposure to environmental substances. Although other federal agencies have established risk assessment guidelines, this report focuses on the guidelines and methodologies established by the U.S. Environmental Protection Agency. Another document, *Reviews of Exposure Assessment Guidelines*, presented issues concerning exposure assessment . Therefore, these issues will not be discussed in the present document. This report was prepared by Dr Kowetha A. Davidson with help from Dr Robert A. Young (draft of the neurotoxicity sections) and Dr Carol S. Forsyth (draft of the developmental toxicity sections), all of the Oak Ridge National Laboratory, and was reviewed by members of the HRAM Working Group.

The ERAP Advisory and Coordinating Committee endorses the information contained within this document with the understanding that the end user is responsible for its application. This means that users are responsible for obtaining any internal scientific and policy reviews required prior to its acceptance within other organizations.

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1. INTRODUCTION

1.1. Overview of Risk Assessment Guidelines

Regulation of chemical and physical substances in the environment is mandated by federal legislation and is implemented by the U.S. Environmental Protection Agency (EPA) and other federal agencies. The objective of the legislation is to protect human health and the environment from the possible adverse health effects of exposure to these substances. The process of evaluating the impact of various substances on health requires a risk assessment and, possibly, appropriate management of such substances.

The risk assessment is an analytical process that primarily estimates some probability of an adverse health effect occurring among human receptors when exposed to these substances. Based upon a finding of unacceptable human risk (a risk management decision), the risk management process will evaluate and apply remedial technologies to lower exposure or remove these substances and correspondingly reduce the risk to an acceptable level.

The risk assessment process has been criticized by the scientific community, industry representatives, and the lay public as not being systematic and, at times, appearing arbitrary. Additionally, decisions made in the risk assessment process may yield overly conservative risk estimates that can sometimes lead to stringent remedial objectives and unusually high remedial costs. For these reasons, guidelines for estimating the risk of exposure to environmental substances were established and they have undergone considerable changes over the years.

EPA formalized its risk assessment process in 1986 with the publication of assessment guidelines for carcinogenicity, developmental toxicity, mutagenicity, exposure to chemical mixtures, and exposure. In 1988, guidelines for female and male reproductive risks were published. The developmental toxicity guidelines were revised in 1991 and the exposure assessment guidelines in 1992. In 1993, EPA proposed guidelines for neurotoxicity risk assessment. EPA has also established methodologies for assessing risk to noncancer toxicants by route of exposure: reference dose (RfD) for the oral route (U.S. EPA, 1993) and reference concentration (RfC) for the inhalation route (U.S. EPA, 1994a). It should be mentioned that prefacing these guidelines and methodologies were the water quality criteria guidelines detailing the method for estimating acceptable daily intakes (ADI) for noncancer toxicants based on no-observed-adverse-effect levels (NOAEL) and lowest-observed-adverse-effect levels (LOAEL) and deriving slope factors (q_1*) for carcinogens using the linearized multistage procedure (U.S. EPA, 1980). These methodologies are directly related to the present methodologies and guidelines for carcinogen risk assessment and the derivation of RfDs and RfCs for noncarcinogens.

This report will first review some of the major documents contributing to the evolution of risk assessment as practiced today; these include the National Research Council (NRC) report of 1983, the NRC report of 1994, and the Office of Science and Technology Policy report of 1985. These reports are pivotal to describing the way risk assessment is performed by regulatory agencies and are the focus on which to evaluate the risk assessment process. EPA's risk assessment guidelines will be reviewed along with published criticisms, alternatives, and recommendations to the guidelines. This is not a comprehensive report; particularly, chemical-specific issues have not been discussed. Also, the mixtures issues will not be discussed, and the exposure assessment issues are discussed in a separate report. EPA is currently working on revising the mixtures guidelines. Some subjects are discussed in detailed discussion than others, but any subject can be expanded at the request of the working group.

1.2. Risk Assessment in the Federal Government: Managing the Process (Red Book) (NRC/NAS, 1983)

Major contribution of this report: established the four steps of the risk assessment process and their contribution to the whole process.

Under a directive from congress, the U.S. Food and Drug Administration (FDA) contracted with the National Research Council's Commission on Life Sciences, which formed the Committee on the Institutional Means for Assessment of Risk, to study organizational arrangements for conducting the risk assessments in support of regulatory management by the federal government. The regulation of chemical and physical substances to which human are exposed is implemented by four government agencies: the EPA, FDA, Occupational Safety and Health Administration (OSHA), and the Consumer Product Safety Commission (CPSC). During the decade of the 70s, there was increased public concern about health hazards, (cancer and other chronic health hazards) associated with exposure toxic substances. There was also concern about the risk assessment process on which regulators based their decisions as well as the cost and benefits associated with implementing the decisions. The interaction between regulators (risk managers) and scientists (risk assessors) was cause for additional concern by scientist, industry, and the public (NRC/NAS, 1983). The objectives of the NRC's Committee on the Institutional means for Assessment of Risk (referred to as the Committee) were as follows:

1. assess the merits of separating the analytic functions of developing risk assessments from the regulatory function of making policy decisions,

2. consider the feasibility of designating a single organization to do risk assessment for all regulatory agencies,

3. consider the feasibility of developing uniform risk assessment guidelines for use by all regulatory agencies.

The Committee searched for "mechanisms to ensure that government regulations rests on the best available scientific knowledge" and to ensure that scientific integrity is maintained as science and government work together in addressing issues related to adverse effects of environmental substances on human health. The Committee noted that its conclusions and recommendations apply primarily to cancer risk associated with exposure to environmental chemicals, but they also apply to other endpoints of human health (systemic effects, developmental effects, etc.)

1.2.1. Steps in the Risk Assessment Process

The Committee established the four basic steps (hazard assessment, dose-response assessment, exposure assessment, and risk characterization) of the risk assessment paradigm, which is widely accepted today. The Committee's description of the steps and the data (information) sources is presented below:

The Committee defined *hazard identification* as the process of determining whether exposure to an agent can cause an increase in the incidence or severity of a health condition. The nature and strength of the evidence is characterized. Hazard identification answers the following question: Does the agent cause an increase in incidence or severity of an adverse effect in test animals? If the answer is yes, then the agent may pose a risk (cancer) to humans. *Data sources*: epidemiologic and other human studies (always given primary consideration when characterizing adverse health effects), animal bioassays, short-term studies, genetic toxicity, and absorption, distribution, metabolism, and absorption (ADME) data, structure-activity relationships. *Dose-response assessment* was defined as "the process of characterizing the relation between the dose of an agent administered or received and the incidence [or severity] of an adverse effect in an exposed population and estimating the incidence of the effect as a function of human exposure to the agent." What is the relationship between the dose and incidence or severity of the adverse effect? *Data sources*: human data are given primary consideration, if available, when characterizing adverse health effects, but quantitative data from human studies are usually not adequate; animal data are usually available, but species extrapolation is required. In addition, high occupational exposures of humans are extrapolated to low exposures to the general population or high experimental doses in animals are extrapolated to low doses is required.

The definition given for *exposure assessment* was "the process of measuring or estimating the intensity, frequency, and duration of human exposures to an agent currently present in the

environment or estimating hypothetical exposures that might arise from the release of new chemicals into the environment." What exposures are currently experienced by the population or anticipated to occur under different conditions? Exposure assessments describes the various aspects of exposure including intensity, duration, route, frequency, populations, and uncertainties associated with the estimates. *Data sources*: direct measurements of chemicals in environmental media, models that predict exposures in environmental media, extrapolations from small segment to large general populations.

Risk characterization is the process of estimating the incidence of a health effect under the various conditions of human exposure described in exposure assessment. What is the estimated incidence or severity of the adverse effect that would occur in a given population? Risk characterization was described as a summary of the dose-response and exposure assessments and associated uncertainties. *Data sources*: hazard assessment, dose-response assessment, and exposure assessment provide the information for making predictions for different population groups.

1.2.2. Components in the Risk Assessment

The NRC Committee compiled a list of components in the risk assessment process that arise from attempts to bridge the gap between inherent uncertainties (missing or ambiguous data or gaps in current scientific theory) and the need to conduct the assessment. The list compiled by the Committee was not exhaustive, but 25 components were associated with hazard assessment and 13 with dose-response assessment. These components focused primarily on cancer assessments but can be extended to evaluate other adverse effects in humans. The following section includes a condensed versions of these components.

1.2.2.1. Components of hazard identification – The components related to performing a hazard assessment are listed below (organized according to the data sources of a hazard assessment).

Epidemiologic studies:

- the relative weight given to different types of studies or to studies with different results
- the level of statistical significance required for positive results
- the significance of positive findings in studies in which route of exposure is different from the one of interest
- how to combine different types of responses

Animal studies:

- the level of confirmation required for positive results (two or more studies?); weight given to negative studies
- the quality and statistical power of the studies as the basis for weighing studies
- the handling of differences in metabolism and pharmacokinetics between animals and humans and the incorporation of the differences in the results
- the weight given to rare tumors, especially when the incidences are not statistically significant
- the weight given to studies when tissue damage or other toxic effects accompany a carcinogenic effect
- the combining of benign and malignant tumors
- the weight given to decrease latency

Short-term studies:

- weight given to short-term results
- level of evidence required for addition to weight of evidence
- weight given to different types of tests
- weight given positive versus negative results

Structural-activity analysis:

- weight given to results with structurally similar compounds.

1.2.2.2. Components of Dose-Response Assessment – The components related to the dose-response assessment are listed below. The data sources for dose-response assessment are epidemiologic (human) studies and animal bioassays. The components associated with human data are encountered only when these data are available, which in not often. The two major components of dose-response assessment are extrapolation from high to low doses and interspecies dose conversion. These components focus primarily on cancer assessments, but can be applied to other adverse effects in humans.

Epidemiologic studies:

- selection of a dose-response model to extrapolate to low environmental doses
- Selection of best estimate of risk or upper confidence limit of risk
- method for adjusting for comparatively short follow-up period in epidemiologic studies
- health effects on which to derive estimates; for example should consideration be given only to cancers unequivocally related to exposure or all types of cancer
- how to account for exposures to other potential carcinogens
- how to account for differences in the temporal pattern of exposed population and the population in question (lifetime risk function of total dose no matter when it was received during the lifetime; weight given to the most recent exposure)
- how to account for possible physiological differences between exposed population and population in question.

Animal data:

- selection of mathematical models used to extrapolate from experimental animal doses to environmental human exposures
- Selection of best estimate of the risk or the upper confidence limit of the risk
- the dose scaling method for converting animal doses to human doses
- incorporation of metabolism data into the assessment
- how data on more than one nonhuman species or strain be used in the assessment; use most sensitive species or strain; combine data on different species and strain and method for combining
- how to use data on more than one tumor type; combine data or use tumor type most affected by exposure
- interpretation of statistically significant decreases in tumor incidences at specific anatomical sites.

The Committee noted that choices made for each component when missing or ambiguous data are encountered are called inference or "default options". Inference or default options are based on both scientific and policy judgements. Science and policy judgments in risk assessment can determine the outcome of the assessment, because the judgements determine the defaults options chosen in a risk assessment, and the choices of default options affect the conservativeness of the assessment. If policy judgments are the basis for choosing defaults, then the policy should be grounded, as much as current advancements allows, in scientific knowledge. The committee

further noted that, when conservatism drives the choices for default options, the risk assessment policies may be driven more by risk management considerations than by science. Risk management takes into account the non-science aspects (political, social, economic, and other considerations) of regulating chemical exposures. In addition, within the framework, there should be clear separation of default options based on scientific judgement and those based on policy.

The Committee also described the need to establish guidelines that direct the risk assessor in conducting the assessment. Guidelines allow the separation of risk assessment and risk management by laying down a formal procedure for conducting risk assessments. Guidelines would aid in the quality control over risk assessments, ensuring that the assessment conforms to the scientific judgements of experts in the diverse fields encompassing risk assessments. Guidelines also ensure that the assessments are clear, complete, and comprehensive. Additionally, the guidelines ensure that risk assessments are consistent and predictable for different chemicals. The Committee noted that the guidelines must be comprehensive in detail, but not so inflexible as to limit scientific interpretation of data or change of a default option in the face of reliable scientific evidence. The Committee stated that uniform guidelines for all aspects of risk assessment, except exposure assessment, are feasible and desirable for governmental agencies.

1.3. Chemical Carcinogens: Review of the Science and its Associated Principles (OSTP, 1985)

Major contributions: reiterated the risk assessment paradigm established by NRC/NAS (1983) and listed principles on which to build risk assessments.

The Office of Science and Technology Policy (OSTP) presented its general scientific view of carcinogenesis and general principles on which federal agencies tailor their risk assessment guidelines to meet legislative requirements. OSTP divided risk into two parts: hazard and exposure. Hazard being the toxicity deduced from a variety of studies of humans or animals, and exposure being the contact of individuals with a substance. There were a total of 31 principles put forth by OSTP; all are not listed below, particularly those related to exposure assessment.

1. Carcinogenesis is a multistage process involving direct or indirect effects on the genome; the process may be influenced by a number of factors, such as age, sex, diet, hormonal status, and genetic background.

2. Carcinogenicity can be influenced by induction of nonphysiological responses (excessive organ damage, hormonal disruption, metabolic saturation, etc) that may affect the relevancy of the test system for evaluating human carcinogenicity.

3. Mechanistic considerations, such as DNA repair or damage, do not prove or disprove the existence of a threshold for carcinogenesis.

4. Short-term tests, particularly genotoxicity tests, are useful in providing information for interpreting carcinogenicity studies, but are limited in their ability to predict carcinogenicity, and thus, cannot substitute for long-term animal or epidemiologic studies.

5. The statement,"In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard chemicals for which there is sufficient evidence of carcinogenicity in animals as it they presented a carcinogenic risk to humans", is a reiteration of the IARC principal. Other relevant information must be considered in reaching a conclusion based on long-term animal studies.

6. Animal models having high background tumor incidences pose special problems, and such data must be interpreted carefully.

7. Long-term animals studies should achieve adequate biological and statistical sensitivity and adequate biological and statistical specificity to avoid producing false negatives and false positives. High doses for animal studies are required to achieve statistical significance, but doses should be compatible with normal life span (except that due to cancer) and minimal organ toxicity.

8. Evidence of carcinogenicity should consider relevant biological and biochemical data.

9. Evidence of probable reproducibility of long-term studies (independent confirmation of results), evidence of a dose-response, increased tumor incidence at multiple sites, and decreased tumor latency increase confidence in the study.

10. Biological plausibility of a neoplastic response may be increased when the incidence of the corresponding preneoplastic lesions is increased.

11. Well-designed and well-conducted cohort or case-control epidemiologic studies can provide data for causal association of exposure with cancer in humans.

12. Well-designed and well-conducted negative epidemiologic studies, while useful, cannot prove the lack of an association between exposure and human cancer.

13. The exposure routes in animal studies should be comparable to human exposure routes.

14. Evaluations on carcinogenicity should be based on relevant data, whether it comes from animal studies, epidemiologic studies, *in vitro* or *in vivo* short-term tests, metabolic and pharmacokinetic studies, mechanistic studies, or structure/activity analysis.

15. Mathematical models for low-dose extrapolation should be consistent with the evidence, but when evidence is limited, low-dose linearity is the preferred method.

16. Quantification of uncertainty is an important issue in risk estimation; sources of uncertainty include the model selected for low-dose extrapolation, statistical uncertainty associated with the risk estimate, and the use of animal models as test organisms.

17. Clear distinctions should be maintained among facts (statements supported by data), consensus (statements generally held by the scientific community), assumptions (statements made to fill data gaps), and science policy (statements made to resolve points of current controversy).

18. Because the human population varies in their susceptibility to chemical exposures, consideration should be given to identifying high risk groups.

1.4. Science and Judgment in Risk Assessment (NRC/NAS, 1994)

Major contributions of this report: reevaluated EPA's risk assessment guidelines in light of the Clean Air Act Amendment of 1990; proposed that EPA define default options and establish principles for moving beyond default options,

modify its hazard assessment classification, and establish a more rigorous uncertainty analysis in risk assessments.

The U.S. Congress charged the National Research Council (NRC) with the following tasks:

1. review the methods used by EPA in determining the carcinogenic risk associated with exposure to hazardous air pollutants,

2. include in its review, evaluation of the methods used for estimating the carcinogenic potency of hazardous air pollutants and for estimating human exposures to these air pollutants, and

3. evaluate, to the extent practicable, risk-assessment methods for noncancer health effects for which safe thresholds might not exist.

To perform this task, the NRC established the Committee on Risk Assessment of Hazardous Air Pollutants consisting of 25 members representing various disciplines. The Committee evaluated EPA's current risk assessment practices and noted that EPA generally followed the recommendations of the 1983 NRC report, but as new information has become available over the years, criticisms of EPA's practices have come from various groups including industry, environmental organizations, and academia. The major criticisms of EPA's risk assessment practices have been related to the lack of quantitative data, the different scientific interpretations of pertinent data, the level of uncertainty, and the incorporation of conservative default options. The committee addressed in detail six issues of EPA's risk assessment process: default options, data needs, validation, uncertainty, variability, and aggregation.

"Default Options": The Committee was concerned that EPA did not clearly identify all its "default options", nor did EPA fully explain the basis for default options. Further, EPA allows departure from default options, but has not identified the criteria for the departures. The Committee recommended that EPA continue to use default options as a means for dealing with "uncertainty about underlying mechanisms in selecting methods and models for use in risk assessment." However, EPA should identify each use of a default option and present the scientific and policy basis for the default option. The Committee also stated that EPA should formalize in its guidelines, principles for departing from default options, so as to prevent *ad hoc* undocumented departures that could damage the credibility of the assessment. The Committee identified the following objectives that EPA should consider in establishing its default options and principles for departure: protecting the public health, ensuring scientific validity, minimizing serious errors in estimating risks, maximizing incentives for research, creating an orderly and predictable process, and fostering openness and trustworthiness. The Committee, however, could not agree on which principles EPA should adopt.

Methods, Models, and Validation: The Committee expressed concern that the predictive accuracy and uncertainty in the models (exposure and toxicity assessments) EPA uses in its risk assessments are not always clearly explained or understood. The Committee noted that EPA classifies potential carcinogens based on the strength-of-evidence [should have said weight-of-evidence] associated with levels of evidence ("sufficient", "limited", "inadequate", or "no data") achieved from human and/or animal studies; the levels of evidence are grouped into A, B, and C categories based on a combination of evidence levels for humans and animals. The Committee criticized EPA's classification scheme because chemicals showing strong evidence for carcinogenicity in humans (class A) could pose a low risk due to low exposure or potency, whereas a chemical showing strong evidence only in animals (class B2) may pose a high risk due to high exposure or potency, yet the class A chemical may be viewed as a greater hazard (The Committee referred to this situation as "accidents of fate"). The Committee failed to note that it is likely that the basis for the A classification is due to cancer mortality (a less sensitive endpoint than diagnosis of cancer for estimating risk); thus, the risk of dying from cancer is an inherent underestimation of the risk of developing cancer. The Committee recommended a scheme using four categories, each of which had two to four subcategories and a descriptive narrative. The descriptive narrative would include "relevance information" based on the animal model and exposure.

The Committee also noted that EPA uses the linearized multistage model (default model) for extrapolating from human occupational exposures or experimental animal doses to low exposures for human populations to estimate the carcinogen potency of a substance. The potency is based on upper bound estimates of the risk. The Committee recommended that EPA include data on mode of action in its quantitative models and that pharmacokinetics data be incorporated into its models to extrapolate from animal to human doses, extrapolate between routes, and to link exposure to dose. The Committee recommended that EPA validate models used in risk assessments. The Committee further recommended that EPA continue to use the linearized multistage model as its default options for extrapolating to low doses, but EPA should develop criteria for using alternative models. The upper bound estimate should continue to be used to estimate the risk for developing cancer due to lifetime exposure.

The report noted that EPA uses the NOAEL/LOAEL approach for establishing safe doses for noncarcinogen effects. The Committee recommended that EPA should continue to explore the use of pharmacokinetic models for establishing target tissue doses and biologically-based quantitative models for linking exposure and noncancer effects.

Data needs: The Committee recommended that EPA develop a two-level plan for risk assessments: screening and full risk assessment. A screening assessment would require only a minimal data set, whereas a full risk assessment would require a rich data set. The Committee suggested requirements for gathering and assessing toxicologic data for the screening and full risk assessments. Generic and acute toxicity data should be collected on all chemicals as a starting point; and toxicokinetic, genetic, subchronic and chronic animal data, human toxicity data, and mechanistic data should be collected on chemicals for which there is a cause for concern. Other factors (emissions, environmental fate and transport, and exposure data) in addition to toxicity determine the level of priority for conducting a full risk assessment. The Committee recommended that data gathering and assessment for either a screening or full scale risk assessment be an iterative process.

Variability: According to the Committee, EPA has not addressed the issue of variability (age, sex, race, ethnicity, lifestyle, etc.) in its cancer risk assessment guidelines. The Committee, therefore, recommended that EPA sponsor research to study variability in susceptibility to cancer, adopt default options to account for differences in susceptibility, validate or improve the default assumption that all humans have the same susceptibility as those in epidemiologic studies and/or the most sensitive animals tested, assess risk to infants and children when their risk appear to be greater, clearly state default assumptions for nonthreshold low-dose linearity of genetic effects, and maintain a distinction between uncertainty and variability.

Uncertainty: The Committee noted that EPA's current approach to uncertainty analysis is a qualitative description of the model uncertainty rather than a quantitative analysis of model parameters. The Committee recommended that EPA develop a formal process of uncertainty analysis as part of its risk assessment process, include an analysis of other models that may be more "accurate", and present risk managers a range of risk values rather than a single point estimate.

Aggregation: Risk assessment usually addresses the hazards and risks associated with single chemical exposures. However, populations or individuals are exposed to multiple chemicals by more than one pathway. In addition, bioassay data often reveal that tumors develop at more than one anatomical site. The Committee recommended that EPA use appropriate statistical procedures to aggregate exposures to multiple chemicals and add individual potency estimates for each relevant tumor types in cases of multiple anatomical targets.

2. OVERVIEW OF EPA's 1986 CANCER RISK ASSESSMENT GUIDELINES

EPA's 1986 guidelines appear to be an extension of the 1980 guidelines for deriving water quality criteria based on threshold and nonthreshold effects (U.S. EPA, 1980). The water quality criteria guidelines first introduced the linearized multistage model as a procedure for low-dose extrapolation of cancer incidence data. The water quality criteria guidelines also provided default practices for interspecies scaling, estimating internal doses from feeding and inhalation studies, and adjusting potency estimates when the duration of the study is less than the theoretical life span of the species. EPA's 1986 carcinogen risk assessment guidelines introduced the four step paradigm identified by the NRC/NAS (1983) as the foundation of its risk assessment process (U.S. EPA, 1986). Two steps of this process, hazard identification and dose-response assessment, will be discussed in this report.

2.1. Hazard Identification

Hazard identification (qualitative aspect of carcinogen risk assessment) consists of an evaluation of the pertinent data to establish the link between exposure to a substance and adverse effects or hazards in humans. The data sources listed by EPA include epidemiologic and other human studies, animal toxicologic studies (particularly long-term exposure studies), genetic toxicity studies, short-term (or subchronic) toxicity studies relevant to cancer, metabolism and pharmacokinetics studies, and physicochemical data (U.S. EPA, 1986). The evidence from human and long-term animals studies is evaluated based on strengths and weaknesses of the studies with the evidence classified as "sufficient", "limited", or "inadequate", depending on the level of causality determined from human and animal studies separately. Evidence from other types of studies may add to or subtract from the weight of evidence.

Hazard identification concludes with an overall weight of evidence consisting of a short narrative on the strength of evidence and a classification based on the A, B, C scheme, similar to that of the International Agency for Research on Cancer (IARC). The five weight-of-evidence categories as established by EPA are: Group A: sufficient evidence from human studies, any level of evidence from animal studies; Group B: limited evidence from human studies and any level of evidence from animal studies (B1) or sufficient evidence from animal studies and inadequate of no evidence from human studies (B2); Group C: no data or inadequate evidence from human studies and limited evidence from animal studies; Group D: no data or inadequate evidence from human and animal studies; and Group E; evidence of no carcinogenicity from human or animal studies.

2.2. Dose-Response Assessment

According to the 1986 guidelines (U.S. EPA, 1986), *dose-response assessment* (quantitative aspect of risk assessment) uses cancer incidence data from humans or animals studies and exposure data (a presumed surrogate for dose) to estimate an upper bound on risk (q_1* or slope factor) using the linearized multistage model (default model). The multistage procedure can be used to estimate risk in the experimental dose range or at low dose levels. Models other than the linearized multistage may be used for low-dose extrapolation if the data suggest that it may be more plausible. Most epidemiologic data are obtained from studies of humans exposed to high concentrations of a substance in occupational environments; thus, low-dose extrapolation would be necessary to estimate the risk to humans at low environmental exposures. For most substances, however, quantitative human data are not available; thus, interspecies and low-dose extrapolations are necessary to estimate potential cancer risk to humans. For interspecies extrapolation, animals doses are scaled to human equivalent doses, averaged over an entire lifetime (70 years (default value for humans), and expressed as a daily dose. According to the 1986 cancer guidelines, animal doses are scaled to human equivalent doses based on surface area expressed as of the ratio of the body weights to the ⅔-power (default); this practice was described in EPA's water quality criteria guidelines (U.S. EPA, 1980).

When several data sets are available, EPA's carcinogen risk assessment guidelines (U.S. EPA, 1986) state that all relevant animal data should be evaluated for quantitative risk estimations, but the most emphasis should be placed on studies showing the greatest sensitivity (default position). However, due regard should be given to statistical and biological considerations in choosing this approach. When the environmental route of exposure is different from that of the dose-response data, route-to-route extrapolation is conducted in accordance with existing pharmacokinetic and metabolism data on the chemical. When more than one tumor site shows statistically significant elevated incidences, the individual incidences can be pooled for risk estimation (default position). The guidelines also relied on a qualitative description of the uncertainties associated with quantitative estimates of risk assessment.

3. OVERVIEW OF EPA's 1996 CANCER RISK ASSESSMENT GUIDELINES

The current external review draft of EPA's proposed carcinogen risk assessment guidelines is the result of several workshops sponsored by EPA, a workshop sponsored by the Society of Risk Analysis, a review by the Office of Science and Technology Policy, and the NRC's comments in *Science and Judgment in Risk Assessment* (U.S. EPA, 1996b) The gradual evolution of the 1986 guidelines into the 1996 proposed guidelines has produced several key changes in the hazard identification and dose-response assessment phases of the carcinogen risk assessment process. These changes will be discussed below.

3.1. Hazard Assessment and Characterization

The purpose of hazard assessment is to present and evaluate pertinent data to determine whether an agent poses a carcinogenic hazard to humans and under what circumstances the hazard may be expressed. The assessment consists of an evaluation of all pertinent data, not just human and animal cancer data, to arrive at a conclusion regarding the carcinogenicity of an agent. In addition to the traditional assessment, which included human and animal cancer data, toxicokinetic, metabolism, and an analysis of structurally related compounds, an analysis of mode of action has become an important aspect of the proposed hazard assessment and characterization process.

Mode of action information provides insight into the relevance of animal data to human carcinogenicity, the conditions under which carcinogenicity may be expressed in humans, and the selection of a dose-response approach. Questions to be answered by analyzing the mode of action include:

- Does the agent affect DNA directly or indirectly?
- Does the agent affect cell proliferation, apoptosis, gene expression, immune surveillance, or other cellular mechanisms not involving DNA?
- Does the agent act by a mode of action reasonably anticipated to occur in humans or by one known not to occur in humans.

Animal data often provide clues as to possible modes of action. For example, agents that induce tumors at multiple site and in multiple species are likely to be mutagenic, whereas agents that affect tumors with high spontaneous incidence rates or induce only late-developing benign tumors suggests a growth promoting mode of action. Therefore, all data related to the mode of action should be evaluated very carefully and incorporated into the weight of evidence analysis.

The weight of evidence analysis uses the entire body of evidence to make a sound judgement as to the potential carcinogenicity of the agent for humans. Consideration is given to quality, consistency, and volume of data. The most weight is given to multiple well-conducted human and animal studies showing consistent responses across studies combined with strong data sets on toxicokinetic, metabolic fate, mode of action, structural activity relationship, and other key evidence such as physicochemical properties. The entire evaluation of all key data elements are combined to develop a conclusion regarding the carcinogenicity of an agent. EPA listed several factors for weighing the totality of evidence.

- Evidence of human causality
- Evidence of animal effects relevant to humans
- Coherent inferences
- Comparable metabolism and toxicokinetics between species
- Mode of action comparable across species

Decreases in the strength of evidence in these categories can result in decrease weight as to the carcinogenicity of an agent to humans.

The proposed guidelines do not categorize substances within the A,B,C weight-of-evidence groups. EPA has established three categories of descriptors for human carcinogenic potential: "*known/likely*", "*cannot be determined*", and "*not likely*." The categories are proposed to be route specific. The *known* category is used when a definite causal association between human exposure and cancer can be established based human data. A subcategory "treated as if they were *known*" human carcinogens is used for agents for which human evidence is not strong enough to show a definite causal association, but experimental animal evidence is strong. The *likely* category is used when strong animals data show carcinogenicity by a mode of action considered to be relevant or assumed to be relevant to human carcinogenicity; human evidence in this case may be weak or absent. Two subcategories to *likely* are *high end* of the totality of evidence and *low end* of the totality of evidence. In the latter subcategory, the evidence is decidedly weak, but still showing potential evidence of carcinogenicity to humans. The *cannot be determined* category is used when the evidence of potential carcinogenicity is based on data that are *suggestive*, *conflicting*, or *inadequate*; it is also used when *no data* are available to perform an assessment. The *not likely* category is used (1) when more than one well-conducted study in at least two appropriate species show no evidence of carcinogenicity, (2) when the mode of action is not relevant to humans, (3) for a particular route when evidence shows route specificity, (4) for a specific dose when evidence

shows dose limitations, and (5) when extensive human experience shows no evidence of a carcinogenic effect.

The weight of evidence analysis is followed by the hazard narrative, which summarizes the results of the hazard assessment. The narrative explains the likelihood of hazard in humans, the conditions under which the hazard would or would not be expressed, strengths and weaknesses of the evidence, the mode of action, and the impact of the mode of action on dose-response assessment.

3.2. Dose-Response Assessment

The dose-response assessment proceeds in two steps. The first step is to model the data in the range of experimental observation; the second step is to extrapolate below the experimental range to lower environmental exposures using specific models or default procedures. When data are available, biologically-based or case-specific models are used for both the experimental range and extrapolation to low doses. However, rarely will there be sufficient data or resources to employ either biologically-based or case-specific models for dose-response assessments. Instead, curve-fitting models are used for dose-response assessment of animal data in the range of experimental observation and estimating the point of departure for extrapolating to low doses. The selection of the curve-fitting model should be consistent with data for which it is applied. The point of departure is the lower 95% confidence limit on dose associated with 10% extra risk (LED_{10}). The point of departure (LED_{10}) is a matter of science policy that will result in consistency among different assessments and consistency with the benchmark approach for noncancer assessments. When sufficient data are available, the point of departure may be set below the LED_{10}. Comments are being sought on using others points on the dose-response curve for the point of departure. Modeling of human data are conducted on a case by case basis.

If data are not available for applying biologically based or case-specific models, one of three science policy default procedures is selected for extrapolation of doses below the point of departure. The three procedures are linear, nonlinear, and both. The mode of action is a primary factor in selecting the default procedure.

The default linear procedure involves straight line extrapolation from the point of departure to the origin or zero response. When the evidence suggests a mode of action involving gene mutation due to DNA reactivity or another mode action anticipated to be linear, a linear procedure is selected. A linear procedure is also selected when there is insufficient evidence for applying either the linear or nonlinear procedure.

When there is sufficient evidence to support a nonlinear dose-response or evidence of a threshold response and no evidence for a linear dose-response, a nonlinear procedure is selected for the dose-response assessment. Probabilistic dose-response functions are not fitted to nonlinear dose-response data for low-dose extrapolation; instead the margin of exposure is calculated (science policy). The margin of exposure is the point of departure, usually the LED_{10}, divided by the environmental exposure of interest. If the dose-response data suggest a threshold response, as seen when carcinogenicity is a secondary response to toxicity or cell proliferation, the margin of exposure procedure is conducted similar to what procedure is used for noncancer endpoints including estimating an RfD or RfC for the primary effect. The guidelines proposed that factors accounting for intraspecies differences (accounting for human variability) and interspecies differences (accounting for differences in sensitivity between humans and test species) can be employed in the margin of exposure analysis. In deciding what constitutes an acceptable margin of exposure the following factors should be considered: (1) slope of the dose-response curve at the point of departure; (2) nature of the response, tumors, frank toxicity, or precursor effect; (3) nature and extent of human variability in sensitivity; (4) persistent of the agent in the body; and (5) human sensitivity compared with that of animals.

When data are available to support both linear and nonlinear procedures, as may be the case for multiple tumor sites, appropriate dose-response procedures are applied.

The selection of data for dose-response assessment first considers the positive quantitative data from well-designed, well-conducted epidemiologic studies. If adequate human data are not available, then priority is given to data from the animal species showing the greatest similarity to humans. If this cannot be determined, the quantitative evaluation should consider all animal data and base the risk estimate on the data set best representing the response in humans. Biological plausibility and the mode of action should receive major consideration when deciding on the data set(s) to be incorporated into the risk assessment. The risk estimate may be the result of (1) a single data set, (2) combined data sets from different experiments, (3) a range of estimates from several data sets, (4) pooled data sets from a single experiment, (5) an analysis of different data sets based on different modes of action, or (5) a combination of the above.

The default measure of exposure to carcinogens is the cumulative lifetime dose expressed as the average daily dose. The dose data applied to the dose-response assessment may be estimated from human studies or from animal experiments. Nevertheless, decisions have to be made on whether to use applied dose, internal dose, or delivered dose to the target organ and whether the doses are to be expressed in terms of parent compound or metabolite. Estimation of

doses from human studies are conducted on a case-by-case basis. If dose estimates from human studies are not available, the preferred method is to use toxicokinetic and toxicodynamic data to estimate human equivalent doses from animal experimental doses. Toxicokinetic and toxicodynamic data can be used to build agent-specific models for scaling internal or delivered animal doses to equivalent human doses. These models require comprehensive data sets, which are seldom available.

In the absence of toxicokinetic data, doses for oral exposure are estimated using the default procedure for interspecies scaling, in which the daily dose applied over a lifetime is scaled proportionally to the ¾-power of the body weight ($BW^{3/4}$). Documentation for scaling daily doses based on $BW^{3/4}$ was published in a Federal Register report (U.S. EPA, 1992b). For inhalation exposure, default methods for estimating human equivalent concentrations for particles and gases are described in the methodology for deriving RfCs (U.S. EPA, 1995).

Quantitative route-to-route extrapolation may be conducted when the route of interest is not the same as the route for which data are available. In the absence of data to the contrary, EPA's default assumption is an agent that causes internal tumors by one route may be carcinogenic by another route if the substance is absorbed by a second route to give an internal dose. Therefore, route extrapolation should be supported qualitatively before attempting quantitative extrapolation. The site of tumor formation (should be distant from the portal of entry), absorption similarities or differences between the two routes, and available toxicokinetic and toxicodynamic data must be evaluated for each case. Regardless of the qualitative support for route-to-route extrapolation, quantitative extrapolations are problematic because of first pass effects, which may alter biological responses.

Toxicity equivalence factors (TEF) are sometimes used to derive quantitative risk estimates for agents within classes of compounds. One class member serves as the reference by which other members are indexed according to shared characteristics. Although the guidelines did not refer to TEFs as screening risk values, they should be considered as such, because TEF are replaced with defensible values when sufficient data become available. So far, dioxins and furans are the only classes of compounds with adequate data to support TEFs. Criteria for developing TEFs have been presented by EPA (U.S. EPA 1991a) and are being developed and expanded in the revision of the mixtures guidelines (ILSI, 1996, in preparation).

The dose-response assessments concludes with a dose-response characterization that describes the judgments and rationales made in selecting the approach for the analysis. Plausible

alternative approaches may be presented, but the preferred approaches should be described. The uncertainties are described and quantified where practicable. Two types of uncertainties are usually encountered, model uncertainty and parameter uncertainty. Model uncertainties are not amenable to quantitation and are described qualitatively. Parameter uncertainties are described quantitatively using sensitivity analysis and statistical uncertainty analysis. Dose-response estimates are presented to one significant figure, along with an indication of whether the values are upper bounds or central tendency. Additionally, the characterization should include a discussion on likely overestimation or underestimation of the results.

3.3. Default Assumptions

In response to the NAS/NRC (1994) report, the EPA addressed the basis and justification for using default assumption in hazard and dose-response assessments. The guidelines addressed the issues and major default assumptions common to risk assessments. The major default assumptions are described within the framework of the following questions. Some of these issues will be discussed in further detail in Section 5 (Issues)

- **Is the presence or absence of effects observed in a human population predictive of effects in another exposed human population?**

Human data are typically obtained from occupational exposure studies, where the working population is different from that of the general population by sex, age, and general health and is not representative of the general population. EPA has two default assumptions concerning this issue. The first is that *when cancer effects in exposed humans are attributed to exposure to an exogenous agent, such data are predictive of cancer in any other human population exposed to the same agent.* This default is not considered to be public health conservative. EPA (U.S. EPA, 1996b) further states that when specific data on sensitive subpopulations are available, these data should be used in the assessment. The different types of susceptibilities are quite numerous and may be related to age (children may be more susceptible that adults), sex (male/female differences), ethnic or racial background, nutritional status or diet, and genetics (e.g., slow and rapid acetylators). Pertinent questions related to this issue are: How do we identify and quantitate the number or proportion of susceptible individuals in a population? How do we quantitate the cancer risk of a susceptible population? How is this information incorporated into the hazard and dose-response assessments and characterizations?

Because null results maybe obtained from studies of worker populations, the second default assumption is as follows: *When cancer effects are not found in an exposed human population, this information by itself is not generally sufficient to conclude that the agent poses no carcinogenic*

hazard to this or other human populations exposed to the same agent. How much weight can one place on well-conducted epidemiologic studies producing null results? Should these results carry more or less weight than positive animal studies? According to the 1996 proposed cancer guidelines, studies of worker populations producing null results may not have the power to detect effects in sensitive population, suggesting that null results carry very little weight in hazard assessments. The 1996 proposed guidelines also stated that quantitative data obtained from null results may be used to estimate upper bounds on human risk for comparison with estimates from animal data.

- **Is the presence or absence of effects observed in an animal population predictive of effects in exposed humans?**

The default assumption states that *positive effects in animal cancer studies indicate that the agent under study can have carcinogenic potential in humans.* This assumption is considered to be public health conservative. This default assumption has several subparts that are addressed by additional default assumptions. (1) *Effects seen at the highest dose tested are appropriate for assessment, but it is necessary that the experimental conditions be scrutinized.* To improve the detection power of animal studies, high doses are used that often cause toxicity manifested by effects such as cell killing and compensatory cell proliferation. If excessive toxicity is observed, a study may be discarded, but expert judgement is required in this decision process. (2) *Target organ concordance is not a prerequisite for evaluating the implications of animal study results for humans.* This is a public health conservative science policy option. The carcinogenic targets for animal and human studies may or may not be concordant. Nevertheless, data that do not support site concordance should be considered when available. Site concordance is inherently assumed when toxicokinetic modeling is used to estimate target doses for humans. (3) *Include benign tumors observed in animal studies in the assessment of animal tumor incidence if they have the capacity to progress to the malignancies with which they are associated.* This is a science policy decision more public health conservative than the alternative. (4) *Benign tumors that are not observed to progress to malignancy are assessed on a case-by-case basis.* It should be noted that animal studies are usually terminated after 2 years, but humans receive continued exposure until death. Also some tumors do not progress because of a short duration of exposure, but they would probably progress if exposure had been continued for a longer time.

- **How do metabolic pathways relate across species?**

The default assumption is that *there is similarity of the basic pathways of metabolism and the occurrence of metabolites in tissues in regard to the species-to-species extrapolation of cancer hazard and risk.* There may be quantitative differences in metabolic pathways; unless data show

qualitative differences, metabolism is assumed to be similar in animals and humans.

- **How do toxicokinetic processes relate across species?**

The default option for oral exposure is that *a human equivalent dose is estimated from data on another species by an adjustment of animal oral dose by a scaling factor of body weight to the 0.75 power* ($BW^{0.75}$). Scaling based on body weight makes the assumption that the area under the curve (AUC) is equivalent across species for dosimetric purposes. This value is based on scaling of metabolic processes across species of different sizes. The default for inhalation exposure is that *a human equivalent dose is estimated by default methodologies that provide estimates of lung deposition and of internal dose.* As new data becomes available, both defaults can be replaced. The default for route-to-route extrapolation is that *an agent that causes internal tumors by one route of exposure will be carcinogenic by another route if it is absorbed by the second route to give an internal dose.* This is a public health conservative default option that assumes qualitative similarity of metabolic processes across routes of exposure; adequate data are required for route specific designation.

- **What is the correlation of the observed dose-response relationship to the relationship at lower doses?**

Biologically-based or case-specific models are used when sufficient data are available. In the absence of sufficient data the default procedure is to *use a curve-fitting model for the observed range of data when the preferred approach cannot be used.* There are three default procedures to consider when extrapolating dose-response data to low doses (linear, nonlinear, and both). Mode of action is a primary feature in selecting the best approach to use. *A linear default approach is used when the mode of action information is supportive of linearity or there is insufficient data to support a nonlinear mode of action.* For linear extrapolation, a straight line is drawn from the point of departure to the zero response or zero dose. The default point of departure is the LED_{10} (public health conservative) when data are not available to support a lower point of departure. The straight line extrapolation gives an upper bound on risk at low doses. *When adequate data on mode of action show that linearity is not the most reasonable working judgement and provide sufficient evidence to support a nonlinear mode of action, the default changes to a margin of exposure analysis, which assumes that nonlinearity is more reasonable.* The point of departure is the LED_{10} unless data are available to support a lower point. *Both linear and margin of exposure procedures can be used when the mode of action data indicate that the dose response may be adequately described by both linear and nonlinear approaches.*

4. OVERVIEW OF NONCANCER RISK ASSESSMENT GUIDELINES

In 1980, EPA published guidelines for deriving water quality criteria for threshold toxicants (U.S. EPA, 1980). These guidelines were established to determine the concentration of toxicants in water that do not pose significant risks to the general population. Water quality criteria are derived for carcinogenic and noncarcinogenic effects. The guidelines for noncarcinogenic effects were based on selecting appropriate NOAELs or LOAELs and applying safety factors as deemed necessary to account for the uncertainty in using animal models as surrogates for toxic risk for humans. The water quality criteria guidelines evolved into the present day methodology for derivation of reference doses (RfDs) (U.S. EPA, 1993) and reference concentrations (RfCs) (U.S. EPA, 1994a) for assessment of noncarcinogenic systemic effects. The RfD/RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of exposure to the human population that is likely to be without appreciable risk of noncancer health effects during a lifetime (U.S. EPA, 1994a). The concept of threshold is inherent in the definition of the RfDs and RfDs; the concentration below which there is no observable adverse effect is considered to be a threshold (U.S. EPA, 1993). A threshold is assumed to exist for both the individual and the population (U.S. EPA, 1993). RfDs/RfCs are derived for chemicals that cause noncancer (or systemic) health effects. All effects except cancer and gene mutations comprise noncancer health effects; these include effects on portal-of-entry organs (gastrointestinal and respiratory tracts and skin), remote sites (internal organs such as liver, kidney, bone marrow, brain, reproductive organs), and the developing fetus. Risk assessment guidelines have been established for general noncancer endpoints (U.S. EPA, 1993, 1994a), development toxicity (U.S. EPA, 1991c), and neurotoxicity (U.S. EPA, 1994b). Guidelines were proposed for male (U.S. EPA, 1988a) and female reproductive toxicity (U.S. EPA, 1988b); the guidelines for male reproductive toxicity are under discussion. In addition to the above citations, EPA's RfC methodology has been discussed in several reports by Jarabek and coworkers (Jarabek et al., 1990, Jarabek, 1995a,b).

The general guidelines for deriving RfDs and RfCs are based on the risk assessment paradigm established by the NRC/NAS (1983): *hazard identification, dose-response assessment, exposure assessment*, and *risk characterization*.

4.1. Hazard Assessment

4.1.1. General Guidelines for RfD/RfC Derivation (U.S. EPA, 1989a, 1991b, 1993, 1994a)

Hazard identification concerns the careful evaluation of all relevant human and animal data and identifying the principal studies that best describe the statistically and biologically significant effect(s) expected to occur in the general population. The data sources for detailing systemic effects likely to occur in humans after exposure to environmental substances are the same as those identified for evaluating potential carcinogenicity. They include human studies (epidemiologic and case studies), long-term animal studies, short-term studies used to identify targets for long-term studies, toxicokinetics studies, and studies on mechanism of action and structure-activity relationships. For deriving RfDs, the evaluation focuses primarily on oral exposure studies and on inhalation studies for deriving RfCs.

EPA has established guidelines for assessing the quality of individual human and animal studies and for assessing the overall quality of the database (U.S. EPA, 1989a, 1991b, 1993, 1994a). Human studies are always given priority over animal studies for assessing the potential hazards of environmental exposures to the general population. Identification of sensitive populations is a critical aspect of hazard assessment. Epidemiologic and clinical studies conducted on potentially sensitive groups are helpful, as well as identification of genetic and other risk factors that may contribute to increased risk. Sensitive groups may include, groups in prenatal and postnatal developmental stages, those with respiratory diseases, circulatory conditions, and liver diseases, and the elderly.

When adequate human studies are not available, animal studies are used. Assessing the validity or appropriateness of animal models requires consideration of the study design (ideal studies are those that follow established procedures and protocols for conduct and analysis of results), elements of exposure definition (concentration, duration, frequency, route, etc.), relevance of exposure levels tested, similarities and differences between the test species and humans. The appropriate or most relevant species is identified based on comparable metabolism, pharmacokinetics, etc. The most sensitive species is not selected as a priority, because the effects produced may not be toxicologically relevant to human. If the most relevant species cannot be identified, then the most sensitive species is selected as a science policy option (U.S. EPA, 1994a). Route-to-route extrapolation of hazard concerns is permissible; EPA's view is that the toxicity potential manifested by one route can be indicative of potential toxicity via any other exposure route unless convincing evidence to the contrary exists.

The overall evaluation identifies the critical effect and the effect levels (NOAEL and LOAEL) associated with exposure to a substance. In addition, the overall conclusions (weight of evidence) regarding the likelihood of an environmental substance posing a hazard for humans is enhanced by the following factors (U.S. EPA, 1989a, 1991b): (1) a clear dose-response relationship, (2) similar effects across species, sex, strain, exposure routes, multiple experiments, (3) biological plausibility of the effect of concern, (4) similar effects in structurally related compounds, and (5) a link between the chemical and evidence of the effect of concern in humans. These factors also increase the confidence in the weight of evidence (U.S. EPA, 1989a). Other factors that increase the confidence in the weight of evidence concerns the completeness of the database. EPA has established criteria by which the completeness of the database can be judged when human data are not available. a complete database consists of (1) two long-term inhalation studies in different species, (2) a mammalian two-generation reproductive toxicity study, and (3) two mammalian developmental toxicity studies in different species. The minimal requirement for deriving an reference values is one long-term (preferred) or subchronic (acceptable) study. These factors also contribute to the confidence in the selection of the critical effect and effect levels.

4.1.2. Developmental Toxicity Guidelines

The U.S. EPA (1991c) defines developmental toxicology as "the study of adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation". Developmental toxicity may be manifested as one or more endpoints: (1) death; (2) structural abnormality; (3) altered growth; and (4) functional deficit.

Data from all available sources, with primary emphasis on human and experimental animal data, are used to evaluate the developmental toxicity potential of a substance (U.S. EPA, 1991c). Human data are often inadequate for evaluating the potential of a chemical to cause developmental toxicity; therefore, it is often necessary to rely on experimental animal data. When using animal data for hazard identification of potential developmental toxicants, several assumptions are made: (a) a substance that causes developmental toxicity in animals will potentially pose a hazard to humans; (b) all four of the manifestations of developmental toxicity are of concern; (c) the types of developmental effects seen in animals are not necessarily the same as those that may occur in humans; and d) the most sensitive species is appropriate for use (U.S. EPA, 1991c).

Maternal toxicity endpoints considered for risk assessment include mortality, body weight and body weight change, food and water consumption, clinical evaluations, gestation length (if animals are allowed to deliver), mating and fertility indices, organ weights, and gross observations

(U.S. EPA, 1991c). Comparison of maternal toxicity in developmental toxicity studies with data from other toxicological studies establishes differences in the responses of pregnant animals to nonpregnant adults.

Developmental toxicity studies generally evaluate death, structural abnormality, and altered growth. Endpoints evaluated for risk assessment include implantation sites, corpora lutea, live and dead offspring, resorptions, pre- and postimplantation loss, altered offspring (external, visceral or skeletal malformation or variations), sex ratio, and fetal body weight (U.S. EPA, 1991c). Testing for functional deficits, which may occur between conception and sexual maturation, is not routinely required. Most of the work involving functional evaluation has been in the area of developmental neurotoxicity and testing protocols and data interpretation are beginning to be standardized (Francis, 1992). Analyses of postnatal renal development have detected what has been interpreted as either apparent hydronephrosis (a variation) (Woo and Hoar, 1972) or hydronephrosis (a malformation) (U.S. EPA, 1991c). Other systems that are not as well studied include the cardiovascular, respiratory, immune, endocrine, reproductive, and digestive systems (U.S. EPA, 1991c).

Dose levels of a substance that results in maternal toxicity may differ from those that result in developmental toxicity. Of greatest concern, are those agents that result in developmental toxicity with no apparent maternally toxic effects. For most substances however, the exposure situations of concern are those potentially near the maternally toxic dose level, but developmental effects should not be considered secondary to maternal toxicity. At dose levels of a substance that result in marked maternal toxicity, stress associated with disruption of homeostasis can occur. Fetal anomalies commonly linked to maternal toxicity include bent or wavy ribs, reduced weight, and death (Black and Marks, 1992; Khera, 1984). However, such fetal effects are toxic manifestations and are considered in regulation and risk assessment since maternal effects may be reversible, whereas effects on the fetus may be permanent (U.S. EPA 1991c).

Substances are categorized based on evidence for developmental toxicity. *Sufficient human evidence* includes data from epidemiological studies that provide strong scientific evidence for a causal relationship. *Sufficient experimental animal evidence/limited human data* includes animal studies and/or limited human data that provide convincing evidence of the potential for developmental toxicity. The *insufficient evidence* category includes substances for which there is limited data upon which to base a scientific judgement, because of no studies or inadequate studies.

4.1.3. Neurotoxicity Guidelines

Hazard identification for neurotoxicity evaluates relevant data to determine if a substance is likely to cause an adverse effect in the nervous system. As for the RfD and RfC, hazard identification for neurotoxicity may involve evaluation of data from various sources including epidemiologic studies, clinical evaluations in humans, short- and long-term animal studies, mechanism-of-action studies, and structure-activity relationship studies (U.S. EPA, 1994b). Generally, hazard identification will evaluate mechanism of action data as well as human and animal toxicity data. Hazard identification for neurotoxicants is complicated by the difficulty in specifically defining an adverse neurological effect, thereby resulting in widely varying estimates of the number of neurotoxic chemicals. Tilson (1990) noted that neurotoxicants are those agents that adversely affect the neurophysiological, neurochemical or structural integrity of the nervous system or the integration of nervous system function expressed as modified behavior.

According to U.S. EPA risk assessment guidelines for neurotoxicity (U.S. EPA, 1994b), direct identification of hazard may be obtained from human studies but human data are often anecdotal, involve acute exposures causing overtly toxic or lethal effects, or (especially for epidemiologic studies) are often complicated by confounding factors such exposures to multiple agents or imprecise exposure characterization. For human studies, identification of neurotoxicity may include evaluations of neuromuscular strength, alterations of sensory-motor function, learning and memory deficits, personality and mood alterations, and alterations in autonomic functions.

Animal studies using models that measure behavioral, neurophysiological, neurochemical, or structural changes are available and can be used to extrapolate to humans for the purpose of hazard assessment. With the exception of behavioral changes, it is generally assumed that neurological processes are fundamentally similar among most species and, therefore, the effects observed in one species are likely to occur in another species including humans. Uncertainties (e.g., sex-dependent differences, species specificity, etc.) are inherent in such extrapolations, however, and special issues often arise regarding how these differences impact on hazard identification in humans.

Assessing the validity and appropriateness of human and animal studies for neurotoxicity hazard identification is generally similar to the methods and procedures described for the RfD/RfC. However, concern has been expressed regarding the fact that identification and acceptance of a NOAEL from a single study may not necessarily be indicative of an absence of neurotoxicity risk.

The objectives of neurotoxicity testing include: (1) determining if the nervous system is affected by the toxicant (detection), (2) characterizing alterations of the nervous system that are associated with exposure to the toxicant, (3) ascertaining if the nervous system is the primary target, and (4) determining the dose-effect and time-effect relationships relative to establishing a no-observable-effect-level (NOEL) (Reiter, 1987).

4.2. Dose-Response Assessment

4.2.1. Guidelines for NOAEL/LOAEL Approach (U.S. EPA, 1989a, 1991b, 1993, 1994a)

4.2.1.1. General Methodology – A dose-response relationship is seen when increased dosage of a toxicant results in increases in a response to the toxicant; the response may be in the form of an increased incidence (quantal response), increased severity (graded response), or increased incidence and severity of an effect. Dose-response assessment is dependent initially on hazard assessment activities: selecting the principal study and selecting the critical effect. In selecting the principal study, human studies are considered first if quantitative exposure and incidence or severity information can be obtained from these studies, because human data eliminate the need for species extrapolation. If a suitable human study is not available for quantitative assessment, then the principal study is selected from the available animal studies. The study using an animal model (species) most relevant to humans (based on mechanism of action, pharmacokinetics, route of exposure, etc.) is given first priority. If no relevant animal model can be identified, then the most sensitive animal species is chosen (default position), because there is no assurance that humans are not as sensitive as the most sensitive species. The most sensitive species is one showing a toxic effect at the lowest tested dose. Therefore, the critical effect is the effect occurring at the lowest dose in either the most relevant or most sensitive species; the NOAEL corresponding to the critical effect is selected for deriving the RfD or RfC. The dose or exposure concentration may be measured as the applied dose or concentration, absorbed dose, or target organ dose. It should be noted that, for portal-of-entry effects, the absorbed dose may not be a relevant expression of exposure. If the critical effect is prevented then all other toxic effects should be prevented (science policy). Exposures less than the reference value are considered to be without (but not categorically) risk of adverse effects in humans.

The RfD or RfC is calculated by the following simple equation:

$$\text{RfD or RfC} = \text{NOAEL}/(\text{UF} \times \text{MF}),$$

where UF is the uncertainty factor and MF is the modifying factor. The RfD or RfC can be derived from a LOAEL when an NOAEL cannot be identified from the principal study. The RfD is expressed in mg/kg/day and the RfC in $mg/m^3/day$.

Dose scaling for species extrapolation in quantitative assessment for noncancer toxicants does not take the same form as for carcinogens. Uncertainty factors are used in noncancer assessments to account for the pharmacokinetic and pharmacodynamic differences between humans and animals, in contrast to the dose scaling procedures (body weight to ⅔ or ¾ power) used for cancer risk assessments.

For the inhalation exposures, human equivalent concentrations (HEC) are calculated according to the following steps: (1) conversion of exposure units from ppm to mg/m^3, (2) adjustment of experimental exposure to 24-h continuous exposure for a lifetime of 70 years for humans, and (3) adjustment of doses for the type of substance (particle/aerosol or gas/vapor) and the anatomical site of the effect (respiratory or extrarespiratory). Pharmacokinetics data are be used for dosimetric adjustments when available. Physiological parameters including surface areas for the different regions of the respiratory tract for humans and experimental animals, body weight, and ventilatory values are used to calculate the HEC. The HEC is calculated according to the following equation:

$$NOAEL_{[HEC]}\ (mg/m^3) = NOAEL_{[ADJ]}\ (mg/m^3) \times DAF_r,$$

where $NOAEL_{[ADJ]}$ is the effect level adjusted for discontinuous exposure and DAF_r is the dosimetric adjustment factor for respiratory region of concern based on the regional deposited dose ratio (RDDR) for particles or the regional gas dose ratio (RGDR) for gases. The RDDR or RGDR is the ratio of the regional deposited dose or regional gas dose (RDD_A or RGD_A) to a target in animals and the regional deposited dose or regional gas dose (RDD_H or RGD_H) to a target in humans. These ratios adjust the exposure concentrations used in animal studies to estimate the dose delivered to a target region in humans. A detailed discussion on the methods for calculating regional doses is found in EPA's reference concentration methodology (U.S. EPA, 1994a).

Dosimetric adjustments for particles and vapors takes into account particle size and size distribution of the particles. Dosimetric adjustments for gases is determined by the physicochemical and uptake characteristics of the gas. Category 1 gases are highly water soluble, rapidly irreversibly reactive, and does not accumulate in blood. Category 2 gases are moderately water soluble, rapidly reversibly reactive, and may accumulate in blood. Category 3 gases are

insoluble in water, unreactive in the extrathoracic and tracheobronchial regions, and their site of action are remote to the respiratory tract (extrarespiratory region or systemic). Default equations for estimating doses to the respiratory region of concern have been derived for both particles and gases (U.S. EPA, 1994a). These equations can be used when chemical specific data are not available for dosimetric adjustments. More details about the regional deposition of particles or regional gas effect/uptake of substances in the respiratory tract and the ratios between humans and animals for calculating human equivalent concentrations are presented in EPA's guidelines (U.S. EPA, 1989a, 1994a) and other reports (Jarabek et al., 1990, Jarabek, 1995a,b; Shoaf, 1991).

Uncertainty factors are applied to the $NOAEL_{[HEC]}$ to derive the RfD/RfC. The uncertainty factors account for the following: (2) variations in sensitivity among the human population, (2) extrapolating from animal data to human exposures, (3) less than lifetime exposures when subchronic studies are used instead of chronic studies, (4) extrapolating from a LOAEL to a NOAEL when a suitable NOAEL cannot be identified, and (5) an incomplete database. Uncertainty factors of 10 are usually applied to derivation of RfDs. For deriving RfC, uncertainty factors of 10 are applied for variation in human sensitivity, up to 10 for a subchronic study, LOAEL, and incomplete database, and 3 for animal to human extrapolation. In addition, a modifying factor of 10 or less can be applied to account for scientific uncertainties in the study or database not accounted for by uncertainty factors.

4.2.1.2. Approach Used for Developmental Toxicity – If developmental toxicity occurs as a result of exposure a substance, developmental endpoints are evaluated quantitatively using a protocol similar to those for deriving RfDs or RfC. An oral or dermal reference dose (RfD_{DT}) or an inhalation reference concentration (RfC_{DT}) for developmental toxicity is derived (U.S. EPA, 1991c). The RfD_{DT} or RfC_{DT} is derived by dividing the NOAEL or LOAEL by the total uncertainty factor. NOAELs or LOAELs are determined from the most sensitive, or critical, developmental effect from the most sensitive animal species. If the NOAEL or LOAEL for maternal toxicity is lower than that for developmental toxicity, this should be noted in the risk characterization and the value compared with data from other experiments of adult exposure. Uncertainty factors generally include a 10-fold factor for interspecies variation and a 10-fold factor for intraspecies variation (3 for RfC) ; an uncertainty factor is not applied for duration of exposure.

The dose-response relationship is evaluated in standard animal studies using three dose groups and a control. For developmental toxicity studies, a threshold is assumed for the dose-response relationship (U.S. EPA, 1991c). Three general patterns of response have been described for agents that cause developmental toxicity (Manson, 1986). Substances that have high

developmental toxicity potency can cause malformations of the entire litter at dose levels that do not cause embryolethality. Conversely, some substances may result in growth retardation and death without malformations. The more common dose-response pattern includes some embryolethality with growth retardation and malformations evident in surviving fetuses. Generally, as dose levels increase causing embryolethality to increase, an observed decrease in malformations may result.

4.2.1.3. Approach Used for Neurotoxicity – If chronic or subchronic toxicity studies show evidence of neurotoxicity and batteries of neurotoxicity test are conducted, it may be possible to derive an RfD or RfC for neurotoxicity. The dose-response assessment is conducted as using the RfC/RfD approach as described above.

Alternately, the benchmark dose concept has been suggested because it makes use of the dose-response curve rather than single dose (i.e., NOAEL) for estimating potential risk. Rather than extrapolating to doses far below the experimental dose range, the benchmark dose estimates a the dose corresponding to a specific incidence of an effect (e.g., 10%) based on the upper confidence limit of the particular dose-response curve (Farland and Dourson, 1993).

4.2.2. Benchmark Approach (U.S. EPA, 1995; Barnes et al., 1995)

The benchmark dose (BMD) has been defined as the statistical lower confidence limit for a dose that produces a predetermined change in response rate of an adverse effect (benchmark response or BMR) compared with background (Crump, 1984a). The benchmark approach can be applied to general noncancer dose-response data, as well as developmental, reproductive, and neurotoxicity data. It has many features in common with deriving the LED_{10} for cancer data. The BMD is calculated using dose-response data fitted to mathematical curve-fitting models with appropriate statistical procedures. There is no extrapolation to doses below the experimental range, but to a predetermine BMR of 1, 5, or 10%.

The EPA is developing guidelines for applying the BMD approach to noncancer dose-response assessments, but the guidelines are not presently available. A overview and description of the benchmark approach has been presented by EPA's Risk Assessment Forum (U.S. EPA, 1995), and a workshop organized to discuss the feasibility and implications of the BMD approach for derivation of reference values (Barnes et al., 1995).

There were several reasons for developing an alternative approach for deriving reference values for noncancer health effects. The primary reason deals with the limitations of the

NOAEL/LOAEL approach for RfD/RfC derivation. These limitations include the following (Crump, 1984a; U.S. EPA, 1995; Barnes et al., 1995):

- The NOAEL is based on scientific judgment and may be the source of controversy.
- Experiments involving a few animals tend to produce large NOAELs, and consequently, large RfDs/RfCs.
- The slope of the dose response plays little role in determining the NOAEL.
- The NOAEL is limited to the experimental doses tested and is dependent on the statistical power of the study.

The benchmark approach to deriving an RfD/RfC involves three steps that are discussed below:

(1) Selection of the response or group of responses from the experimental data set. The responses from animal studies are selected based on toxicological relevance to humans and convincing evidence of a dose-related effect for the responses. Mathematical curve-fitting should be applied to all relevant responses.

(2) Calculation of the BMDs for the selected responses. Calculation of the BMD may involve transforming the data to a form that can be fitted by a mathematical model. Fitting data presented in the quantal format (dependent only on presence or absence of a response), such as incidence data, is a straightforward process using most mathematical models (Barnes et al., 1995). Categorical data in which effects are described in terms of severity of effect (mild, moderate, severe, etc.) and continuous data (e.g. body and organ weights, enzyme levels, etc.), which can be transformed into a quantal format or modeled without being transformed is a more complex process (Barnes et al., 1995). Sometimes data are presented in more than one format, i.e., the severity of liver lesions may presented along with the incidence of the lesion. The mathematical model chosen to estimate the BMD depends on the format of the data. For quantal data, the probability of a response or the dose (BMD) corresponding to a specific response (BMR) can be estimated. For continuous data, the mean response corresponding to dose can be estimated. Different types of mathematical models, such as quantal, quadratic, and polynomial regression can be fitted to both quantal and continuous data; Weibull and log-normal can be fitted to quantal data and linear-quadratic and continuous models can be fitted to continuous data (U.S. EPA, 1995). The model selected should adequately describe the biological response, such as the slope transitions near the threshold. After the model is selected, the BMR level is selected; the value is generally in the range of 1 to 10%.

(3) Choosing an appropriate BMD and calculating the RfD/RfC. Because multiple studies with multiple responses may be available or a single response may be subjected to multiple curve-fitting models, multiple BMDs can sometimes be calculated for a single assessment. Three options that have been recommended for selecting the BMD are as follows: (1) calculate an average or geometric mean of the BMDs, (2) use the most appropriate species and/or sex, or (3) select the smallest BMD. After the BMD is selected, the RfD/RfC is calculated by applying uncertainty factors. Several options were presented for choosing the uncertainty factors: (1) use uncertainty factors similar to those applied to NOAELs, (2) use uncertainty factors applied to NOAELs modified by the average ratio of BMD/NOAEL, (3) use-risk based uncertainty factors to extrapolate to 10^{-4} or 10^{-5}, which would represent a virtually safe dose (Kimmel and Gaylor, 1988), (4) use uncertainty factors dependent on the choice of BMR and size of confidence limit, and (5) use uncertainty factors that incorporate the slope of the dose-response and/or other biological considerations.

5. ISSUES IN HUMAN HEALTH RISK ASSESSMENT

This report has focused on the EPA's cancer and noncancer risk assessment guidelines and methodologies. There are similarities and differences in the assessment procedures used for the two types of hazards. The benchmark approach for noncancer assessments has been harmonized with the dose-response analysis within the range of experimental observation for carcinogen assessments; the methodology for dosimetric adjustments for inhaled particles and gases are the same for cancer and noncancer hazards; and the criteria for assessing the adequacy of human and animal data are similar. Some of the issues can be applied to both cancer and noncancer assessments: route-to-route extrapolation, pharmacokinetic modeling, target organ concordance involving non-analogous sites, and including the sensitive population in the LED_{10}. Nevertheless, the following issues are discussed as they relate to either the cancer and noncancer risk assessments. The issues are discussed in detail in the following sections, and some key issues are summarized briefly in Section 6.

5.1. Carcinogen Risk Assessment

The following sections address some of the issues concerning the cancer risk assessment process. Some of these issues were specifically discussed in the EPA's1996 proposed cancer guidelines in its discussion on default assumptions (U.S. EPA, 1996b).

5.1.1. Relevance of Animal Models to Human Carcinogenicity

EPA's 1986 guidelines state that each animal study should be reviewed as to the relevance of the evidence for humans. In a working paper (draft), EPA stated that ".....tumors at any animal tissue site support an inference that humans may respond at some site" (science policy) (U.S. EPA, 1992a). The agency further stated in the same report that if the information suggests a mechanism unique to an animal species or strain, then the evidence does not support a carcinogenic hazard to humans. This concept was slightly modified in the 1996 guidelines, which stated that "Information on an agent's potential mode(s) of action is important in considering the relevance of animal effects to assessment of human hazard."

Munro (1988) studied the relevancy of animal models to human carcinogenicity and proposed eight criteria by which relevancy could be judged. Evidence would not be considered as relevant to human carcinogenicity if these criteria can be applied to the data gathered on a particular substance (Munro, 1988).

1. The test chemical or mixture does not represent that to which humans are exposed.

2. The route of exposure of the test animal is vastly different from that of humans (route-to-route extrapolation).

3. The only tumor response of the test species occurs at a site having high background incidence (high spontaneous incidence).

4. The tumor response occurs only at high doses that produced toxicity incompatible with normal physiological function (carcinogenicity only at high doses).

5. Tumors are produced only at an anatomical site not found in humans (target organ concordance).

6. Tumor induction is closely linked to chronic physical irritation, physiological perturbations, or a marked derangement of endogenous metabolism.

7. Pharmacokinetic studies show vast differences in the disposition or fate of the test material between the animal model and humans or between the low and high doses.

8. Epidemiologic evidence suggests that the substance is not carcinogenic in humans under normal conditions of exposure.

Most of these items were addressed in EPA's 1996 proposed cancer assessment guidelines. However, EPA's guidelines and Munro's criteria differ in some respects. According to the guidelines the only criteria for judging whether hazard can be extrapolated to a second route is absorption of the agent by that route to give an internal dose. The 1996 guidelines further state that adequate data are necessary to demonstrate that an agent will act differently by a second route. Therefore, the burden of proof is on showing that an agent will act differently rather than acting similarly by a different route. First pass effects, particularly those occurring as a result of liver alterations after absorption from the gastrointestinal tract, are not taken into account. The default assumption allowing route-to-route extrapolation for hazard assessment is very conservative. Quantitative route-to-route extrapolation is conducted on a case-by-case basis. In most cases, however, sufficient data will not be available to conduct quantitative route-to-route extrapolation. Therefore, the issue is: should qualitative route-to-route extrapolation be allowed when sufficient data are not available for quantitative extrapolation?

Target organ concordance a carcinogen assessment issue, particularly for organs or tissues not shared by animals and humans. EPA's view is that target organ concordance is not assumed *a priori* for establishing potential carcinogenicity for humans (U.S. EPA, 1996b). Gregory (1988) stated that site-specific tumors should not be unique to the species tested and should be correlated with carcinogenicity in humans. According to Goodman and Wilson (1991), site concordance is more likely to occur if the routes of exposure of the animal models and humans are similar. Munro (1988) listed anatomical sites not shared by test species and humans as one of the criteria for

judging the evidence as not relevant and gave as an example the production of forestomach tumors in rats fed butylated hydroxyanisole (BHA). Munro (1988) also considered other criteria (high doses, irritative hyperplasia, and lack of genetic toxicity) in questioning the relevancy of this evidence for potential carcinogenicity of BHA in humans. Questions related to this issue are: Should target organ concordance be considered in establishing relevancy of animal models for human cancer assessments? Should organs unique to animals, such as the forestomach and Zymbal's gland be given special consideration? Should target organ concordance be considered when carcinogenicity is seen only in organs such as the forestomach? Does forestomach tumors occurring after oral exposure suggests that exposure by another route would result in tumor development in the contact organs? For example, would skin tumors develop after dermal route of exposure or the respiratory tract tumors after inhalation exposure? Although the default assumption described in the 1996 proposed cancer guidelines states that target organ concordance is not a prerequisite for establishing potential carcinogenicity to humans, carcinogenicity occurring only in target organs unique to animals poses a problem for weighing evidence of carcinogenicity.

In 1986, EPA's position regarding high background tumors was that an increased incidence of tumors with a high background constituted "sufficient" evidence of potential carcinogenicity, but when other evidence (replicate studies, malignancy) is considered the conclusion may be changed (U.S. EPA, 1986). According to the 1996 proposed guidelines, tumors with high background incidences are considered in a hazard assessment, but are given less weight (U.S. EPA, 1996b). Liver tumors in male mice, testicular interstitial cell tumors in male rats, and pituitary tumors in male and female rats occur with high background rates (Gregory, 1988; Goodman and Wilson, 1991). Mouse liver tumors are often used as evidence of potential carcinogenicity in humans. The background incidence of liver tumors the $B6C3F_1$ male mouse was reported to be as high as 31.1% (Pereira, 1985); the corresponding incidence in females was only 6.2%. A different pattern of activated oncogenes is seen in spontaneous liver tumors compared with those induced by chemicals (Reynolds et al., 1988). Therefore, in cases where the background tumor incidence is high, it may be possible to distinguish between increased incidences due to stimulation of existing lesions and the induction of new tumors (Anonymous, 1993).

Another issue related to relevance of animal models to human cancer risk is carcinogenesis by a mode of action known not to occur or unlikely to occur in humans. EPA's position is that all mode of action information should be considered when assessing risk, and if the mode of action has been shown not to occur in humans, the agent is *not likely* to be carcinogenic to humans. One example is tumorigenesis in male rat kidney due to accumulation of $\alpha_{2\mu}$-globulin, a mode of action

that does not occur in humans. EPA has established a science policy and guidance for assessing the carcinogenicity of chemicals inducing renal tubule tumors (U.S. EPA, 1991a). The science policy is as follows: *Male rat renal tubule tumors arising as a result of a process involving $\alpha_{2\mu}$-globulin accumulation do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk.* The guidance state that the assessor first evaluate renal tubule tumor data to determine if $\alpha_{2\mu}$-globulin is involved in the carcinogenic process. If so, then the extent to which $\alpha_{2\mu}$-globulin accounts for the carcinogenicity as opposed to other mechanistic processes is evaluated (U.S. EPA, 1991a).

In addition, urinary bladder tumors associated with formation of calculi or implantation of foreign bodies into the bladder lumen should be evaluated carefully as well as tumors formed after exposure by routes not likely to be encountered by humans (intraperitoneal or subcutaneous injection).

The susceptibility of rats and mice to urinary bladder carcinogenesis induced by endogenous formation of a bladder calculus or a foreign body implanted into the bladder lumen has been documented (Cohen and Ellwein, 1992). Chemicals producing calculi are generally nongenotoxic, induce tumors above a threshold (Cohen and Ellwein, 1992), and involve a mode of action not likely to occur in humans. Consequently, such tumors are not likely be relevant to evaluating potential carcinogenicity to humans. Bladder tumors induced by implantation of substances into the bladder lumen should also be considered as not relevant to human carcinogenicity. OSTP (1985) also stated that such tumors may not be relevant to human oral exposure.

There are some routes of exposure by which humans are unlikely to be exposed, such as subcutaneous, intraperitoneal, intratracheal instillation. OSTP (1985) noted that subcutaneous sarcomas formed by subcutaneous injection of a substance may not be relevant to human exposure. How much weight should be given to evidence of carcinogenicity by unusual routes of exposure when evaluating potential carcinogenicity, particularly in cases where local tumors are formed? Should evidence from a route unlikely to be experienced by humans be considered in a different light from evidence of carcinogenicity induced in animals at high doses not likely to be experienced by humans?

5.1.2. Genotoxic vs Nongenotoxic Modes of Action

Distinguishing between genotoxic and nongenotoxic modes of action in tumor induction is a critical element in assessing the potential carcinogenicity of agents to humans. Purchase (1994) defined genotoxic carcinogens as those chemicals or their metabolites that alter DNA or genetic information by producing point mutations, insertions, deletions, or changes in chromosome structure or number. The outcome of evaluating the genotoxicity of a substance is to determine if the substance interacts directly with DNA resulting in damage to DNA or heritable changes in the DNA (Cohen and Ellwein, 1992). The DNA damage could result in activation of oncogenes (e.g. *ras* or *myc*) or alterations of tumor suppressor genes (Purchase, 1994). Carcinogenicity induced by genotoxic substances is considered to have no threshold, i.e., that every exposure no matter how small is associated with a measurable risk. Most chemicals known to be carcinogenic to both humans and animals have genotoxic activity (Cohen and Ellwein, 1992).

Nongenotoxic carcinogens are described as those chemicals whose primary activity does not involve genetic toxicity. According to Purchase (1994), nongenotoxic carcinogens induce mitogenesis and hyperplasia which allows the fixation of DNA damage by oxygen free radicals or different types of mutational events produced by other endogenous or exogenous agents. There are several mechanisms by which nongenotoxic agents induce neoplasia (Purchase, 1994). Some of the more widely studied are (1) excess production of trophic hormone or disruption of homeostasis (thyroid stimulating hormone/thyroid neoplasia; trypsin inhibitors/pancreatic neoplasia; gastrin/ECL gastric neoplasia; luteinizing hormone/Leydig cell neoplasia), (2) receptor binding (dioxin:Ah cytoplasmic binding), and (3) cytotoxicity and mitogenesis (crystalline formations/bladder neoplasia, $\alpha_{2\mu}$-globulin/male rat kidney neoplasia). Purchase (1994) noted that nongenotoxic carcinogens usually display tissue (affect a single tissue or organ) and species specificity (affect only one species or one sex in one species).

Potential carcinogenicity in humans is often assessed based on induction of mouse liver tumors. Pereira (1985) stated that a distinction should be made between genotoxic and nongenotoxic modes of action when assessing potential carcinogenicity to humans based on mouse liver tumor data. The nongenotoxic carcinogens may act by promoting already initiated cells in the mouse liver, and therefore, may promote spontaneously or environmentally initiated cells in human liver. He further stated that the anticipated nonlinear dose-response curve for nongenotoxic carcinogens does not mean that these chemicals pose no risk to humans, but that a safe level may exist. Reynolds et al. (1988) reported that spontaneously occurring mouse liver tumors and those induced by chemical agents have different patterns of activated oncogenes. Different patterns

were also seen in rat tumors that developed spontaneously compared with lung tumors induced by chemical agents.

EPA's 1986 guidelines stated that information on genotoxicity provides supportive evidence of carcinogenicity and may indicate the mode of action. In the 1996 proposed guidelines, the genotoxic nature of an agent is evaluated within the concept of mode of action, which is an integral part of the cancer risk assessment process. The 1996 proposed guidelines also noted that mutagenic chemicals usually induce tumors across species and at multiple sites, with both situations increasing the level of concern about a chemical's carcinogenic potential in humans. Issues of evaluating nongenotoxic substances for potential carcinogenicity in humans are interrelated with issues of cell proliferation and mode of action. The technical review workshop reviewer who evaluated EPA's proposed guidelines listed three types of genotoxic effects as subsets of mode of action: direct mutagenic effects, indirect mutagenic effect, and heritable epigenetic effects (Eastern Research Group, 1994).

To establish the genotoxic and nongenotoxic modes of action, it is necessary to develop criteria for judging the genotoxicity of an agents and determine how much evidence is sufficient to determine that an agent is genotoxic.

5.1.3. Carcinogenicity as a Manifestation of Cell Proliferation or Toxicity

Huff (1993) analyzed site-specific cell proliferation/toxicity and carcinogenic responses in long-term toxicity studies using rodents. He evaluated 53 chemicals tested in male and female Fisher rats and B6C3F$_1$ mice producing a total of 207 carcinogenic responses. He examined proliferative, toxic, and carcinogenic responses to specific chemicals (1,4-dichlorobenzene and furan), and evaluated specific target organs (liver and kidney). Huff (1993) concluded that "toxicity findings from higher exposures in short-term experiments or observed toxicity in long-term experiments (e.g., kidney) cannot be used mechanistically to either predict eventual carcinogenicity or to advocate toxicity and resultant sequelae as a mechanism of tumor development." He, therefore, questioned the notion of using cellular proliferation and toxicity measured for 1 week as a means for establishing the mechanism for liver carcinogenesis; he also noted that the species and sex showing liver toxicity and hepatocyte proliferation may not be the one showing a carcinogenic response. Huff (1993) also noted that spontaneous chronic nephropathy could not be correlated with renal carcinogenesis in rats. The high incidence or severity of nephropathy is not necessarily associated with a high incidence of renal cancer. From his evaluation, Huff (1993) reached six major conclusions concerning toxicity and carcinogenicity in long-term rodent studies:

1. Only 7 of the 53 carcinogenic chemicals produced target organ toxicity at all carcinogenic sites.

2. Only three chemicals showed carcinogenicity at the highest dose without corroborating evidence at lower doses (refutes the "high-dose-only carcinogen" theory).

3. The number of chemicals with a possible "indirect or secondary mechanism" (i.e,, toxicity) is small.

4. There is no uniform correlation between induction of toxicity and carcinogenicity.

5. Chemicals evaluated for long-term toxicity and carcinogenicity fall into three categories:
   a. those causing organ toxicity without cancer,
   b. those causing cancer without associated target organ toxicity,
   c. those causing both site-specific toxicity and carcinogenicity.

6. To separate chemicals by mechanisms of carcinogenicity (e.g., primary and secondary) for risk assessment purposes is premature

According to Huff (1993), it "would be premature and probably incorrect to make public health decisions on the basis of skimpy scientific data regarding the influence of cell proliferation *per se* on the carcinogenesis process." He further stated that cell proliferation had an influence on carcinogenesis, but existing data do not support the hypothesis that increased cell proliferation leads to or causes cancer, and there are no data suggesting that a noncarcinogenic chemical can be made carcinogenic by enhancing cell proliferation of a normal tissue. He asserted that more and better data are needed to establish the relationship between cell proliferation, toxicity, and carcinogenicity.

Ward et al. (1993) also looked at the correlation between toxicity, cell proliferation and carcinogenicity at specific sites (nasal cavity, liver, kidney, skin, and urinary bladder) and showed many instances where toxicity and cell proliferation did not lead to carcinogenesis. Ward et al (1993) suggested several reason for the lack of correlation: (1) cell proliferation does not occur in the stem cell population, which is an important target for carcinogens; (2) the sustained cell proliferation occurs before preneoplastic cells or foci appear, then the effects of cell proliferation are minimized; (3) cell proliferation may not play a role in some specific cases or in all cases in which it occurs.

Haseman (1985) also noted that, when considering the relationship between cytotoxicity and carcinogenicity, tissue damage does not always lead to carcinogenicity. Griesemer (1992) noted the lack of correlation between sites of early or late toxic effects and carcinogenesis in animal studies.

In contrast, Moolgavkar (1993) presented two reasons for a role of cell proliferation in carcinogenesis. First, the increase in cell proliferation leads to an increase in mutation frequency and consequently an increase in the risk of cancer. Second, the increase in cell proliferation relative to cell differentiation or cell death results in a larger population of cells susceptible to malignant transformation. He concluded that programmed cell death and cell proliferation are important determinants of cancer risk.

Cohen and Ellwein (1993) stated that the bladder carcinogen *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) and the bladder and liver carcinogen 2-acetylaminofluorene (AAF) induce cell proliferation in the bladder epithelium at the high doses and are genotoxic after metabolic conversion to a reactive intermediate producing adducts. Carcinogenicity is not detected at doses below that which induces cell proliferation, although with AAF, DNA adducts were detected in the bladder epithelium at very low doses. In the liver, however, carcinogenicity occurred at doses below those associated with cell proliferation. Cohen and Ellwein (1993) concluded that a carcinogenic response was not observed in the bladder at the low genotoxic doses because of the detection power of the bioassay was limited; therefore, a true threshold was not established. In contrast, tumors induced by implantation of a foreign substance or the formation of calculi in the bladder lumen appear to act by a true threshold mechanism associated with the induction of cell proliferation. How can we be sure that detection limit of a bioassay is not a factor in observing a carcinogenic response with other agents considered to have a threshold?

The nasal cavity is another site in which carcinogenesis has been linked to cell proliferation. Monticello et al. (1993) reported that regenerative cell proliferation in the nasal cavity is clearly involved in carcinogenic response to formaldehyde, but it is not the only determinant of nasal carcinogenesis. Other toxicants, such as dimethylamine, that induce cell toxicity, inflammation, and squamous metaplasia, do not induce nasal tumors (Monticello et al., 1993). Additional data are needed to show the relationship between toxicity, epithelial proliferation, and carcinogenesis in the nasal cavity. A simple measurement of cell proliferation is inadequate for determining whether exposure to a chemical may be associated with increased cancer incidence (Short, 1993).

Rapid proliferation of cells has been postulated to increase the probability of a mutation occurring in the cells. Although this hypothesis is widely accepted as a mode of action for carcinogenesis, there is no evidence for an increased frequency of mutations in rapidly proliferating cell populations. Since no mutations have been detected as a result of cell proliferation, what is the role of cell proliferation in carcinogenesis?

5.1.4. Weight-of-Evidence Classification or Hazard Description

Hazard assessment usually concludes with a categorization of the evidence based on the degree to which the risk assessor believes the data support a causal association between cancer and exposure to a substance. Chemical classification has been the subject of concern, particularly the issues of whether chemicals should be classified, at what stage of the risk assessment they should be classified, and the basis for the classification. Anderson et al. (1993) stated that hazard conclusions should be delayed until the hazard assessment, dose-response assessment, and exposure assessment are brought together for an integrated summary. Scientists have questioned the "carcinogen" label placed on substances, because of the societal impact of this label (Harvard Center for Risk Analysis, 1994)

In EPA's 1986 guidelines, the classification of potential carcinogens was based on the a, B, C scheme similar to that used by IARC. This scheme is based on a three step approach, (1) determining the level of evidence in humans and animals (2) combining the level of evidence in humans and animals for a tentative categorization, and (3) applying supporting evidence to determine if the tentative categorization should be changed.

In one of its working paper (U.S. EPA, 1992a), EPA proposed a classification scheme whereby human and animal evidence of carcinogenicity would be classified (or weighed) separately. Briefly the categories for human evidence were described as follows: category 1- plausible evidence; category 2 - suggestive evidence; category 3 - inconclusive evidence; category 4 - evidence of noncarcinogenicity. Similar descriptive categories were proposed for animal evidence. Categorizing the animal evidence included an evaluation of the supporting evidence (short-term studies, genotoxicity data, structure/activity relationships, etc.) . The working group noted that no weight should be given to animal evidence not considered relevant to establishing potential human carcinogenicity. Therefore, relevancy would be included in the initial evaluation of the data instead of later in the classification. The final step in hazard characterization proposed by the working group was an overall route-specific weight-of-evidence scheme that would combine the categories for human and animal evidence. The hazard descriptors for the overall weight of evidence would be "*known*" (category 1 human evidence); "*highly likely*" (category 2 human evidence plus category 1 animal evidence, or strong category 1 evidence for animals, or "known" by one route and absorbed by another route); "*likely*" (category 1 animal evidence that is persuasive or category 2 for both human and animal evidence); "*some evidence*" (category 2 human or animal evidence); "*not likely*" (category 4 human or animal evidence). The hazard descriptor would be accompanied by a short hazard narrative that would characterize the evidence, discuss mechanism of action, and suggest an approach for the dose-response assessment. This

hazard classification scheme is rather confusing and has been replaced by the categories described in the 1996 proposed guidelines.

The NRC/NAS (1994) Committee recommended establishing four categories based on strength of evidence and relevance to humans: Category I - the substance might pose a carcinogenic hazard to humans under any condition of exposure; Category II - the substance might pose a carcinogenic hazard to humans, but under limited conditions of exposure; Category III - not likely to pose a carcinogenic hazard to humans under any conditions; Category IV - available evidence demonstrate a lack of carcinogenicity or no evidence available. This last category Implies that "no data" situations would be equivalent to evidence of noncarcinogenicity. The technical review workshop (Eastern Research Group, 1994) recommended that EPA adopt the scheme proposed by the NRC Committee.

Ashby et al. (1990) proposed a classification scheme consisting of eight categories. The classification considered human studies, animal bioassays, supportive evidence from bioassays, mechanistic studies for establishing potential human cancer hazards. Category 1: *"known human carcinogen"* - sufficient evidence for human carcinogenicity (a causal relationship is demonstrated between exposure to an agent and human cancer). Category 2: "*carcinogenic activity in animals; probable human carcinogen*" - limited evidence of carcinogenicity in humans (a causal relationship is observed, but biases and confounders cannot be ruled out) and sufficient or limited evidence in animals along with evidence showing the carcinogenic response is relevant to human. Category 3: "*possible human carcinogen*" - limited evidence of carcinogenicity in humans and animals with some supporting data (genotoxicity, DNA reactivity, metabolism, mechanism of action, structure/activity relationships, etc.); or inadequate evidence of carcinogenicity in humans, sufficient evidence in animals, inadequate evidence on relevancy; or strong evidence for lack of carcinogenicity in humans (several adequate studies showing no association between exposure and cancer), sufficient evidence in animals with positive evidence that animal studies are relevant to humans. Category 4: "*equivocal evidence for carcinogenic activity*" - inadequate evidence of carcinogenicity in humans and limited evidence of carcinogenicity in animals with little or no evidence of relevancy to human. Category 5: "*evidence inadequate for classification*" - inadequate human evidence and inadequate or suggestive evidence of noncarcinogenicity in animals. Category 6: "*carcinogenic activity in animals; probably not a human cancer hazard*" - human evidence inadequate or suggestive of noncarcinogenicity; sufficient or limited animal evidence with evidence from humans or experimental animals indicating that animal studies are not likely to be predictive for humans. Category 7: "*carcinogenic activity in animals; considered not a human cancer hazard*" - human evidence inadequate or suggestive of noncarcinogenicity; sufficient or

limited animal evidence shown not to be predictive of a human response by strong evidence from human or experimental animal studies. Category 8: "*evidence of noncarcinogenicity*" - valid information exist indicating that the agent lacks carcinogenic activity.

EPA's weight-of-evidence categories in the 1996 proposed guidelines are very different from those describe in the 1986 guidelines; the categories are also simpler and more straight forward than those described in the working paper (U.S. EPA, 1992a). All the evidence is brought together in a hazard narrative that would include the hazard descriptor or category (*known/likely*, *cannot be determined*, and *not likely*) designated by route of exposure. The *known/likely* category includes agents for which there is sufficient causal evidence of carcinogenicity from epidemiologic studies as well as agents for which there are no human data. The guidelines include a subcategory, *treat as if known*, for evidence showing "plausible", but not definitive, causal association between exposure to an agent and human carcinogenicity and strong evidence from animal studies. The question is whether the descriptor, *known*, should be used for agents for which the evidence from humans studies is not definitive. Some exceptions should be considered, such as chromium IV, which should include all chromium IV compounds, arsenic and its compounds, and other similar agents. The guidelines place the descriptors, *known* and *likely,* in the same category. Considering the degree of uncertainty in the levels of evidence for the two descriptors, should these two descriptors be placed in the same category or put into two separate categories? The descriptor, *not determined*, encompasses evidence levels ranging from "suggestive" or "equivocal" to "no data." There is no clear category or descriptor for agents in which the evidence is not sufficient for a *likely* descriptor, but is believed to pose a hazard because the evidence shows a strong reason for concern for potential carcinogenicity (e.g., only one available study in one sex and species showing carcinogenicity and genetic toxicity data are limited). Should the guidelines include an additional category for suggestive evidence or for agents showing strong reasons for concern?

5.1.5. Maximum Tolerated Dose

The concept of maximum tolerated dose (MTD) is incorporated into risk assessments to ensure that animal studies evaluated for carcinogenicity attain the sensitivity to detect a carcinogenic response given that only a limited number of animal can be used for testing. Testing at doses considered to be too high or too low serves as a reason for rejecting studies for risk assessment purposes. Therefore, a discussion on the role of maximum tolerated dose (MTD) in risk assessment deals to a large degree with the science policy of carcinogen testing. The primary basis for testing at the MTD is to minimize the chances a "carcinogen" remaining undetected and to compensate for using a small number of animals because of limited statistical power to detect

a significant increase in tumor incidence (Carr and Kolbye, 1991). However, the highest doses tested should cause no undue toxicity that would confound the interpretation of the study.

Haseman (1985) noted that some of the confusion about MTDs centers around the definition of an MTD. Haseman and Lockhart (1994) presented the definition used as the basis for the dose selection (selecting the top dose) process in the National Toxicology Program's (NTP) testing protocol. The NTP's definition came from Sontag et al. (1976) and is defined as the "highest dose of the test agent during the chronic study that can be predicted not to alter the animals' normal longevity from effects other than carcinogenicity." Haseman (1985) stated that the Sontag et al (1976) definition included the caveat that, in the subchronic study, the doses selected should not cause more than a 10% decrement in body weight compared with appropriate controls. Carr and Kolbye (1991) included a weight loss of no more than 10% in their definition of MTD. Currently, in NTP studies, body weights do not factor as prominently in selecting the MTD as do other signs of toxicity, such as development of nonneoplastic lesions and their prediction to be life threatening during long-term treatment (Haseman, 1985). Haseman and Lockhart (1994) stated that the NTP prefers to use the term "minimally toxic dose" for its dose selection process. Overall, the selection of the top dose for long-term NTP studies includes data on body weight, survival, histopathology, clinical and pharmacologic signs, and metabolism and disposition information obtained from 90-day studies (Haseman and Lockhart, 1994). Some toxicity at the top dose in a long-term study confirms that the animals have been sufficiently challenged (Haseman, 1985).

The definition of MTD as presented in EPA's working paper (U.S. EPA, 1992a) was "a dose which is estimated to produce some minimal toxic effects in a long-term study (e.g., a small reduction in body weight), but should not shorten an animal's life span or unduly compromise normal well-being except for chemically induced carcinogenicity."

In attempting to maximize the ability to detect weak carcinogens, other problems of organ toxicity and possibly tumor promotion can distort the interpretation of the results (Carr and Kolbye, 1991). Therefore, one criticism of using the MTD to select the top dose for carcinogenicity studies is organ toxicity, which is manifested by cell killing and regenerative hyperplasia, producing conditions having no relevance to humans exposed to lower doses (Haseman and Lockhart, 1994).

"Metabolic overloading" and/or "secondary carcinogenesis" may be reasons for rejecting the MTD concept of dose selection (Haseman, 1985). Carcinogenesis associated with metabolic overloading is caused by saturation of the detoxification mechanism. Secondary carcinogenesis is due to induction of excessive nonspecific tissue damage. Haseman (1985) stated that a

distinction should be made between saturation of the activation pathway and saturation of the detoxification pathway. He also stated that, if increases in tumor incidences at high doses are to be explained away because of metabolic overload or secondary carcinogenesis, a direct cause and effect relationship should be established between these factors and tumor induction (i.e., one must show how the overload produces carcinogenic effects). It is not enough just to show that the overload occurs.

Another criticism of testing at the MTD is that positive evidence for carcinogenicity would not have been obtained for two-thirds of the NTP carcinogens if the top dose had been excluded (NTP, 1992). According to Griesemer (1992), this conclusion was based on a misinterpreted report by Haseman (1985). Haseman (1985) concludes that, if the high dose had been reduced from the MTD to ½MTD, then two-thirds of the carcinogenic effects in feeding studies would be eliminated. Eight of 13 chemicals were judged to be carcinogenic based only on effects at the top dose. On the other hand, some equivocal results would have been regarded as real carcinogenic effects if the top dose had been excluded. After evaluating the results of 216 chemicals tested by the National Cancer Institute (NCI) and NTP, Haseman and Lockhart (1994) concluded that, even without the top dose, two-thirds of the carcinogens would have been detected, but not some of the site-specific effects.

A third concern regarding the MTD concept is the overestimation of the high dose resulting in excessive toxicity and mortality. Haseman (1985) countered this argument by noting that the standard dosing protocol for NTP studies is to include three doses (MTD, ½MTD, and ¼MTD), insuring that if unanticipated chronic toxicity is seen at the top dose, the two lower doses provide a margin of experimental safety. In addition, in 31 NTP feeding studies, survival of high-dose animals generally exceeded that of controls, and body weights were reduced in some experiments, but not enough to suggest that the MTD had been exceeded (Haseman, 1985).

Carr and Kolbye (1991) recommended that the MTD be replaced by a minimally toxic or the highest subtoxic dose that can be tolerated over a long period of time. The high dose would not produce life-shortening, weight loss, or demonstrable organ or tissue toxicity. However, long-term studies often serve a dual purpose – documentation of carcinogenicity as well as chronic toxicity. If no effects are produced at the highest dose in long-term toxicity studies, then chronic toxicity cannot be documented. Carr and Kolbye (1991) further stated that nonneoplastic toxicity could be assessed by testing at high doses achieving minimal toxicity; they further stated that carcinogenicity may be grossly exaggerated at minimally toxic doses. OSTP (1985) stated that pharmacokinetics and metabolism should be included in the dose selection process. Butterworth et al. (1991)

proposed that cell proliferation in 90-day studies be used as an additional criterion for selecting doses in long-term studies.

OSTP (1985) stated that the high dose should maximally enhance the sensitivity of the test without introducing qualitative distortion of the results. In its 1986 guidelines, EPA (U.S. EPA, 1986) stated that "long-term animal studies at or near the maximum tolerated dose (MTD) are used to ensure an adequate power for the detection of carcinogenic activity." The agency further noted that carcinogenic responses at doses exceeding the MTD should be review carefully as to their relevancy to humans. In the 1996 proposed guidelines, EPA (U.S. EPA, 1996b) asserted that failure to reach adequately high doses reduces the sensitivity of the study to detect a response, whereas overt toxicity due to excessive doses raises questions about the specificity of the response, whether it is related to exposure or to secondary toxic effects. Findings that confound the interpretation of studies are significant mortality not due to cancer; body weight decrements greater than 10%; and significant toxicity manifested by clinical signs, clinical chemistry and hematological changes, organ weight changes, and gross and histopathologic changes. The 1996 proposed guidelines presented general guidance for evaluating the dosing protocol in animal studies. The guidelines also asserted that studies showing excessive toxicity "are generally not suitable for risk extrapolation." It appears that the guidelines have adequately addressed this issue and provided guidance for evaluating dosing issues in carcinogen risk assessment. It should be noted that long-term animals studies serve a dual purpose, that of documenting systemic toxicity as well as carcinogenicity. Because of the extensive resources required to conduct these studies, it may be necessary to compromise on certain issues. Therefore, definite toxicity at the high dose is necessary to document the two types of responses in a study.

5.1.6. Dosimetry: Pharmacokinetic or Toxicokinetic Modeling

Pharmacokinetic models can be used in both cancer and noncancer assessments to estimate internal and delivered doses and to extrapolate doses across species when empirical data are available. EPA's 1986 cancer guidelines state that "In the absence of comparative toxicological, physiological, metabolic, and pharmacokinetic data for a given suspect carcinogen, extrapolation on the basis of surface area is considered to be appropriate because certain pharmacological effects commonly scale according to surface area." The 1996 proposed cancer guidelines takes the position that available data are evaluated to reach a measure of internal or delivered dose. The inhalation RfC methodology recommends pharmacokinetic models for route-to-route extrapolations and estimating human equivalent doses (U.S. EPA, 1994a). These models are an improvement over using the default dose scaling method for cancinogen dose-response assessments or the default RDDR or RGDR methodology for inhalation toxicants. EPA has not

established guidance for determining when data are sufficient to apply pharmacokinetic models to estimate internal or delivered doses.

The data base on pharmacokinetic models is too extensive to be discussed here. These models are applied in risk assessment on a case-by-case basis.

5.1.7. Dosimetry: Default Dose Scaling Methods

Dose-response data from human studies are seldom available for quantitating risk due to exposure to environmental substances; therefore, it is necessary to use the data available from animal studies. The human dose capable of producing a response similar to that of experimental animals can be estimated by one of two ways: case-specific pharmacokinetics data or in the absence of data, a default method, which extrapolates or scales animal doses to equivalent human doses. In the 1986 carcinogen risk assessment guidelines EPA's default dose-scaling method was based on evidence that "certain pharmacological effects commonly scale according to surface area" (U.S. EPA, 1986) and the proportionality of body surface area to the ⅔ power of the body weight (U.S. EPA, 1980, 1986). The resulting equation for calculating the human equivalent dose is as follows: HED = animal dose (mg/day) × (human weight (kg)/animal weight (kg))$^{2/3}$ or HED = animal dose (mg/kg/day × (animal weight (kg)/human weight (kg))$^{1/3}$. Scaling based on body surface area is considered to be the most accurate method of scaling (Calabrese, 1991); however, measuring individual surface areas is inherently difficult (Calabrese, 1991) and, therefore, not feasible. Calabrese et al. (1992) stated that dose adjustments using surface area results in similar blood levels of the substance reaching potential target organs, but some principal causes for intraspecies difference in response are not addressed (metabolic and pharmacodynamics factors). Travis and White (1988) analyzed data on direct acting carcinogens and reported that interspecies dose scaling should be based on body weight raised to the ¾ power.

In the proposed guidelines (U.S. EPA, 1996b), EPA adopted the default dose scaling procedure based on body weight raised to the ¾ power. The basis for the change in the default scaling method can be found in EPA's 1992 Federal Register report (U.S. EPA, 1992b). This scaling method takes into account both toxicokinetic (area under the concentration curve (AUC) and toxicodynamic (mechanistic data) parameters such that lifetime equivalent doses required to produce lifetime equivalent responses among species are predicted. Calabrese (1991) also presented a detail discussion on cross-species extrapolation. EPA considers dose scaling based on body weights to the ¾-power is a more scientifically defensible default method than the ⅔-power scaling, and it is amenable to incorporation of case-specific data (U.S. EPA, 1996b).

5.1.8. Low-Dose Extrapolation (Estimation of Risk at Low Doses)

Gad and Weil (1986) described three basic steps to low-dose extrapolation: (1) identifying the dose-response data points, (2) selecting a mathematical model to extend to observations from the experimental region to the region of concern, and (3) making a basic assumption about the nature of the dose-response relationship at extremely low doses. Steps 2 and 3 should be reversed; assumptions about the nature of the dose-response relationship at low doses should precede selecting the model to extend the data to low doses. Gad and Weil (1986) stated that the assumption about the low-dose region concerns the question of threshold. EPA's default positions are related to the nature of the dose-response relationship(linear, nonlinear, or both (U.S. EPA, 1996b). A threshold concept is encompassed within the nonlinear dose-response relationship.

Gad and Weil (1986) presented some arguments for and against the existence of a threshold for carcinogens. The arguments against a threshold are as follows: (1) a single molecule of a chemical can mutate a cell, *in vitro*; (2) the presence of other agents in the environment may act as a promoter for the agent of concern or saturate the existing defense mechanism; (3) a threshold would preclude a linear dose response; and (4) there may be thresholds for some or most individuals in a population, but, not for all individuals, i.e., there are no exposures that are absolutely safe for absolutely everyone. The arguments for a threshold are as follows: (1) most carcinogens and mutagens exhibit a dose-response relationship, which show an apparent threshold for some agents; (2) toxicity, including carcinogenicity, is a result of pharmacokinetic processes (absorption, distribution to tissues, reaction with cellular components, adaptation and repair by molecular and cellular components, and clearance from the body by metabolism and/or excretion), which are linear only within particular ranges, and metabolic thresholds may lead to disproportionate increases in toxicity at certain dose levels; (3) a biological threshold is suggested based on probabilistic grounds, because the probability of a "hit" by a carcinogen producing an initiating or promoting effect is low; (4) as the dose decreases the time-to-tumor increases, which could eventually result in a time-to-tumor exceeding the life span of the exposed population; and (5) there are physicochemical factors (or mechanisms) that cause some agents to be carcinogenic above certain doses only. Cohen (1981) stated that the justification for the linear-no-threshold model leaves much to be desired and the evidence does not support it at low doses. Wilkinson (1987) stated that extrapolation below the experimental range often encompasses four to five orders of magnitude and that most areas of science would not attempt such an extrapolation. He further stated that, from a practical standpoint, thresholds must exist.

Low-dose extrapolation is an essential element of quantitative risk assessment, because exposure levels encountered in epidemiologic (usually occupational exposure) and animal studies

do not achieve the low level environmental exposures experienced by the general population. To achieve statistical significance of response to such low levels, animal studies would require vast numbers of animal and economical resources so as to make such studies unfeasible. Consequently, mathematical models have been developed to extrapolate experimental doses to low level environmental exposures. The conceptual basis for the low-dose extrapolation is intermixed with the linear nonthreshold concept of carcinogenesis. In one of it principles, OSTP (1985) stated that "mechanistic considerations such as DNA repair and other biological responses, in general, do not prove the existence of, the lack of existence of, or the location of a threshold for carcinogenesis." OSTP (1985) further noted that no single mathematical model can be used for low-dose extrapolation, but the model chosen should be consistent with the evidence. However, when data are limited, models incorporating low-dose linearity are preferred. In its 1986 guidelines, EPA's default procedure for low-dose extrapolation was the linearized multistage model (a mathematical curve-fitting model based on a nonthreshold concept). The Agency noted in its guidelines, that the model selection should be consistent with the evidence. The linearized multistage model estimates the upper limit on the risk at low doses; however, according to the EPA (U.S. EPA, 1986), the true risk is unknown and could be as low a zero.

In the 1996 proposed guidelines, dose-response assessment is conducted in two steps: modeling within the experimental range to determine the LED_{10} or another point of departure and extrapolation below the point. Biologically-based or case-specific models are used when sufficient data are available for both steps, otherwise curve-fitting models are used for the experimental range and a straight line extrapolation from the point of departure to the origin is used for the linear approach; the margin of exposure procedure is used for the nonlinear approach. The linear approach produces a probabilistic risk of cancer and the nonlinear approach produces a ratio between the dose at the point of departure and actual environmental exposures.

The technical review workshop reviewers (Eastern Research Group, 1994) noted that the linearized multistage or other low-dose extrapolation procedures are generally inappropriate for "extrapolating risk from the upper-bound confidence intervals and dose from the lower-bound confidence intervals....."; the reviewers agreed with EPA's proposal to perform a simple straight line extrapolation to the zero response, use the linear procedure when sufficient data for applying other procedures are not available (default position), and use nonlinear procedure only when extensive data are available to support a nonlinear procedure.

Although the risk manager determines whether the margin of exposure is adequate, the risk assessor should recommend an adequate margin of exposure and the basis for the

recommendation. The 1996 proposed guidelines discussed some issues that should be considered in determining what is an adequate margins of exposure. There are other issues, in addition to those discussed in the guidelines, that should be considered.

The 1996 guidelines discusses the use of factors (as used for RfD and RfC derivation) applied to the point of departure in the analysis of the margin of exposure (no less than 10 for intraspecies variability and 10 for interspecies sensitivity). It should be pointed out that human equivalent doses estimated by either default or toxicokinetic models are incorporated into general curve-fitting or chemical-specific procedures to determine the LED_{10}. Therefore, elements for species sensitivity are already incorporated into the LED_{10}. To derive an RfD, experimental animal doses are usually adjusted by a factor of no more than 10 (a factor of 3 is applied for RfC derivation); therefore, a factor of "no less than 10-fold", as recommended in the proposed cancer guidelines (U.S. EPA, 1996b), may be too large to account for interspecies sensitivity. Additionally, applying a factor of "no less than 10-fold", as recommended by the cancer guidelines (U.S. EPA, 1996b), to account for human variability, implies that the LED_{10} (95% confidence limit on dose) is exclusive of the sensitive population. The factors applied to margin of exposure analysis are similar to the uncertainty factors applied to RfD/RfC derivations. The guidelines did not explain how an analysis of intraspecies and interspecies considerations would be applied to the margin of exposure analysis. Nevertheless, a margin of exposure of 100 would not be protective of sensitive populations, because the environmental dose would correspond to a 10% response in the sensitive population whether the response measured is tumor induction of a precursor lesion. Applying factors of 10 each to account for interspecies and intraspecies variability does not correspond to reduction in the response level, but only for intraspecies and interspecies differences.

5.1.9. Dose-Response Model Selection

In the 1996 guidelines, EPA proposes that curve-fitting models be used to model data in the experimental range when biologically-based or case-specific data are not available. EPA, however, did not recommend a default curve-fitting model to be used. Perhaps a default curve-fitting model should be used except when data is best described by another model. Several mathematical models have been described by Gad and Weil (1986) and Johannsen (1990). These models have been applied to low-dose extrapolation, but could be used to model data in the experimental range. Gad and Weil (1986) discussed seven models and Johannsen (1990) discussed ten. The various models can be placed in the following categories (examples of each model): linear, mechanistic (one-hit, multihit, multistage, linearized multistage), tolerance distribution (probit, logit, and Weibull), time-to-tumor (Weibull distribution), and biologically

motivated (Moolgavkar, Venzon, and Knudson(MVK)). Johannsen (1990) also ranked the conservativeness of some of the mathematical models applied to low-dose extrapolation as follows: one-hit > linear > multistage » Weibull > multihit » logit > probit.

5.1.10. Uncertainty Analysis

NRC/NAS (1983) noted that one of the inherent limitations of risk assessment is the pervasive uncertainty due to incomplete data sets and the estimates of types, probability and magnitude of health effects associated with exposure to chemical agents. The NRC/NAS (1994) Committee placed considerable emphasis on uncertainty analysis in risk assessment. The Committee's main concern regarding EPA's lack of quantitative uncertainty analysis in its risk assessment process appears to be the difficulty in determining the degree of conservatism in the risk estimate. The Committee recommended that EPA conduct a formal uncertainty analysis, identify errors of either overestimation or underestimation, and develop guidelines for quantifying and communicating uncertainty.

In the 1996 proposed guidelines EPA discussed two types of uncertainty: model and parameter. Model uncertainty, which deals with biological questions, is described qualitatively or by presenting alternative results when more than one extrapolation model can describe a particular data set. Parameter uncertainty, which deals with statistical or analytical measures of variance in data set or estimates, are described quantitatively.

5.2. Noncancer Risk Assessment

5.2.1. General Guidelines for RfD/RfC Derivation

The NOAEL/LOAEL approach to deriving risk values for noncancer effects have traditionally made little use of the biological data available for chemical assessments. Very little quantitative use is made of the wealth of information available on some agents, because the assessment, although based on scientific judgment, is reduced to selecting a critical effect (one effect seen at the lowest dose) and applying predetermined uncertainty factors to the largest dose at which the critical effect does not occur. Biological data on mode of action data and dose-response relationships have traditionally been exempt from assessments of noncancer effects. Few epidemiologic studies are available for assessing noncancer studies in humans, and the more detailed scientific analysis of animal models to determine the likely occurrence of a response in humans have not received the attention as in cancer assessments. Part of the problem is that noncancer data are often presented in a format that cannot be readily incorporated into dose-

response models, but could be readily used for the NOAEL/LOAEL approach for noncancer assessments

Reference values are derived by applying uncertainty factors to NOAELs and LOAELs to account for the inherent scientific uncertainty (U.S. EPA, 1991b). They address issues of variability in the human population, the existence of sensitive subpopulations, and the differences (pharmacokinetic and pharmacodynamic) between humans and animals (Calabrese et al., 1992). An uncertainty factor of 10 for interspecies variations (U.S. EPA, 1993) is intended to account for toxicokinetic and toxicodynamic uncertainties (U.S. EPA 1991b). Dourson and Stara (1983) analyzed the 490 probit, log-dose slopes for acute lethality presented by Weil (1972) and concluded that a 10-fold uncertainty factor to account for intraspecies variability was reasonable when chemical specific data are not available. Dourson and Stara (1983) also concluded that a 10-fold decrease in animal dose was adequate to adjust to human doses in the absence of chemical-specific data if the assumed dose equivalence based on surface area is correct. They noted a need for additional investigation on this subject.

Calabrese et al. (1992) compared dose adjustments based on surface area with the application of uncertainty factors. They found that for the mouse, a tenfold uncertainty factor would not be as protective as the surface area adjustment of the dose; whereas a tenfold uncertainty factor for the rat would be more protective than surface area adjustment of the dose. They concluded that humans are likely to be more protected if the critical study for deriving a reference value is based on a large-sized animal. According to Calabrese et al. (1992), EPA's argument that the animal-to-human uncertainty factor incorporates the surface area normalization (1) has inherent toxicologically based contradictions, (2) has an inadequate theoretical foundation, (3) differentially protects the public according to the animal model, and (4) includes no interspecies uncertainty factor for non-surface area normalization for the mouse.

Gaylor (1983) noted that the use of safety factors (uncertainty factors) results in an uneven control of risk depending on the number of animals used in a study. As an example, he noted that if the same percentage of positive responses are seen in a study using 20 animals per dose and one using 60 animals, the study using only 20 animals (lower statistical power resulting in a higher NOAEL) would receive the same uncertainty factor and result in a higher reference value. This example is critical of using uncertainty factors applied to experimental no effect levels. Gaylor (1983) recommended that uncertainty factors should be applied to a dose that would results in a risk below that of the no effect level (e.g., ED_{01}, the dose at which there is a 1% response compared with controls). Because this dose is not easily determined experimentally, Gaylor (1983) suggested that a curve-fitting model be used to determine the upper confidence limit on the risk

at ED_{01}, which could be used as an estimate of the uncertainty reflected by the sample size. EPA uses the lower confidence limit on dose instead of the central tendency in the benchmark approach.

Another major criticism of the present method for deriving reference values is that the NOAEL/LOAEL approach does not use all of the dose-response information. In addition, the NOAEL must come from the experimental data, and dose spacing can greatly influence the outcome of the evaluation (U.S. EPA 1991b). The benchmark procedure for deriving reference values has become a primary focus of the Agency in addressing some of the inherent limitations of the NOAEL/LOAEL approach (U.S. EPA, 1991b). The benchmark approach involves using a dose-response (curve-fitting) model to estimate the lower confidence limit on the dose (generally the LED_{10}, LED_{05}, or LED_{01}). This procedure is similar to that described by Gaylor (1983) and the issues are discussed in more detail in section 5.2.2.

5.2.2. Benchmark vs NOAEL/LOAEL Approach to Dose-Response Assessment

The BMD approach to noncancer risk assessment has the potential of incorporating more biological data into the assessments and is also be more comparable to cancer assessments. The robust databases available for some chemical agents will result in reductions in uncertainties and increases in the confidence in the risk values obtained.

One of the major issues regarding benchmark dose and the NOAEL/LOAEL approaches is the application of uncertainty factors. When animal data are used to derive reference values, two primary uncertainty factors are applied, one accounting for intraspecies variability and the other accounting for interspecies sensitivity. Comparison of the LED_{10} with the ED_{10} (central tendency) may suggest that it is possible to reduce or eliminate the tenfold uncertainty factor accounting for intraspecies sensitivity. The 95% confidence limit on dose should account for some of the population variability of a response, but the dose for supersensitive individuals may fall outside the range of the 95% confidence limit. When a large segment of the population fall with the sensitive subpopulation as may be the case for developmental (fetus or young children) or reproductive effects (women or men during reproductive age), the BMD analysis can be based on these specific endpoints. When studies with adults show strong sex specificity, the BMD should be based on the most sensitive sex. Under these conditions, the RfD/RfC can be directly derived for the sensitive population, thus eliminating the need for an intraspecies uncertainty factor.

The uncertainty factor accounting for interspecies sensitivity may be reduced by using dose scaling to derive human equivalent doses similar to the method used for dose-response

assessments for cancer endpoints. The RfC methodology uses dosimetric adjustments for inhalation of particles and gases; this adjustment accounting in part for differences in interspecies sensitivity, resulted in a reduction of the uncertainty factor from 10 to 3 (U.S. EPA, 1994a; Jarabek, 1995a). EPA has a default dose scaling method ($BW^{3/4}$) when chemical-specific data on pharmacokinetics and metabolism are not available for carcinogen assessments. By using a similar method for noncancer assessments, the uncertainty factor accounting for interspecies sensitivity could be reduced. The precedence for using this method is based on the similarity of the benchmark approach to the nonlinear approach for cancer assessments.

Uncertainty factors accounting for intraspecies and interspecies sensitivities should not assume a reduction in the response level for the resulting doses. Therefore, an additional uncertainty factor should be incorporated into derivation of RfDs/RfCs, that of extrapolating from the LED_{10} to a safe dose, or a dose expected not to have adverse effects. The LEDs, which approximates the 95% statistical bound on the lowest-effect level, are effect levels whether they are based on 1, 5, or a 10% response, and should not be considered as no-effect levels. Therefore, a factor accounting for extrapolation to a non-adverse effect should be applied to the LED_{10}. Just as the slope of the dose-response relationship at the point of departure should be considered in deciding on an adequate margin of exposure (U.S. EPA, 1996b), the slope at the BMD should also be considered in the extrapolation to a dose expected to have no adverse effects.

The BMD has been compared with the NOAEL (Beck et al., 1993; Allen et al., 1994), and it is questionable whether this is a valid comparison. The benchmark method can be compared with the NOAEL/LOAEL method for deriving reference values, but the value of the BMD derived from a study should not be compared with the value of the NOAEL identified in the study. The comparisons may be invalid, because the BMD is a statistically derived value, whereas the NOAEL is empirically derived and is dependent on the statistical power of the study. However, it is valid to compare RfDs/RfCs derived by the NOAEL/LOAEL approach with the value derived using the benchmark approach.

Barnes et al. (1995) addressed a number of issues pertaining to using the benchmark approach to calculate BMDs and reference values. Some of the issues discussed concerned selection of dose-response models, use of a default dose-response model, analysis of non-quantal data, characterization of uncertainties in benchmark estimation, and application of uncertainty factors to the BMD.

The benchmark approach is a different perspective and could be an improvement over the NOAEL/LOAEL approach to deriving risk values for noncancer endpoints. There are advantages and disadvantages to using the benchmark approach; the disadvantages are due in part to the testing protocol, which confirm with the current NOAEL/LOAEL method of deriving noncancer risk values. The implementation of the benchmark approach may result in changes in the testing protocols that would provide data more easily incorporated into the benchmark methodology. The major advantage of the benchmark approach is that more of the available data can be used to derive risk values.

5.2.3. Developmental toxicity: maternal/developmental toxicity

Developmental toxicity effects at dose levels that result in pronounced maternal toxicity may be difficult to interpret. However, for risk assessment whether a developmental effect is secondary to maternal toxicity or not, does not affect the selection of the NOAEL. One approach for ranking substances according to their relative maternal and developmental toxicity involves the calculation of the ratio of the adult toxic dose to the developmental toxic dose (A/D ratio) (Johnson and Gable, 1983). However, comparison of A/D ratios for 14 chemicals found little agreement between four species (Daston et al., 1991).

5.2.4. Developmental toxicity: functional toxicity

Functional developmental toxicity endpoints may be used for establishing the NOAEL when these endpoints are found to be the adverse effect occurring at the lowest dose. Support of the use of behavioral assessment has been shown for human lead exposure data (Annau, 1990). However, debate still exists on weighting tests within the battery of functional neurotoxicity tests and whether observed effects are due to neurotoxicity alone or as part of overall developmental toxicity (Tyl and Sette, 1990).

5.2.5. Neurotoxicity: Endpoint Determination and Dose-response Assessment

Similar to most toxicants, hazard identification for neurotoxicants is complicated by uncertainty regarding the definition of adverse effect. Starting with the NAS definition of adverse effect, various modifications and refinements have been suggested including that of the EPA test guidelines (U.S. EPA, 1985) which states that a neurotoxic effect is "an adverse change in the structure or function of the nervous system following exposure to a chemical agent" and that of Spencer and Schaumberg (1985) defining neurotoxicity as "a consistent pattern of neurological dysfunction in humans, comparable dysfunction in animals, and reproducible lesions in animals/humans that are related to the neurobehavioral dysfunction expressed". Tilson (1990) suggested the following criteria for defining neurotoxicity: (1) side effects or overdose (unwanted

effects), (2) decreased ability to function fully or [provide] compensation in order to function normally, and (3) an alteration that diminishes the ability to survive, reproduce, or adapt to the environment.

Selecting the most appropriate toxicity endpoint for dose-response or exposure-effect determination remains a point of concern. Endpoint selection may be affected by various elements such as species, gender, and dosing regimen. Additionally the selection of an endpoint appropriate for the risk assessment process is often subjective and dependent upon the definition of "adverse effect". Neurotoxic effects reported in humans have been categorized by Tilson and Cabe (1978) and Reiter (1987) as: (1) sensory disorders, (2) cognitive disorders, (3) changes in CNS excitability, (4) autonomic dysfunction, (5) motor disorders, (6) sleep disturbances, (7) affective disorders, and (8) physiological alterations. Stanton and Spear (1990) identified more general groups for assessment of neurotoxicity: sensory, motivational, cognitive, motor, and social functional. Many of these categories may be functionally interrelated and some level of integration would be required for expression of the neurotoxic effect.

The U.S. EPA (1994b) notes that the selection of a toxicity endpoint is dependent upon the level of nervous system organization being investigated - biochemical, anatomical, physiological, or behavioral. General categories of neurotoxicity endpoints include behavioral (learning and memory, altered behaviors, etc.), neurochemical (changes in synthesis, release and uptake of neurotransmitters, alterations in membrane-bound enzymes relevant to neuronal activity, etc.), neurophysiological (changes in nerve conduction parameters, etc.), and structural (accumulation, breakdown, or rearrangement of structural elements, etc.).

Because of the subtleties of neurotoxic effects, and the uniqueness of the nervous system and its responses to toxic insults, the use of biomarkers and dose has been suggested as a method for hazard identification (Gaylor and Slikker, 1990). Specifically, this method involves four steps, the first three of which would pertain to hazard identification: (1) establishing a mathematical relationship between effect or biomarker and dose, (2) determining the distribution of the individual measurements of effect or biomarker around the dose-response curve, (3) establish a level of the effect or biomarker that would be considered abnormal or adverse, and (4) assess the proportion of individuals exceeding the adverse or abnormal level of the effect or biomarker as a function of dose.

Currently, dose-response assessments are made with the assumption that a threshold exists for a toxicologic response and that below this threshold there will be no significant response.

As previously noted, however, the commonly used NOAEL/LOAEL approach may not provide a true measure of the no-adverse-effect-level; there may be toxic responses below the adopted NOAEL or the NOAEL may actually be an overestimation of the true response level. Furthermore, this method does not make use of the complete dose-response curve. Alternative methods have been described (Gaylor and Slikker, 1990; Dews, 1986; Glowa and Dews, 1987; Glowa et al., 1983; Crump, 1984b) that make use of complete dose-response data and mathematical functions to estimate the variability in exposures or dose and effects. Because dose-response curves may not be linear for all chemicals, these alternative methods may provide more accurate and definitive assessments of the dose-response relationship.

5.2.6. Interspecies Extrapolation

Although not unique to neurotoxicants, the issue of interspecies extrapolation presents special problems regarding toxicity endpoints of cognitive function and behavior. For example, there is no direct animal counterpart for psychometric IQ but conceptual analogs can be developed to assess changes in neurobehavioral and cognitive functions in animals (Winneke, 1992). When extrapolating from animal data, quantitative assessments must address three basic problems: 1) identification of a relevant adverse effect, 2) high-to-low dose extrapolations, and 3) assessing dose equivalency (Rees and Glowa, 1994).

5.2.7. Reversibility

Although hazard identification principles are similar for neurotoxic effects as they are for other adverse effects, the phenomenon of reversibility requires special consideration. Unlike many organ systems, the nervous system exhibits considerable redundancy and plasticity but also lacks the repair potential of most tissues. The redundancy and plasticity often results in apparent recovery (reversibility) from a toxic insult when, in fact, damage has occurred that may become manifest at a later time or under different conditions.

5.2.8. Delayed Neurotoxicity

Organophosphate induced delayed neurotoxicity (OPIDN) guidelines have been established for pesticide assessment (U.S. EPA, 1991d). OPIDN assessment focuses on biochemical (e.g., inhibition of acetylcholinesterase and neurotoxic esterase) and behavioral changes that are known to occur several days to several weeks after acute and short-term exposure to organophosphates.

6. KEY ISSUES

A large number of issues pertinent to cancer and noncancer risk assessment have been discussed in this report. The following is a list of the most pertinent or key issues.

(1) Target organ concordance – Rodent species are most often used as surrogate for human carcinogenicity studies. Although there are species specificity in the targets affected by carcinogenic agents, multiple targets in one species often indicate that an agent will elicit a response in another species, but not necessarily at the same sites. Carcinogenic agents eliciting responses in multiple animal species at multiple target are more likely to be associated with carcinogenicity in human studies, no matter the sites affected. Rodent species have anatomical sites that have no analogues in humans (e.g., forestomach and Zymbal's gland) that are the targets for carcinogens. Sometimes these sites may be the only target in a rodent study. Is carcinogenicity occurring only in organs having no analogues in humans predictive of human cancer risk?

(2) Genotoxic carcinogens – Mode of action information is an important aspect of hazard assessment and is used to determine the dose-response procedure to apply to a particular agent, and genetic toxicity is pivotal is determining mode of action. There are many tests available for determining the genotoxicity of an agent. Which tests or endpoints are considered the most important for determining genotoxicity (e.g., DNA adduct formation, positive response in the *Salmonella* test, micronucleus test, dominant lethality, etc.) and how much weight should be given to a particular genotoxicity test. What are the criteria for determining genotoxicity?

(3) Role of cell proliferation in carcinogenesis – Induction of cell proliferation has been implicated in the mode of action of nongenotoxic carcinogens. It has been postulated that cell proliferation increases the probability of a mutational event. However, mutations have not been detected as a result of cell proliferation. Therefore, what is the role of cell proliferation in carcinogenesis? Could the reason for not detecting carcinogenesis at doses below those causing cell proliferation be due to the limited detection power of the rodent bioassay for weak carcinogens. Is cell proliferation alone sufficient for cancer induction?

(4) Route-to-route extrapolation – According to the 1996 proposed cancer guidelines, the only requirement for route-to-route extrapolation of hazard is to show absorption by another route to give an internal dose There are other factors that may determine whether cancer would be induced by another route. Because of first pass effects, metabolism and tissue distribution may

be different. The guidelines also stated that the hazard classification is route specific. Why allow route specific hazard classification along with liberal requirements for route extrapolation? A small change in the hazard categories could address the issue of route-to-route extrapolation of hazard. There is a need for a "*suggestive*" or "*evidence shows reason for concern*" category. Evidence of absorption of an agent by another route to give an internal dose is definite reason for concern for the second route. There are cases in which route-to-route extrapolation of cancer hazard and potency can be conducted with less uncertainty. EPA (U.S. EPA, 1996a) concluded, in its dose-response assessment of PCBs, that a cancer risk by skin contact and inhalation exposure is possible. This conclusion was based on absorption by both route, but most importantly was the slow metabolism rate and accumulation of PCBs in fat, would cause a slow release of PCBs over a prolonged period of time. Therefore, the slow metabolism and fat accumulation of PCBs would reduce the uncertainty associated with first pass effects.

(5) Curve fitting in the experimental range – The 1996 proposed cancer guidelines states that curve-fitting models can be use as the default procedure for modeling data within the range of experimental observation. Curve-fitting models can be used also for deriving BMDs for noncancer assessments. A variety of curve-fitting models available, including the multistage model, most of which can be used to model the data and estimate the LED_{10}. Unless there are compelling reasons to recommend another model, a default curve fitting model could be applied to experimental data, which would provide consistency in the risk assessments.

(6) Low-dose extrapolation: margin of exposure procedure for cancer assessments – A straight line approach is used for extrapolation below the point of departure when data are not available for applying a case-specific model and the mode of action suggest a linear procedure. When the mode of action shows evidence for a nonlinear procedure the margin of exposure approach is used for low-dose extrapolation. The risk manager determines whether the margin of exposure is adequate based on the recommendations of the risk assessor. The recommendations are dependent on a number of factors including shape and slope of the dose-response curve at the point of departure. There are, however, a number of questions concerning the margin of exposure analysis. What is an adequate margin of exposure protective of sensitive individuals? Would an LED_{10} based on a 97 or 99% rather that a 95% confidence limit encompass sensitive populations. The confidence limit explains experimental variability, which includes physical factors (food, water, housing, etc.), dosing measurements, and variability in the response of inbred animals. Is it possible to develop default margins of exposure that take into consideration the shape of the dose-response curve at the point of departure? More guidance is needed on applying margin of exposure analysis in risk assessments.

(7) Pharmacokinetics modeling – Pharmacokinetic modeling is a substantial improvement over the dose scaling method for estimating internal and delivered doses as well as for interspecies dose extrapolation. Constructing a model requires a very rich data set, which is available for only a few agents. Guidance for evaluating accuracy of the models used to estimate doses and guidance for validating pharmacokinetic models may speed up the process for using these models in risk assessments. Specific guidance or criteria could also aid investigators in conducting experiments used to construct models for risk assessments purposes.

(8) Benchmark dose approach to noncancer risk assessment – There are a number of issues related to the benchmark approach to noncancer risk assessment.

(a) Analysis of non-quantal data or transformation into a form easily incorporated into mathematical model – Quantal data expressed as incidence of lesion are easily incorporated, but data presented as increased severity of lesions require transformation. In addition, continuous data can be modeled, but should be evaluated in terms of an adverse and non-adverse categories. The magnitude of a change in enzymes or hematological parameters, the degree of severity in tissue lesions, or the magnitude of body weight decrements can affect data transformation. For example, should a 10% decrement in body weight, which is usually considered to be an adverse effect in animal models be considered an adverse effect for humans? Should the effects seen in animal studies be defined in terms of the degree of hazard to animals or the degree of hazard humans?

(b) Uncertainty factors – There are a number of issues concerning the application of uncertainty factors to the BMD to derive reference values. Should factors of 10 each for intraspecies and intraspecies (3 for inhalation exposures) as applied to NOAELs be applied to the BMD? BMD are based on LEDs, which are effect levels. Should a factor be applied to the BMD to extrapolate to a non-adverse effect level, as is done for LOAELs when deriving reference values?

(c) Validating and testing the BMD approach – A number of studies has compared the BMD to the corresponding NOAEL in a study, but is this a valid comparison? The benchmark approach is seen as an improvement over the NOAEL/LOAEL approach; nevertheless, a method for testing and validating this approach for deriving plausible noncancer risk values for humans should be developed. Deriving BMDs require more data. For most chemicals, sufficient data will not be available. Should the risk assessment be postponed until more data become available, or should the NOAEL/LOAEL approach be used as a default approach when data are not available to use the benchmark approach?

(9) Multiple endpoints and critical effects – Depending on the selection of doses or the type of study conducted, multiple endpoints may be evaluated in a single study. This is particularly true for neurotoxicity and developmental studies. In the case of neurotoxicity studies, behavioral, neurochemical, neurophysiological, and structural effects of a toxicant may be evaluated. Developmental studies may include fetal weight, fetal death, morphological variations and malformations, as well a functional toxicity, which could include neurotoxicity. If only the critical effect (observed at the lowest dose) is evaluated, important information may not be included in the assessment. Should the critical effect be used without considering the severity of the effect? For example, should a decrease in fetal weight be given the same weight as malformations? Should effects that would have a potentially greater impact on the quality of life or on society be given more weight (e.g., structural variations vs malformations or cognitive deficits vs change in nerve conduction)? Should effects such as cancer, which occur after many years of exposure, be given more weight than potentially serious developmental effects that are irreversible throughout life span of an individual? How multiple endpoints or different types of endpoints are handled is an important issue in the risk assessment process.

(10) Establishing guidance for deriving RfD/RfC based on short-term exposures – The manifestation of neurotoxic effects may not be dependent on a long-term duration of exposure, and the manifestation of other effects that may not be dependent on a long-term duration of exposure. For example, adaptation may occur of there may be no increase in the severity of an effect with continued exposure. Whether the NOAEL/LOAEL or benchmark approach is used, guidance should be developed for deriving reference values based on hazards not associated with long-term exposure.

7. Glossary

The following definitions were obtained from the sources presented in this report.

Adverse effect - a biochemical change, functional impairment, or pathological lesion that either singly or in combination that compromise the performance of the whole organism.

Components - points in a risk assessment at with judgments must be made regarding the analytic approach to be taken.

Critical effect - the adverse effect or the known precursor to the adverse effect that first appears in the dose scale as the dose is increased.

Inference Options/Default Options - choices made among several scientifically plausible options.

LOAEL (lowest-observed-adverse-effect level) -the lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.

NOAEL (no-observed-adverse-effect level) - an exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control group. Effects may be produced at this level, but they are not considered to be adverse, nor precursors to specific adverse effects.

NOEL (no-observed-effect level) - an exposure level at which there are no statistically or biologically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control group.

Risk Assessment - qualitative or quantitative characterization of the potential health effects of particular substances on individuals or populations.

fD or RfC (reference dose or reference concentration) - an estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime.

Risk Assessment Policy - choices made based on both science and broader areas of social and economic considerations.

Risk Management - process of evaluating alternative regulatory actions and selecting among them; entails political, social, economic, and technological considerations; and involves the use of value judgements related to acceptability of risk and reasonableness of cost.

Uncertainty factors - generally 10-fold factors (but may be less) representing specific areas of scientific uncertainty inherent in the extrapolation from the available data (sensitive subpopulations, species extrapolation, less-than-lifetime extrapolation, LOAEL to NOAEL extrapolation, and insufficient database).

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Tri-Service Procedural Guidelines for Ecological Risk Assessments

VOLUME 1

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13. ABSTRACT *(Maximum 200 words)*

The purpose of this report is to provide guidance for conducting ERAs for use by risk assessors at Navy, Air Force, and Army installations. Each of the three services has a support center which is available to provide guidance and programmatic services. The three members are: U.S. Army Environmental Center (AEC), Naval Facilities Engineering Service Center, and the Air Force Center for Environmental Excellence. Using this approach will provide the Tri-Service Centers with cost-effective, tiered procedures with which to direct and coordinate the scientific and technical efforts of contractors involved in ERA. Employing a common framework across sites will assure that the requirements of state and federal regulators are satisfied. The process described in this report follows the paradigm put forward in the 1992 Environmental Protection Agency Report entitled "Framework for Ecological Risk Assessment." Volume 2 contains information on more than 100 environmental models and test methods.

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FOREWORD

In an effort to improve efficiency within DOD, the Air Force Center for Environmental Excellence (AFCEE), the Army Environmental Center (AEC), and the Naval Facilities Engineering Service Center (NFESC) have combined to coordinate projects of mutual interest. One such project has been the development of procedural guidelines for ecological risk assessment. The product of this effort will maximize the transfer of programmatic and technical information in ecological risk assessment to the Tri-Service Centers.

The purpose of this report is to provide guidance for conducting ERAs for use by risk assessors at Navy, Air Force, and Army installations. Each of the three services has a support center which is available to provide guidance and programatic services. The three members are: U.S. Army Environmental Center (AEC), Naval Facilities Engineering Service Center, and the Air Force Center for Environmental Excellence. Using this approach will provide Tri-Service Centers with cost-effective, tiered procedures with which to direct and coordinate the scientific and technical efforts of contractors involved in ecological risk assessment.

The Procedural Guidelines for Ecological Risk Assessments is representative of the DOD trend toward partnership, and the goal to use increasingly scarce DOD dollars as efficiently as possible. With this vision in mind, the tri-services have joined efforts to produce this procedural guidance document that will benefit each of the services equally.

FOR

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PREFACE

The work described in this report was authorized under MIPR No. 2372 from the U.S. Army Environmental Center (AEC) and work order No. 56015408-05-0000 from the U.S. Army Edgewood Research, Development and Engineering Center. This work was started in January 1996 and completed in May 1996.

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Acknowledgments

The authors are indebted to the Tri-Service Ecological Risk Assessment Working Group members who are under the Tri-Service Environmental Support Centers Coordinating Committee.

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TRI-SERVICE PROCEDURAL GUIDELINES FOR ECOLOGICAL RISK ASSESSMENTS

1. INTRODUCTION

The objective of Ecological Risk Assessment (ERA) is to employ available chemical, toxicological, and ecological information to estimate the probability of undesirable ecological effects[1] and to provide a systematic means of balancing and comparing risks associated with environmental problems[2]. More specifically for the Superfund program, ERA "refers to a quantitative and/or qualitative appraisal of the actual or potential impacts of a hazardous waste site on plants and animals, other than humans and domesticated species. A risk does not exist unless: (1) the stressor has the ability to cause one or more adverse effects and (2) it co-occurs with or contacts an ecological component long enough and at sufficient intensity to elicit the identified adverse effect"[3].

The purpose of this report is to provide guidance for conducting ERAs for use by risk assessors at Navy, Air Force, and Army installations. Each of the three services has a support center which is available to provide guidance and programatic services. The three members are: U.S. Army Environmental Center (AEC), Naval Facilities Engineering Service Center, and the Air Force Center for Environmental Excellence. The purpose of the Committee is to exchange programmatic, regulatory, and technical information and to develop and coordinate joint Service activities relative to the programs, activities, and matters of concern to the Tri-Service Environmental Support Centers. This document addresses an installation restoration pillar concern for National Priority List (NPL) sites, sites listed under the Base Realignment and Closure (BRAC) program, and any other sites required to perform an ERA (i.e. RCRA corrective action). This project is needed because ERA has recently been proposed to meet requirements under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) at NPL sites. The report is designed to enhance an understanding of the requirements under CERCLA. Using this approach will provide the Tri-Service Centers with cost-effective, tiered procedures with which to direct and coordinate the scientific and technical efforts of contractors involved in ERA. Employing a common framework across sites will assure the Tri-Service Centers that requirements of state and federal regulators are satisfied. The process described in this report follows the paradigm put forward in the 1992 Environmental Protection Agency (EPA) report entitled "*Framework for Ecological Risk Assessment*"[4]. This framework, although similar to the human health risk assessment framework, recognizes the differences between ecological and human processes. These differences include the variety of endpoints and terminology. This ERA

widely accepted as the proper procedure for ecological risk assessments. Currently, guidance documents are in preparation to provide procedural details for ecological risk assessments. The EPA Environmental Response Team, Edison, NJ, recently published a draft document on ERA guidance for Superfund[3]. The EPA document "provides a process for designing and conducting technically defensible ERAs within Superfund. It is not the intent of this document to determine the appropriate scale or complexity of an ERA to be conducted at a hazardous waste site. Additionally, this document is not intended to direct the user in the selection of specific protocols or investigation methodologies". This document, for the Tri-Service Centers, compliments the EPA Superfund report by providing more detailed procedures, thought processes, a tiered approach, sources of information, and methodologies. This report reinforces important points presented in the EPA Superfund report, such as planning and coordination steps in the Problem Formulation phase and risk characterization issues. The U.S. Army Corps of Engineers (ACE) also produced a draft document entitled "*Risk Assessment Handbook, Volume II: Ecological Assessment*"[5]. It addresses the managerial oversight for ERAs performed by U.S. ACE contractors.

1.1 Report Objectives

There is a critical need for a technical document which combines the theory of ERA with available ecological effects and exposure assessment methods to provide a guide to the scientific process of ERA. This document is a technical "road map", with examples and discussion of the thought process to lead environmental scientists through this effort. We realize that the field of ERA is in a dynamic flux and recognize that this report cannot address all of the ERA issues. Ecological risk quantification requires a multi-disciplinary approach, typically involving interaction among experts in biochemistry, biology, ecology, environmental chemistry and toxicology[6]. Ecological risk assessments, at any level of effort, have at least two phases:[2] 1) the first requires a conceptual understanding of the environmental problem; 2) the second requires quantification of spatial and temporal variances in exposure to the hazard. Success in this phase requires understanding both exposure and ecological effects.

Ecological risk assessment requires an appropriate, albeit varied, methodology to assess the variety of aquatic and terrestrial ecosystems. To effectively assess critical habitats, populations, and contaminant transfer through trophic levels, the methodology should 1) predict and isolate ecological risks from point and non-point sources; 2) distinguish changes caused by anthropogenic sources from those related to natural stresses or cycles; 3) be non-destructive (i.e., not add to the perturbation of species or the environment); 4) promote a rapid turn-around

from data collection to decisions on status of the environment and remediation; and 5) protect the existing biological communities at sites within the larger ecosystem. This information should be collected in such a manner that the risk assessment can distinguish responses in organisms caused by anthropogenic stressors from those caused by natural stressors or seasonal cycles.

This document uses the framework developed by the EPA[4] as the vehicle for conducting an ERA. The framework presents the ERA process in a technically accurate format and in a format understandable for environmental scientists. Suter[7] provides an excellent presentation of the theory of ERA and presents examples of ERA. We highly recommend developing an understanding of information presented in Suter[7]. Other publications on ERA were used to provide viewpoints and approaches for ERA. These publications were combined with the expertise of scientists experienced in research approaches to ERA through trial and error at various sites[8,3,9,10]. Those scientists are also developing new scientifically defensible technology to provide useful information for risk assessments[11].

1.2 History of Risk Analysis

1.2.1 Application of Risk to Environmental Issues

The history of the field of risk analysis is the history of the development and use of various techniques for gathering and analyzing information about potential hazards. Many different qualitative and quantitative analytic techniques have been employed, most of which are borrowed from other disciplines, including actuarial accounting, economics, biology, geology, geography, and engineering.

Risk assessment is appropriate as an analytical tool to help identify problems, set regulatory priorities, compare effectiveness of risk management options, communicate to the public, and identify research needs. Because the purpose of environmental regulations is to protect human health or the environment, risk assessment will quantitatively or qualitatively estimate needed protection levels. Risk assessment is often involved in the generation of health or environmental criteria used in the regulations. Typically, risk assessment alone will not provide, **and is not intended to result in**, a hard and fast number for regulation.

1.2.2 Initial Activities of Federal Agencies

Federal agencies began to use chemical risk assessment in the 1970's to estimate the cancer-causing potential of chemicals in commerce. By the mid 1970's, agencies had begun efforts to

improve coordination among programs and to ensure consistent use of uniform risk assessment procedures within and across agencies of the federal government. Formal procedures for extrapolating research results to human health effects, i.e., chemical risk assessment, were adopted in the late 1970's. In 1976, the EPA established an internal working group, the Carcinogen Assessment Group (CAG), which published the first interim guidelines for assessing risks of suspected carcinogens. In 1977, the EPA, Consumer Product Safety Commission (CPSC), Food and Drug Administration (FDA), and Occupational Safety and Health Administration (OSHA) formed the Interagency Regulatory Liaison Group (IRLG). The IRLG met as a forum for voluntary coordination and information exchange until 1980. One of its products was a "cancer policy" that attempted to present the scientific basis for determining a substance's carcinogenicity.

The efforts of agencies in the field of risk analysis were encouraged when, in 1980, the U.S. Supreme Court issued an opinion that required OSHA to perform risk assessments of toxic chemicals as a basis for regulating occupational exposures. Agencies' subsequent use of risk assessments in the development of regulations continued to be controversial, in part because authorizing legislation provided little guidance about how risks should be balanced against other factors. Thus, for example, in 1987, a federal appeals court vacated an EPA rule restricting emissions of vinyl chloride because the agency had not used risk assessment properly. Other risk assessment decisions also have been overturned by federal courts because the assessment was judged to be of insufficient technical quality.

1.2.3 National Academy of Science (NAS) Framework

The efforts of federal agencies to systemize risk analysis were criticized by some scientists and industrial representatives who were concerned that policy judgements were influencing scientific judgements and thus, the risk assessment process. In response, Congress requested a study by the National Academy of Sciences (NAS) of institutional arrangements to improve the agencies' use of risk assessments. This led to the landmark NAS report in 1983 entitled "*Risk Assessment in the Federal Government: Managing the Process*"[12]. This report presented the initial framework for conducting risk assessment. It discussed the need to distinguish risk assessment from risk management and recommended that uniform risk assessment guidelines be established by the federal government. Although such a uniform guide for the federal government has not yet been adopted, the Office of Science and Technology Policy (OSTP) in the Office of the President, produced a report in 1985 on chemical carcinogens and proposed a method to assess their hazards. The 1985 report described the state of the science on which decisions could be made. More recently, the National Science and Technology Council Committee on Environment

and Natural Resources established a subcommittee on risk assessment to coordinate risk assessment procedures.

1.2.4 Cancer and Non-Cancer Guidelines

Following the 1983 NAS framework, various agencies, including the EPA have promulgated their own risk assessment guidelines for carcinogens. The EPA guidelines for cancer risk assessment were finalized in 1986 and are now in the process of being revised (in a related report the EPA published a classification guide for carcinogens in 1986).

The risks of non-cancer effects of chemical exposure to humans also have been recognized and analyzed. As early as 1980, just before it was disbanded, the IRLG was developing guidelines for the risk assessment of effects on reproduction and human development. To date, the EPA has promulgated final guidelines for risk assessment of effects on human development and reproduction. No guidelines have yet been established for assessing risks of reproductive failure, nervous system damage, respiratory effects, or damage to the immune system.

1.2.5 Council, Committee, and Society Actions

Other groups are also active in risk assessment and risk management. The National Research Council (NRC) of the NAS has a permanent Committee on Risk Assessment Methodology (CRAM). They are working to consider changes in the scientific foundation of risk assessment that have occurred since the 1983 NAS report. CRAM issued their first report entitled "*Issues in Risk Assessment*"[13] in 1993. As a result of congressional discussions on the use of risk assessment in the Clean Air Act Amendments (CAAA) of 1990, Congress directed the EPA to contract with the NAS to review EPA's risk assessment methods for cancer and non-cancer health effects of exposure to hazardous air pollutants. In response, the NAS established the Committee on Risk Assessment for Hazardous Air Pollutants (CRAHAP) which completed its deliberations in 1992. A report was published in February, 1994.[14] A Commission on Risk Assessment and Management was also authorized by CAAA. The Commission will conduct meeting on various risk issues and produce a report in early 1996. In addition, professional organizations, such as the Society of Environmental Toxicology and Chemistry and the Society for Risk Analysis, were formed to bring together the various scientific disciplines interested in this field.

1.2.6 Ecological Risk Assessment

Ecological risk assessments were initiated by the EPA in order to develop water quality criteria required under the Clean Water Act of 1977. The first ecological risk assessments were done in the

late 70's and early 80's. Ecological risk assessments initially followed the 1983 NAS framework, but in 1991 several workshops were held by the EPA and NAS to reassess the procedures for ecological risk assessment. In 1992, the EPA published a report entitled "*Framework for Ecological Risk Assessment*"[4]. This new framework, although similar to the human health risk assessment framework, recognizes the differences between ecological and human processes. These differences include terminology and the diversity incumbent in ecological assessments. Ecological risk assessments must consider many species with various endpoints such as protection of bald eagles (*Haliaeetus leucocephalus*) or maintaining species diversity in an aquatic system, while human health is the only endpoint in health risk assessments. This ERA framework has been widely accepted as the proper procedure for ecological assessments. Currently, guidance documents are in preparation to provide procedural details for ecological risk assessments.

1.3 Comprehensive Environmental Response Compensation and Liability Act (CERCLA) and Ecological Risk Assessment

The Comprehensive Environmental Response Compensation and Liability Act (CERCLA), as amended by the Superfund Amendments and Reauthorization Act of 1986, requires the EPA to ensure the protection of the environment via the selection of remedial alternatives, and assessment of the degree of cleanup necessary. The CERCLA makes reference to protection of health and the environment as parts of a whole. Sections call for methods to evaluate and remedy any substance release into the environment or threats of releases which pose substantial danger to public health or the environment. The CERCLA further directs the EPA to attain a degree of cleanup which assures protection of both human health and the environment[15]. The National Oil and Hazardous Substances Pollution Control Plan (NCP) also calls for the identification and mitigation of environmental impacts at hazardous waste site. The NCP calls for the selection of remediation methods to protect organisms, populations, communities, and ecosystems. In response to these regulations the Superfund program established the remedial investigation and feasibility study (RI/FS) process. The RI/FS process characterizes the nature and extent of contamination and the resulting risks posed by the site[4].

Under the CERCLA, sites are initially evaluated for their hazard to humans or the environment by the hazard ranking system (HRS). Substances designated as hazardous under the CERCLA (40 CFR 302.4) usually are the stressor of concern. The HRS uses a health assessment along with exposure and persistence data on the chemicals to rank sites for inclusion as a Superfund site. Hazard ranking system values above 28.5 require the site to be listed on the National Priorities List (NPL). Risk assessment is

used in two ways at an NPL site. First, a baseline risk assessment of health and ecological concerns is conducted to determine if the risk justifies mitigation. Second, in the remedial investigation/feasibility study (RI/FS), risk assessment is used to establish risk levels for different areas of the Superfund site.

Health and ecological risk are both critically evaluated under the CERCLA. However, depending upon site-specific characteristics (e.g., waste site location near relatively high density human population in an urban setting versus relatively low density population in a rural location; waste site near endangered species habitats), human health and ecological risk assessments may receive different levels of effort during the RI/FS process. Regardless of the level of effort, the EPA stresses a preference toward permanence, which EPA has defined as clean-up to background levels. For example, to help establish clean-up goals under the CERCLA, applicable, relevant, and appropriate requirements (ARARs) such as the Clean Water Act, the Endangered Species Act, and other federal or state environmental statutes may be used, when available, to set these clean-up criteria.

Risk assessment can identify areas of the site that have elevated risk. These risks can be related to chemical levels necessary for remediation. An example of the use of risk assessment would be to support the Endangered Species Act. An effects (or hazard) assessment could be used to establish the chemical concentration (with uncertainty bounds) to protect a given organism. The results of the risk assessment are used with other risk management tools to determine mitigation of a site. Risk assessment is also used to evaluate alternate remedial actions.

The CERCLA directs the EPA to notify the appropriate Federal and State natural resource trustees promptly about potential dangers to natural resources. The Federal natural resource trustees include: the U.S. Fish and Wildlife Service (USFWS), the National Park Service (NPS) and the Bureau of Land Management (BLM) of the Department of Interior; the National Oceanic and Atmospheric Administration (NOAA) of the U.S. Department of Commerce; and the Forest Service of the U.S. Department of Agriculture. State agencies and Indian tribes are also designated trustees for natural resources under their jurisdiction. The trustees determine if a natural resource damage assessment (NRDA) should be conducted at a site. An ERA is a necessary step for an NRDA because it establishes the causal link between site contaminants and adverse ecological effects. When a non-CERCLA ERA is initiated, for instance under BRAC, these same agencies may be contacted to provide continuity and as a source of substantive information for the ERA.

The proposed National Contingency Plan (NCP) refers throughout to

health and environment as aspects of the evaluation and remediation processes. For example, in discussing the baseline risk assessment in a Remedial Investigation (RI), the purpose is defined as to determining "whether the site poses a current or potential risk to human health and the environment in the absence of any remedial action". The exposure assessment in the RI "is conducted to identify the magnitude of actual or potential human or environmental exposures" and considers the types of adverse health or environmental effects associated with chemical exposure. In addition, the proposed NCP states that "Superfund remedies will.. be protective of environmental organisms and ecosystems"[15].

The proposed NCP would require the lead Agency to review the remedial action every five years to ensure continued protection of the environment[15]. If, after the remedial action is completed, any hazardous substances remain on a site above levels that allow for unlimited use and unrestricted exposure for human and environmental receptors, CERCLA directs that the Superfund remedial actions meet federal and state ARARs.[15] Federal environmental statutes and regulations that may be ARARs for a particular site include: the Resource Conservation and Recovery Act (RCRA); the Federal Water Pollution Control Act; the Clean Air Act (CAA); the Toxic Substances Control Act (TSCA); the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); the Endangered Species Act of 1973; and the Clean Water Act (CWA).

1.4 Risk Assessment Framework

The EPA document "*A Framework for Ecological Risk Assessment*"[4] presents a concise explanation and outline of what an ERA should contain. The framework is presented in Figure 1. The authors stress the importance of preliminary discussions between the risk manager (RM) and the risk assessor (RA). These discussions should set time and funding limits and address policy issues that may affect the selection of assessment endpoints. In the Framework document, the Problem Formulation phase establishes the path the ERA will take. It includes a preliminary characterization of exposure and effects, site history, and various surveys and studies. This information is used to determine assessment and measurement endpoints. Working hypotheses are formulated on how the stressor(s) may affect ecological components.

In the Analysis phase, two interrelated activities occur: 1) characterizing exposure and 2) characterizing ecological effects. The spatial and temporal distribution of the chemicals of concern and their interaction with the ecological system are addressed. In addition, the impact of the chemicals on individuals, populations and communities is quantified. Data requirements should be part of the work plan (see below) for inclusion in the

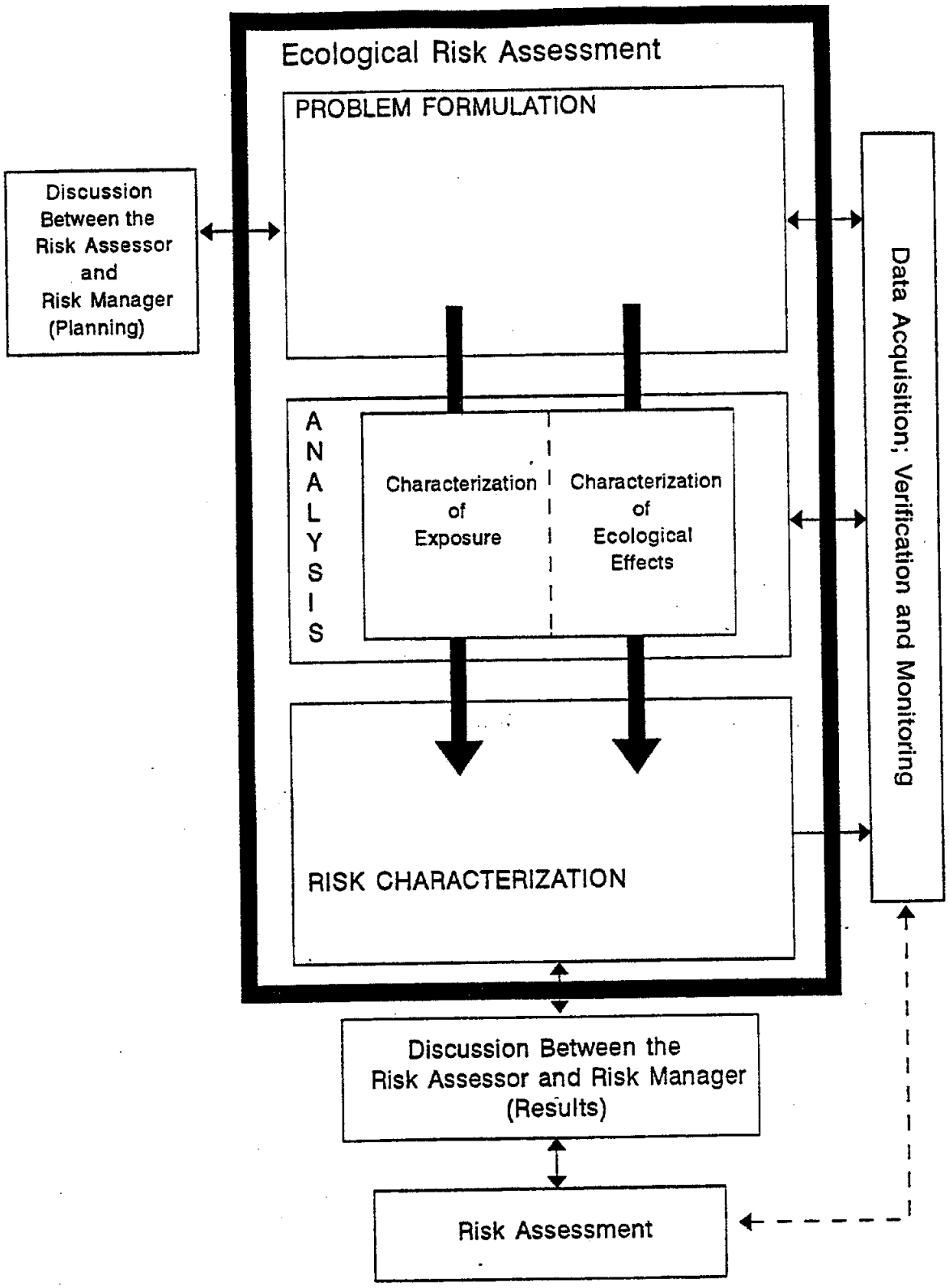

Figure 1. Framework for Ecological Risk Assessment.[2]

remedial investigation. As a required component of the work plan, it is necessary to address data gaps and data quality objectives[4].

The Risk Characterization phase uses input from the Analysis phase to determine the likelihood of chemical exposure resulting in adverse ecological effects. Various issues including: cause and effect, strength ("robustness") of the data, and scientific uncertainties are used to judge the ecological significance of the risk. The results from this process are interpreted by the risk assessor in an understandable and useable format. This information should be included in the work plan for inclusion in the RI. Within the framework for ERA[4], the guidance for Superfund contains eight steps and several scientific management decision points (SMDPs) (Figure 2). The guidance stresses meetings between the primary risk managers and risk assessors to evaluate and approve or redirect the assessment up to that point. A group decision is made on whether or not the risk assessment is proceeding in a manner acceptable to the risk assessor and the managers. The guidance emphasizes the importance of SMDPs to build consensus, minimize cost, and speed up the assessment process. The first four steps are in the Problem Formulation phase of the ERA and emphasize the importance of planning and coordination at the beginning of the ERA process. These steps limit the need for repeated studies or delays. Steps 5 and 6 support the Analysis phase of ERA, with site assessments and field investigations. The final two steps are risk characterization and risk management.

1.5 A Tiered Approach to Ecological Risk Assessment

A tiered or phased approach has been put forward as a rational procedure by several authors[7,9]. The purpose of a tiered approach is to do the necessary and sufficient amount of work to characterize the risk to an ecological system with an acceptable degree of uncertainty. The definition of "necessary and sufficient work" should be agreed on early in the Problem Formulation phase, with agreement among the "principal responsible party" (PRP), site manager, environmental monitors, the public, and regulatory groups. Field data requirements for the ERA should be conducted in an overall RI work plan. The level of effort in the RI can act as a guide for the level of effort required for the ERA. Limiting factors such as time or funding constraints that could influence the ERA should be acknowledged early in Problem Formulation discussions with the risk manager.

The tiered-analysis process consists of three tiers, each structured similarly, with a Problem Formulation (PF) phase, Analysis phase, and Risk Characterization (RC) phase (Figure 3). Data collected in the Analysis phase of each tier is evaluated

1.	Preliminary Problem Formulation and Ecological Effects Evaluation	
2.	Preliminary Exposure Estimate and Risk Calculation	SMDP (a)
3.	Problem Formulation: Assessment Endpoint Selection Testable Hypothesis	SMDP (b)
4.	Conceptual Model Development: Conceptual Model Measurement Endpoint Selection and Study Design	SMDP (c)
5.	Site Assessment to Confirm Ecological Sampling and Analysis Plan	SMDP (d)
6.	Site Field Investigation	
7.	Risk Characterization	
8.	Risk Management	SMDP (e)

SMDP = Scientific/Management Decision Point

(a) Early Regional decision in the Superfund Accelerated Cleanup Model (SACM) concerning priority of the site.

(b) Initial agreement on scope of the assessment and work plan.

(c) Signing approval of the work plan and sampling and analysis plan for the ecological risk assessment.

(d) Approval of any changes to the work plan or sampling and analysis plan.

(e) Signing the record of Decision

Figure 2. Steps in the ecological risk assessment process and the corresponding decision points for Superfund (EPA 1994).

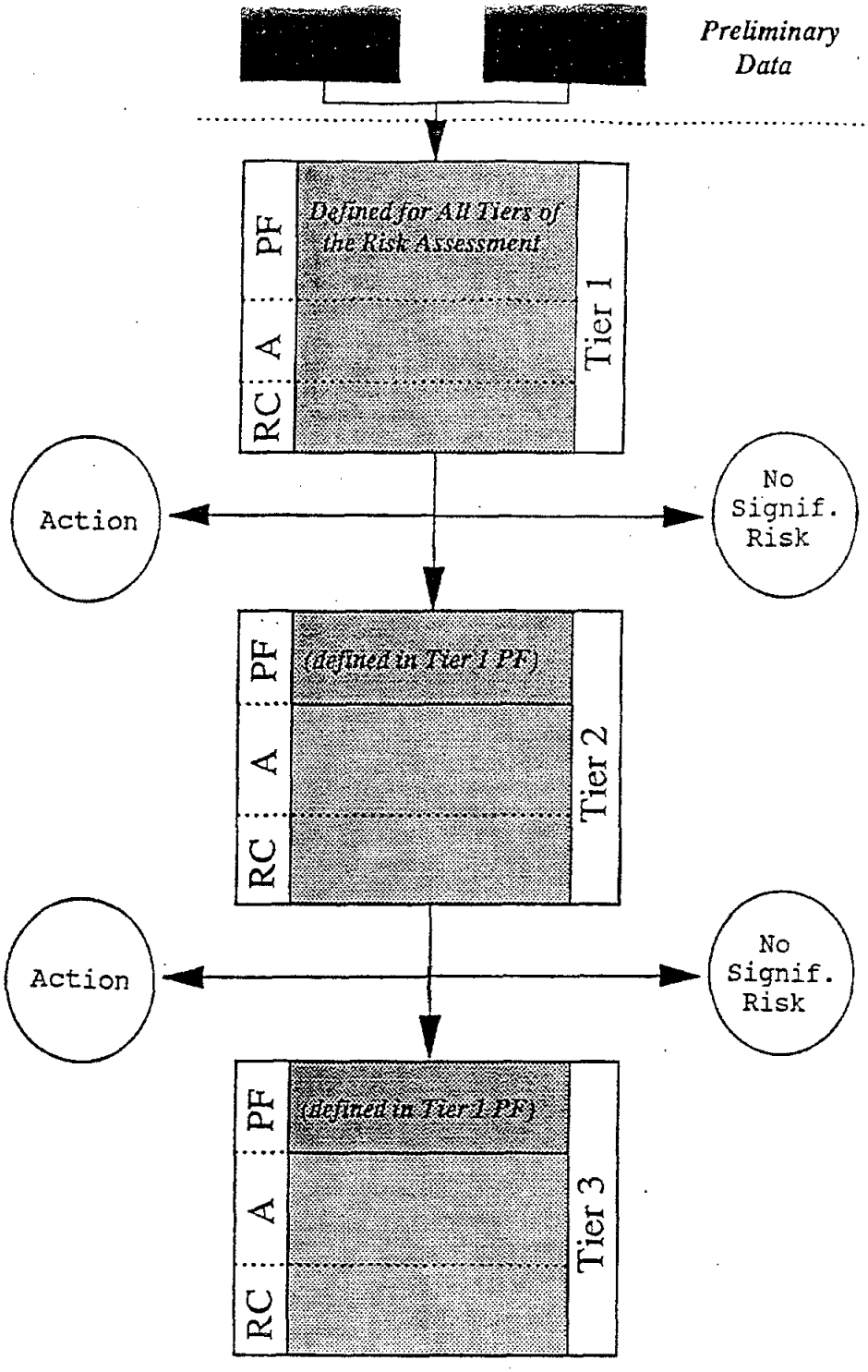

Figure 3. Generalized schematic of the tier structure for Ecological Risk Assessments. Abbreviations are: PF= Problem Formulation, A= Analysis, RC= Risk Characterization.

and a decision made concerning the potential for risk to occur in the RC phase, after which a decision will be made whether to proceed to testing at higher tiers. The assessment should proceed if the probability of risk is apparent, but complete characterization of risk cannot be determined due to significant data gaps. The assessment should not proceed if no risk is apparent, or if the risk is so great that action (e.g., remediation, containment, etc.) is warranted immediately. Proceeding to higher tiers in these situations would be a waste of time and money. Tiers are defined on the basis of progressive increases in the level of concern or in levels of manpower and monetary inputs in each successive tier.

Tier 1 (Figure 4) involves primarily a literature study, but adds RI results, historical site information, existing field data, literature and output from fate and effects models, and previous field surveys on the biota (including endangered and threatened species). These studies can be conducted by personnel from the installation, the USFWS, or other governmental agencies. Measurement endpoints rely on available data with underlying conservative assumptions and infer protection for assessment endpoints. These data and results may be used to develop preliminary hazard indices (risk quotients). The purpose of higher tiers (Figure 5) is to address data gaps and reduce uncertainty in the risk characterization and lessen the need for the use of conservative assumptions. This does not necessarily mean that laboratory studies are conducted in Tier 2 and field studies in Tier 3. In many cases, a laboratory study in Tier 3 will answer data gaps in the ERA with more precision than would field studies.

Tier 2 should address site-specific issues, limiting reliance on literature-cited values. This may include more models, laboratory tests, or limited field studies to address data gaps in exposure or ecological effects, and use more sophisticated analyses to develop more rigorous hazard indices to prioritize various locations at the site for potential risk. Measurement endpoints should be more complex, relying on specific laboratory or field studies that address data gaps identified in Tier 1, to better relate to assessment endpoints.

Tier 3 involves increased complexity, combining site-specific field observations with laboratory and field data to refine exposure and ecological effects characterization. Studies may include population- and ecosystem-level complexity and involve substantially longer-term investigations. The uncertainty associated with measurement endpoints is reduced, resulting in stronger data and greater confidence. At this point, the risk characterizations rely on distribution of exposure and effects results to facilitate understanding and interpretation of hazard indices at the site.

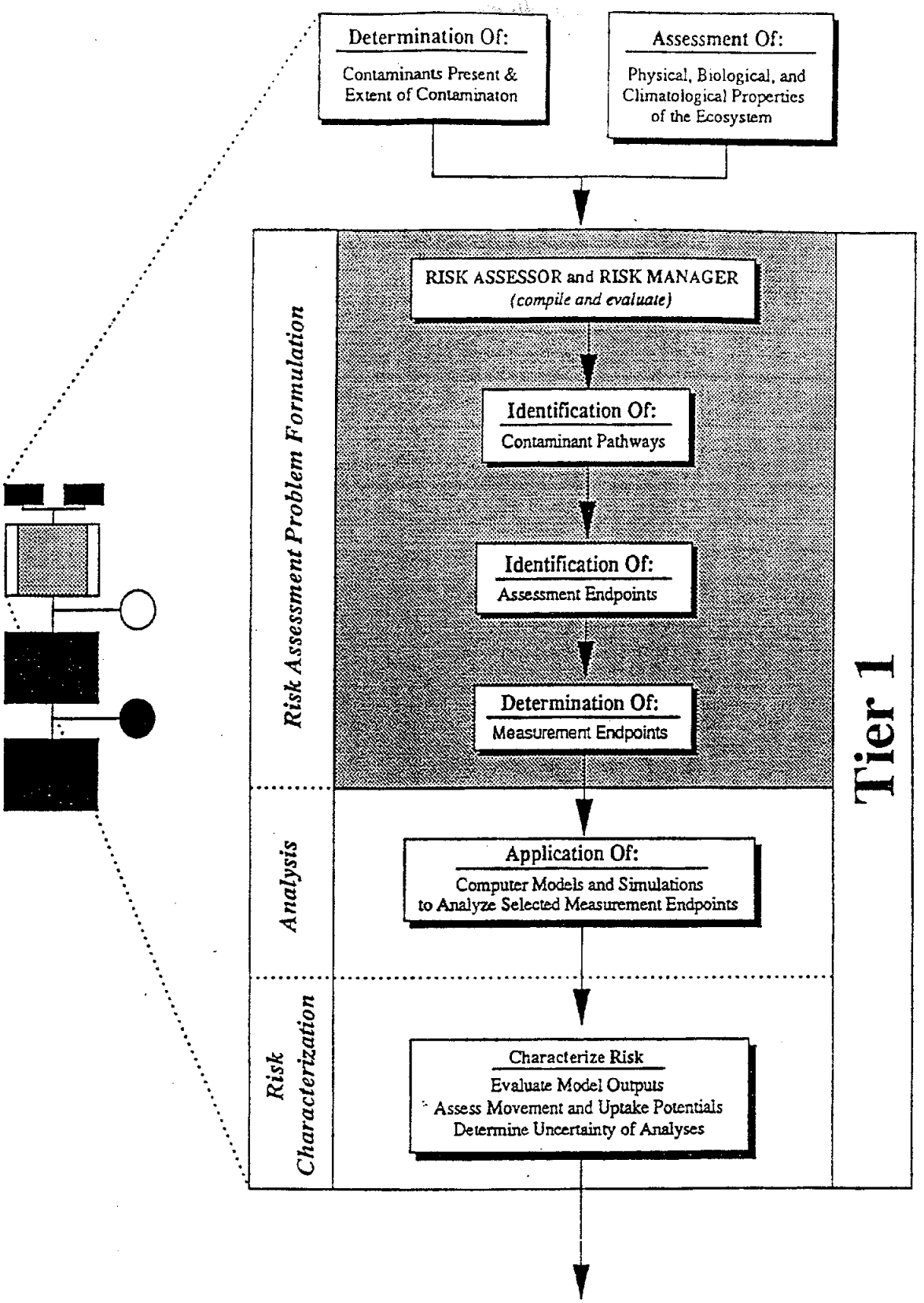

Figure 4. Tier 1 analysis.

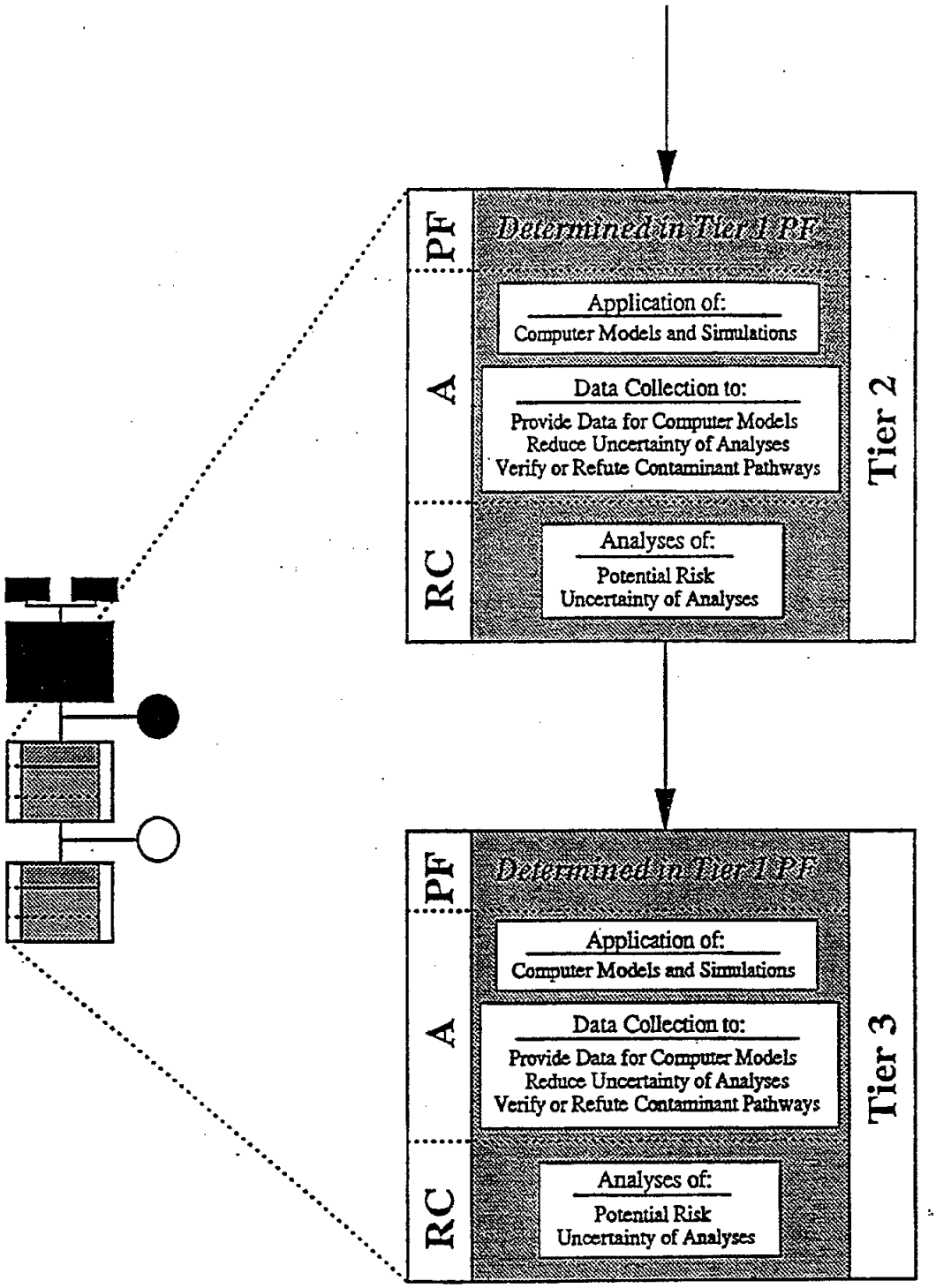

Figure 5. Tier 2 and Tier 3 analyses.

Although each tier is, in essence, an evaluation by itself, it is important that if testing proceeds to higher levels, there exists continuity in the risk assessment among tiers. Continuity is provided by establishing assessment endpoints. The measurement endpoints employed will change if the ERA progresses to higher tiers; however, the focus on assessment endpoints remains intact. For example, for an investigation of dieldrin residues in soils on a population of coyotes, one measurement endpoint in Tier 1might be "dieldrin concentration in soil and in resident field mice". In Tier 2, measurement endpoints might be "analysis of coyote feeding habits on resident field mice and dieldrin concentrations in coyote tissue". In Tier 3, the procedure might involve a detailed analysis of coyote home range, time spent feeding, reproductive behavior, etc. In each tier, the measurement endpoints differ while the assessment endpoint remains the same. Further, if the assessment were stopped at Tier 1, estimates of risk would have to be conservative (e.g., broad "safety factors"). As the ERA process gathers more data on actual exposure and effects, the conservative assumptions may be relaxed.

2. PROBLEM FORMULATION

2.1 General Overview

In the Problem Formulation phase (Figure 6), policy and regulatory discussions with the risk manager establish the goals and focus of the risk assessment. The views and values of the various stakeholders concerned with the management of the site are discussed, coordinated and prioritized. In this phase, the major factors to be considered are identified for the particular assessment, and working hypotheses are developed.

The process begins by characterizing exposure and ecological effects, including evaluating the stressor characteristics, the ecosystem potentially at risk, and the ecological effects expected or observed. Assessment and measurement endpoints are then identified. A conceptual model is constructed from this information that describes how a given stressor might affect the ecological components in the environment. The model also describes the relationships among assessment and measurement endpoints, the data required, and the methodologies that will be used to analyze the data. The conceptual model serves as input to the analysis phase of the assessment[4].

Problem Formulation (PF) should clearly define the goals of the assessment (i.e., what are we trying to protect) and develop a scope that is appropriate for achieving those goals within the constraints of available resources and the overall uncertainties of the analyses. To accomplish this, the problem formulation should ensure that the assessment focuses on the stressors, ecological components, and endpoints that are most appropriate for determining whether a cause and effect relationship exists and for making ultimate management decisions. Reviewers of risk assessment case studies[16] observed that establishing cause and effect is especially critical when resources are limited by fiscal constraints. Strengths and weaknesses of the case studies seemed to originate, in large part, from decisions made during the preliminary planning stages.

Steps 1-4 presented in the EPA draft report on an ecological risk assessment process for Superfund sites (Figure 2), are addressed in the PF phase of EPA (Figure 6). After stressor characteristics, ecological effects, and ecosystem parameters have been initially reviewed (after step 2 in the EPA Superfund draft report) a scientific/management decision point (SMDP) is reached to decide whether the data warrants further study. After each of the two remaining parts of the PF phase, endpoint selection and development of the conceptual model, the EPA Superfund report[3] calls for SMDPs to formally agree to the results from these two key planning parts of PF. The use of SMDPs stresses good communication among all parties involved and

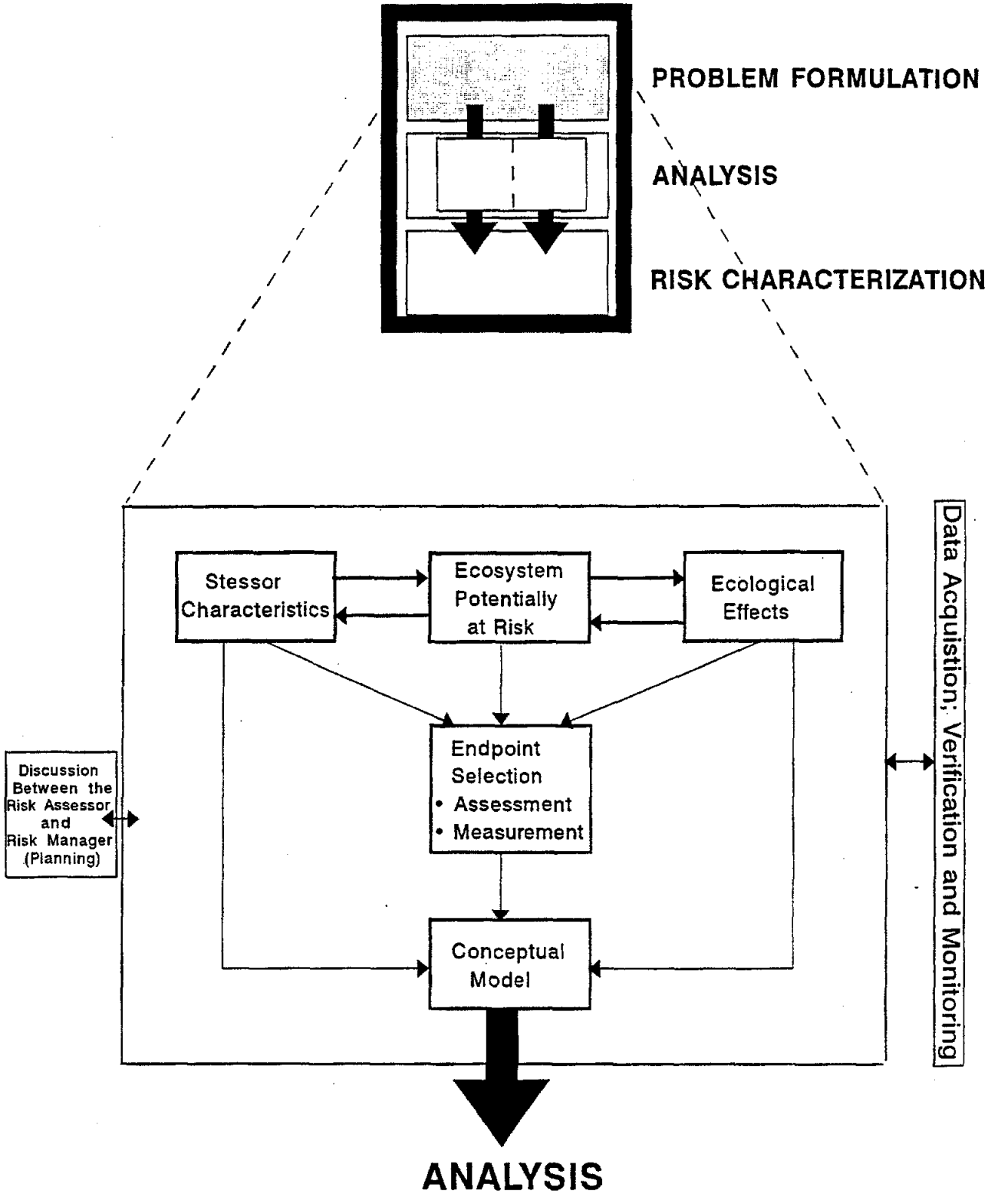

Figure 6. Problem Formulation Phase

keeps the risk assessment process focused and efficient.

2.2 Discussion Between Risk Assessor and Risk Manager

Establishing a two-way dialogue between the risk assessors and risk managers during the problem formulation phase is essential to achieving societal, regulatory, and scientific goals. Risk managers can ensure that the risk assessment will provide answers for questions related to protection of societal values, selection of remediation technologies, policy concerns and cost, whereas, the ecological risk assessor ensures that the assessment addresses important scientific concerns. Both perspectives are necessary to efficiently utilize resources to produce scientifically sound risk assessments that are relevant to management decisions and public concerns[4]. Establishment of SMDPs, as described above, is a good method to ensure that all policy and scientific issues are addressed.

The National Crop Loss Assessment Network (NCLAN) case study[16] was a good example of an assessment where the ultimate management issue was clear from the onset; the stressor, ecological components, and endpoints were clearly defined; and the design of the study was structured around a clear set of hypotheses amenable to scientific inquiry. This level of clarity was achieved, in part, through frequent meetings and interactions among researchers and others involved with the risk assessment/risk management process. The author and reviewers of the case study stressed the importance of this type of communication for clarifying issues and goals.

2.3 Stressor Characteristics

Stressors are chemical, physical or biological influences causing negative impact on the populations or ecosystems at risk. Chemical stressors include not only the contaminants of concern (COCs), but inorganic and organic chemicals inherent in the environment as well. Secondary stressors may arise as a result of primary COCs, such as increased concentrations of chlorofluorocarbons causing stratospheric ozone depletion which, in turn, results in increased exposure to ultraviolet radiation. Physical stressors are generally the abiotic environmental conditions under which the biota find themselves. These include such factors as seasonal and diurnal variance in atmospheric temperature, soil characteristics (soil type, parent material, climate, pH, organic matter content, management practices, etc.), the hydrologic regime (seasonal flooding, tidal influences, etc.) and habitat alterations (logging, construction, urbanization, etc.). Biological stressors also exist and are often important in determining survivorship of populations. Examples of biological stressors include competitor and predator species, introduced pests, such as the gypsy moth and various fungal

pathogens of tree species, or cholera epidemics in bird species. Changes in the physical/chemical environment may lead to subtle changes in competitive abilities of a species or may lead to changes in abilities to avoid predators, infestations, or disease epidemics. Therefore, biological stressors may assume larger roles in determining the maintenance of a population if the habitat has been altered chemically or physically. Stressors may also result from management practices such as harvesting of fishery or forest resources, or cultivation techniques during crop production.

Any stressor cannot be judged as such without reference to the species or community under stress. One cannot isolate the stressors from the species response, as they are interrelated. The degree to which stressors influence the survivorship of species depends on the magnitude of the stress (the intensity), the duration of the stress (how long the species is exposed, relative to its own life history characteristics), the frequency (how often a stress of a particular intensity occurs), the timing (when the stress occurs, relative to critical life history stages of the species). A complex of stress factors influence species responses; hence, creating a map of direct or indirect influences of contaminant stressors onto the "mosaic" pattern of normal stressors involves considerable thought.

The task of the RA in the PF phase is to analyze a suite of previously compiled chemical, physical and biological data. Literature data bases contain a variety of environmental toxicology data for chemicals. A partial listing of such data bases is given in Table 1. Defense Technical Information Center (DTIC), DoD research laboratories and DoD scientists may also be able to guide the RA to relevant toxicity data.

With this information, the RA then evaluates site-specific stressor characteristics in the PF phase of the Tier 1 analysis. During Tier 1, the RA identifies which chemical, physical, and biological stressors are present based on available information and estimates the nature, extent and potential interaction of these stressors. This information may be obtained from databases listed above but also from information previously collected from the site, such as record searches or Installation Assessments, reports on chemical storage, use and distribution, or from DTIC. Information on chemical properties of the contaminants should be examined in the context of biological, chemical, and physical characteristics of the ecosystem.

The manner in which contaminants interact with the physical and biological ecosystem components are predictable, within certain constraints. Interactions among site-specific soil and biotic characteristics influence contaminant distribution, fate and, importantly, allow the RA to estimate the likelihood of the contaminants remaining *in-situ* rather than moving off-site or

through the ecosystem. For example, fairly simple models (SESOIL, EXAMS; see Volume 2) may be called upon in Tier I to estimate the distribution of contaminants downstream or in soils on the site. The input data (e.g., soil moisture, pH, particle size, percent organic matter) for these types of models, if not measured directly, are available from detailed county soil surveys (Soil Conservation Service), USGS topographic maps, or state resource agencies. When more detailed and site-specific information is available, more sophisticated models may be used (CMLS, LEACHM; see Volume 2).

Table 1. Listing of databases available for information on contaminant fate and effect.

1. Chemical Information System (CIS)

AQUIRE - Aquatic Information Retrieval
CERCLIS - CERCLA Information System
CHRIS - Chemical Hazard Response Information System
ENVIROFATE - Environmental Fate
ISHOW - Information System for Hazardous Organics in Water

OHMTADS - Oil and Haz. Materials/Tech. Assist. Data System
PHYTOTOX - Toxic Effects on Plants

2. National Library of Medicine's Database Selection Menu

HSDB - Hazardous Substances Data Bank
IRIS - Integrated Risk Information System
EMICBACK - Environmental
EMIC - Environmental
ETICBACK - Environmental

3. Dialog Databases

Oceanic Abstracts
Enviroline
Pollution Abstracts
Aquatic Sciences and Fisheries Abstracts
Environmental Bibliography

Bioavailability of chemical constituents should also be considered at this point. For example, is the chemical hydrophilic or hydrophobic?; is it available in the soil water and subject to surface runoff and leaching, or is it tightly bound to soil particles and organic matter?; and how do site specific soil characteristics affect the contaminants' bioavailability?

At the end of Tier 1 PF, the risk assessor should have a good understanding of the stressor characteristics for the particular site under study. Data gaps should be addressed in Tier 2 if the assessment proceeds that far.

2.4 Identifying the Ecosystem Potentially at Risk

Identifying the ecosystem potentially at risk from a stressor depends in part on how the risk assessment was initiated. Once a stressor is identified, information on the spatial and temporal distribution patterns of the stressor can be helpful in identifying ecosystems potentially at risk. Similarly, if the risk assessment is initiated by observing effects, these effects can directly indicate ecosystems or ecological components of the system that may be considered in the assessment.

Ecosystem properties should be analyzed during PF. These properties include ecosystem structure (including types and abundances of different species and their trophic level relationships), ecosystem function (i.e., ecosystem energy source, pathways of energy utilization, and nutrient processing), bioavailability, and aspects of the abiotic component (see Section 2.3 above). In addition, types and chronology of historical disturbance should be determined to help predict ecological responses to stressors.

At this point, it is important to emphasize that not all aspects of ecosystem structure and function need to be analyzed in every risk assessment. The extent to which ecosystem properties are analyzed depends upon the nature of the stressors and ecosystem components, bioavailability, and the resources available. Analyses should concentrate on those ecosystem components that are determined to be at greatest risk. Knowing the stressor characteristics can help to narrow the focus of the investigation on the components of the ecosystem that are potentially most susceptible.

Once stressor characteristics and the ecosystem potentially at risk have been identified, potential pathways for contaminant(s) through the ecosystem must be identified. Contaminant pathways may be simple and straightforward or complex and highly branched. Pathways are generally defined by naturally occurring physical, chemical, and biological components of the ecosystem. As an example, consider the evapotranspiration potential,

precipitation, soil type, slope, local vegetation, and ground squirrels (*Citellus* sp.) foraging on the vegetation in a given ecosystem. In this example, the movement of an organic contaminant might be a function of the seasonal food source sought by the rodent species. In other seasons, the ground squirrels are absent or dormant; hence, they would not be subject to exposure by the same pathway.

The origin of each contaminant pathway is typically from soil or water, at the site of contamination and the end of each pathway is a component of the ecosystem where adverse effects may occur (such as threatened or endangered species, a resident small mammal population, or fish species in a downstream lake or reservoir). Several assessment endpoints (see Section 2.6 below) may exist at the end of a contaminant pathway because pathways will seldom be unidirectional or linear. Chemical pathways generally branch and proceed in multiple directions; for example, a contaminant may have the potential for moving from a contaminated site into an aquatic system, with no potential impacts (branches) en route to a pond. However, once the contaminant enters the pond, potential contaminant pathways may include uptake of the contaminant by aquatic vegetation, by aquatic organisms (e.g., mollusks, gastropods, aquatic insects), uptake by fish, or amphibians, or transport back to the terrestrial environment via birds or mammals that feed on aquatic organisms.

The number of contaminant pathways are determined by the characteristics of the contaminant and the complexity of the ecosystem. Contaminant pathways must by identified on each Army Superfund site; however, similarities in pathways will likely exist among many sites resulting from similar ecosystems. Greater definition (closer focus) of specific contaminant pathways will be a function of Tier 2 and Tier 3 chemical analyses. Ultimately, however, if a pathway is incomplete or does not exist at a particular site, no cause and effect relationship exists and there is no associated risk.

2.5 Ecological Effects

Ecological effects in Tier 1 of the PF phase should be derived from studies in the literature that are applicable to the stressors and ecological components of concern in the assessment, and from reports of previous studies (e.g., RI/FS) conducted at the site. Published data may come from a variety of sources including field observations (e.g., fish kills, changes in aquatic community structure), laboratory tests (e.g., single species or microcosm bioassays), and chemical structure-activity relationships. Home range, feeding area, and migratory patterns of the biota of concern at the site should be determined from USFWS, site specific sources (i.e., state fish and wildlife agencies, military installation records, etc.) or the open

literature. These data, together with spatial and temporal patterns of the COC within the site can help characterize the extent of ecological effects. Analysis of this information can help focus the assessment on specific stressors and on ecological components relevant to the site.

Caution must be taken so that the ecological effects data are properly utilized in Problem Formulation. For example, applicability of laboratory-based tests may be affected by extrapolations to various field conditions, whereas the interpretation of field observations may be influenced by site-specific factors such as natural variability or the presence of stressors other than the COCs. Ecological effects data obtained in PF can then be used to identify data gaps and to characterize ecological effects in the Analysis Phase of the assessment.

2.6 Endpoint Selection

Ecologically based endpoints are selected after the societal, regulatory, and biological goals have been established following review of stressor characteristics, the ecosystem potentially at risk, and the potential ecological effects. It is important that the RA and RM collaborate and agree on the endpoints selected before proceeding to the Analysis phase. An endpoint is defined as a characteristic of an ecological component (e.g., increased mortality in fish) that may be affected by exposure to the stressor[17]. Two types of endpoints, assessment and measurement, are used in the ERA to determine risk to the ecosystem.

An assessment endpoint is defined as:

> An explicit expression of the environmental value to be protected.[4]

For best use, assessment endpoints should have biological as well as societal value so that scientific information can be linked to the risk management process (e.g., policy goals). For an ERA to produce sound, acceptable results, there are five criteria necessary for choosing assessment endpoints[7,4]:

1) policy goals and societal relevance;
2) ecological relevance;
3) unambiguous operational definition;
4) accessibility to prediction and measurement; and
5) susceptibility to the hazardous agent.

When choosing assessment endpoints, two general questions must be answered: (1) what valued components of the environment are considered to be at risk; and (2) how should effects be defined? Some assessment endpoints are mandated legally or politically; however, the RA should also determine what endpoints should be

selected on technical grounds. Suter[7] suggests performing one of the following formal analyses of the relationship of components of the action being assessed and components of the receiving environment:

1. Create a matrix of exposure alternatives (e.g., soil contamination by munitions, spilling a product during shipment, etc.) and environmental receptors (fish, terrestrial plants, aquatic heterotrophic microflora, etc.) that are potentially affected. Environmental receptors are then checked off and possibly scored for the intensity and duration of the exposure and relative sensitivity to the toxic material.

2. A receptor identification exercise is valuable to identify which organism will be most exposed to a chemical. This consists of two steps: (1) performing a rapid quantitative exposure assessment to determine what media are most contaminated (note: this may be from a fate model determination), and (2) determining what communities, trophic groups, populations and life stages are most exposed to those media.

3. Indirect effects of stressors can be identified by developing models, including "event trees" showing causal linkages between site contaminants and various environmental components (Figure 7).

4. Existing data can be reviewed to determine the sensitivity of species or processes to the contaminant or to similar contaminants. These may include data from toxicity testing or from biological monitoring of prior releases.

We remind the reader that the primary objective of the Problem Formulation phase is to focus on appropriate endpoints within the risk assessment, coordinating frequently with the risk manager. Of course, the resources expended must be considered in light of the potential loss of the management resource. Often assessment endpoints cannot be directly measured. When this occurs measurement endpoints are selected that are related to assessment endpoints. Measurement endpoints are defined as:

A measurable ecological characteristic that is related to the assessment endpoint.[4]

Relating measurement to assessment endpoints is important to produce risk estimates that are scientifically sound and address policy goals. Measurement endpoints must accurately measure indicators of effects that will reflect the assessment endpoints and are often expressed as the statistical or arithmetic summaries of the observations that comprise the measurement.

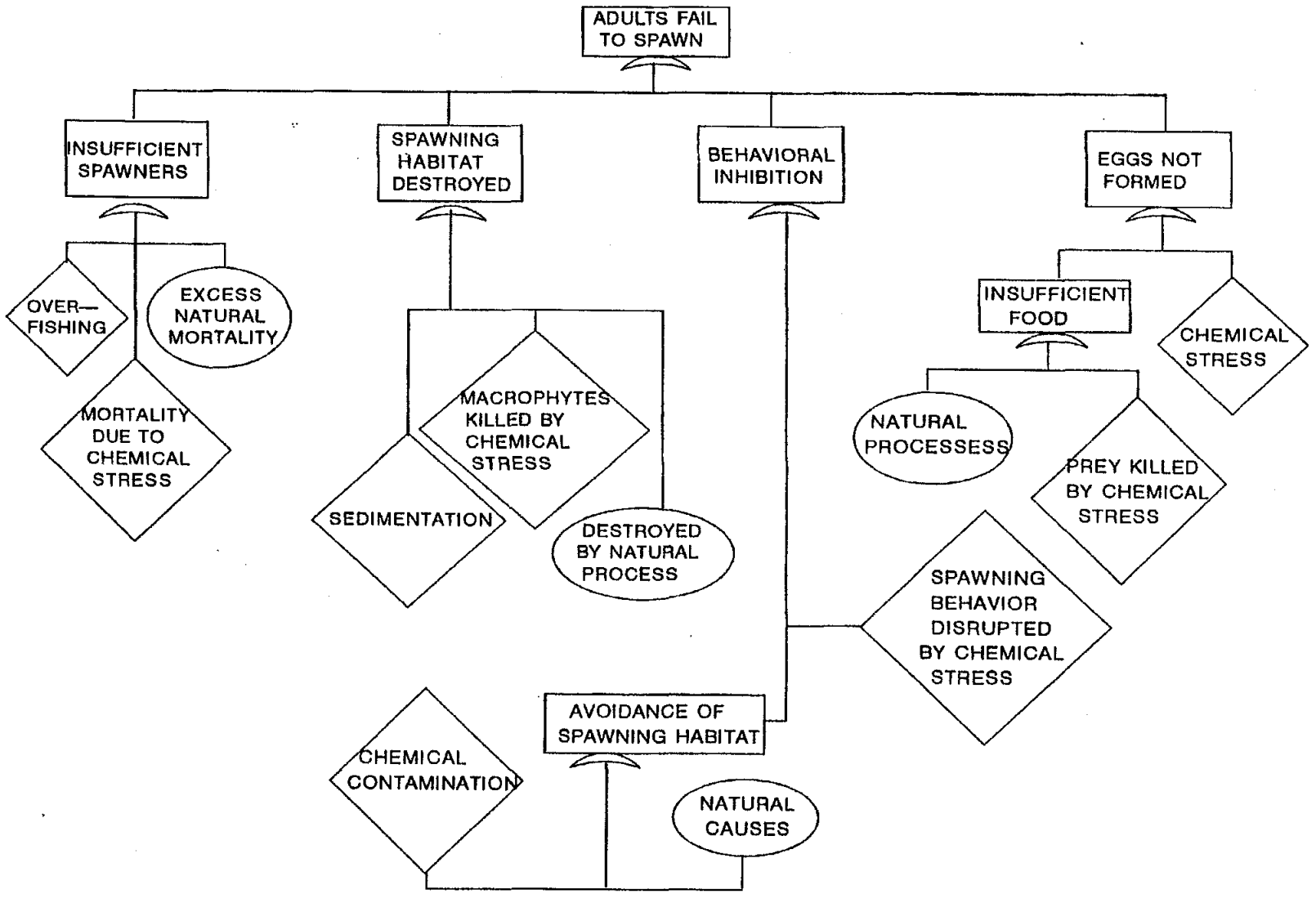

Figure 7. Event tree for failure of fish to spawn. This tree would be a branch of larger tree used to determine how toxicants might cause reductions in fish populations.[7]

Measurement endpoints may be single numbers such as LC_{50}'s or biomass measurements. Less common but more useful measurement endpoints are multidimensional descriptive models such as concentration-response functions. Selection of measurement endpoints must be carefully thought out prior to undertaking an ecological risk assessment. Considerations in selecting measurement endpoints are discussed in Table 2. If a tiered approach is applied, the types and number of measurement endpoints depend on the level of effort (i.e., personnel and cost) required to address data gaps in each tier (see Section B.2.). Ideally, a suite of assessment and corresponding measurement endpoints at different levels of biological organization (e.g., organism, population, community, food web) is preferred because it reduces the level of uncertainty and ensures that all relevant assessment endpoints are evaluated. However, time and monetary constraints may limit the types and quantity of measurement endpoints. Therefore, endpoints from models can sometimes be used to extrapolate across scales of time, space, and biological organization.[18] For example, measurement endpoints acquired from a sub-population (e.g., mortality, reproduction, and growth) could be used to predict effects on an assessment endpoint in a larger population (e.g., viability of a trout population in a stream). Sloof et al. (see Suter[7]) developed a simple statistical model to grossly estimate effective concentrations for ecosystem tests from a single organism-level test endpoint. Examples of assessment endpoints, indicators, and measurement endpoints are presented in Table 3.

2.7 The Conceptual Model

Once the stressors and potential receptors have been identified and characterized, and the assessment and measurement endpoints have been determined, a series of working hypotheses should be formulated on how the stressor(s) may affect ecological components.[4] At this point, the RA is at the stage between Problem Formulation and Analysis phases. The conceptual model (Figure 6) includes descriptions of the ecosystem potentially at risk and the relationship between measurement and assessment endpoints. Exposure scenarios should be constructed to include spatial and temporal distribution of the chemicals of concern and their interaction with the ecological system. Each scenario is defined in terms of the stressor, the type of biological system and principal ecological components, how the stressor will interact within the system, and the spatial and temporal scales.

Table 2. Considerations in selecting measurement endpoints.[4]

Relevance to an Assessment Endpoint

When an assessment endpoint cannot be directly measured, measurement endpoints are identified that are correlated with or can be used to infer or predict changes in the assessment endpoint.

Consideration of Indirect Effects

Indirect effects occur when a stressor acts on elements of the ecosystem that are required by the ecological component of concern. For example, if the assessment endpoint is the population viability of trout, measurement endpoints could evaluate possible stressor effects on trout prey species or habitat requirements.

Sensitivity and Response Time

Rapidly responding measurement endpoints may be useful in providing early warning of ecological effects, and measurement endpoints also may be selected because they are sensitive surrogates of the assessment endpoint. In many cases, measurement endpoints at lower levels of biological organization may be more sensitive that those at higher levels. However, because of compensatory mechanisms and other factors, a change in a measurement endpoint at a lower organizational level (e.g., a biochemical alteration) may not necessarily be reflected in changes at a higher level (e.g., population effects).

Signal-to-Noise Ratio

If a measurement endpoint is highly variable, the possibility of detecting stressor-related effects may be greatly reduced even if the endpoint is sensitive to the stressor.

Consistency With Assessment Endpoint Exposure Scenarios

The ecological component of the measurement endpoint should be exposed by similar routes and at similar or greater stressor levels as the ecological component of the assessment endpoint.

Diagnostic Ability

Measurement endpoints that are unique or specific responses to a stressor may be very useful in diagnosing the presence or effects of a stressor. For example, measurement of acetylcholinesterase inhibition may be useful for demonstrating responses to certain types of pesticides.

Practicality Issues

Ideal measurements endpoints are cost effective and easily measured. The availability of a large database for a measurement endpoint is desirable to facilitate comparisons and develop models.

Table 3. Examples of assessment endpoints. Possible indicators of effects on those endpoints, and possible endpoints for measurements of those indicators.[7]

Hazard/Policy Goal	Assessment Endpoints	Indicators of Effects	Measurement Endpoints
Herbicide used for weed control in southern lakes/No acceptable loss of fisheries	Probability of >10% reduction in game fish production	Laboratory toxicity to fish	Fathead minnow LC_{50} Larval bass concentration/mortal ity function
		Laboratory toxicity to food-chain organisms	*Daphnia Magna* LC_{50} *Selenastrum capricornutum* EC_{10}
		Field toxicity to fish	Percent mortality of caged bass
		Populations in treated lakes	Catch per unit effort Size/age ratios by age class
Agriculture insecticide associated with bird kills/No acceptable reductions in avian populations function	Proportion of raptors killed within the region of use	Laboratory toxicity to prey	Rat LD_{50} Japanese quail dietary LC_{50}
		Laboratory toxicity to raptors	Sparrow hawk dietary concentration/respon se Japanese quail dietary LC_{50}
		Avian field toxicity	Number of prey carcasses per hectare Number of dead moribund raptors per hectare
	Increase in rates of decline of declining bird populations within the region of use	Avian laboratory toxicity	Japanese quail dietary LC_{50}, Starling dietary LC_{50}
		Avian field toxicity	Number of bird carcasses per hectare by species
		Trends in populations of declining birds	Rates of decline in areas of use as proportions of reference areas

At this stage of the RA, the conceptual model should be used to predict the impact of the chemicals on individuals, populations and communities. The exposure scenario for chemical stressors usually involves consideration of sources (e.g., explosives burning ground), environmental transport (e.g., rate of movement through soil column), partitioning of the chemical among various environmental media (e.g., soil particles vs. organic matter), chemical/biological transformation or speciation processes (e.g., photolysis, biodegradation), and identification of potential routes of exposure (e.g., ingestion, plant root absorption, etc.). Exposure scenarios for non-chemical stressors such as soil compaction, or habitat alteration describe the ecological components exposed and the general temporal and spatial patterns of their co-occurrence with the stressor. For example, the exposure scenario may describe the extent and distributional pattern of compacted and disturbed soil in a field used for military training with tracked vehicles, the soil microflora, vegetation and wildlife occupying or using this field, and a comparison of the size and distribution of these populations with those in adjacent undisturbed fields[19].

The hypotheses formulated must first be "weeded out" for those considered most likely to contribute to risk. Then the risk assessor should further narrow down the choices to focus only on those hypotheses that can be addressed with available resources. These hypotheses are then evaluated in the Analysis phase. It is important that any hypotheses not originally used in the Analysis phase be re-visited when uncertainty is addressed in the Risk Characterization (RC) phase. Uncertainty considerations of model predictions in the RC phase may require that previous hypotheses explaining the assessment endpoint be reviewed. Professional judgement is needed to select the most appropriate risk hypotheses; further, it is needed to document the rationale underlying the selection process[4].

A detailed work plan should then be written describing objectives, data requirements (including assessment and measurement endpoints), experimental design, procedures and methods, quality assurance objectives, and a time schedule to estimate duration and completion dates of various phases of the assessment. Work plans will vary according to the specific needs of each assessment but should be formulated and agreed upon by all parties involved. The work plan should be included in the remedial investigation. In formulating a work plan, it is critical to address how data gaps will be handled and to explicitly state the data quality objectives[4]. The conceptual model describes the approach that will be used for the Analysis phase and the types of data and analytical tools that will be needed.

2.8 Evaluation of Problem Formulation

At the conclusion of PF, it is important for the risk assessors and risk managers to determine the attributes and focus of the rest of the assessment and to decide if indeed the assessment should continue. The EPA[3] has compiled a list of scientific/management decision points (Figure 2) that include factors that should be agreed upon before proceeding further with the risk assessment such as:

(1) Deciding whether or not the risk assessment should proceed further based on available information;

(2) Selecting assessment endpoints, testable hypotheses, and measurement endpoints;

(3) Agreement upon the exposure pathways;

(4) Selection of specific investigation methodology;

(5) Selection of data reduction and interpretation methods.

Agreement by all involved parties on the decisions and methodologies shown above will help to keep the risk assessment focused and save time and money.

3. ANALYSIS PHASE

During the Analysis phase (Figure 8), the working hypotheses developed during the PF phase link exposure assessment to ecological effects. This phase acknowledges that the abiotic and biotic characteristics of the ecosystem of concern will impact the ecological effects and the exposure profile. The various steps in this phase lead to the development of a stressor-response profile and an exposure profile. These profiles are used as the basis for risk characterization.

The most effective tool available to the ecological risk assessor is a site visit. During this visit the ecosystem is ualitatively assessed to determine potential receptors present at the site, determination of routes of exposure, and other stressors present (e.g., dredging activity, prop wash, lack of riparian habitat on the banks of a stream, etc.). Signs of direct effects may be noted during the site visit such as stressed vegetation around a seep.

On the basis of this site visit as well as existing data for the site, the risk assessor has to determine what additional data are necessary. Ecological risk assessment is commonly performed using a "weight of evidence" approach. An excellent description of this approach applied to a terrestrial ecosystem can be found in Menzie et al.[20]. They utilized predictive modeling based on measured surface water, sediment and soil concentrations of COCs, laboratory toxicity tests, field toxicity tests, and other field methods to assess potential ecological impacts.

It is important to realize that many potential hazardous waste site assessments have been designed by engineers without consultation with risk assessors. What often results is a large amount of data, none of which is of value to the risk assessor. For example, many metal water quality criteria are dependent upon site-specific water hardness, but water hardness is often not analyzed, or even thought of as important for analysis by the workplan author. Another important data quality often overlooked is the required detection limits necessary to perform risk assessment. The CLP procedure does analyze for polycyclic aromatic hydrocarbons (PAH), however CLP reporting limits are much above concentrations at which one may expect potential ecological impacts. Listed below are parameters commonly overlooked and chemicals which alternative analytical methods which provide lower detection limits may be appropriate:

* Parameters Commonly Overlooked

Hardness in surface water,
Total organic carbon in sediment and soil,
Lipid content in biological samples

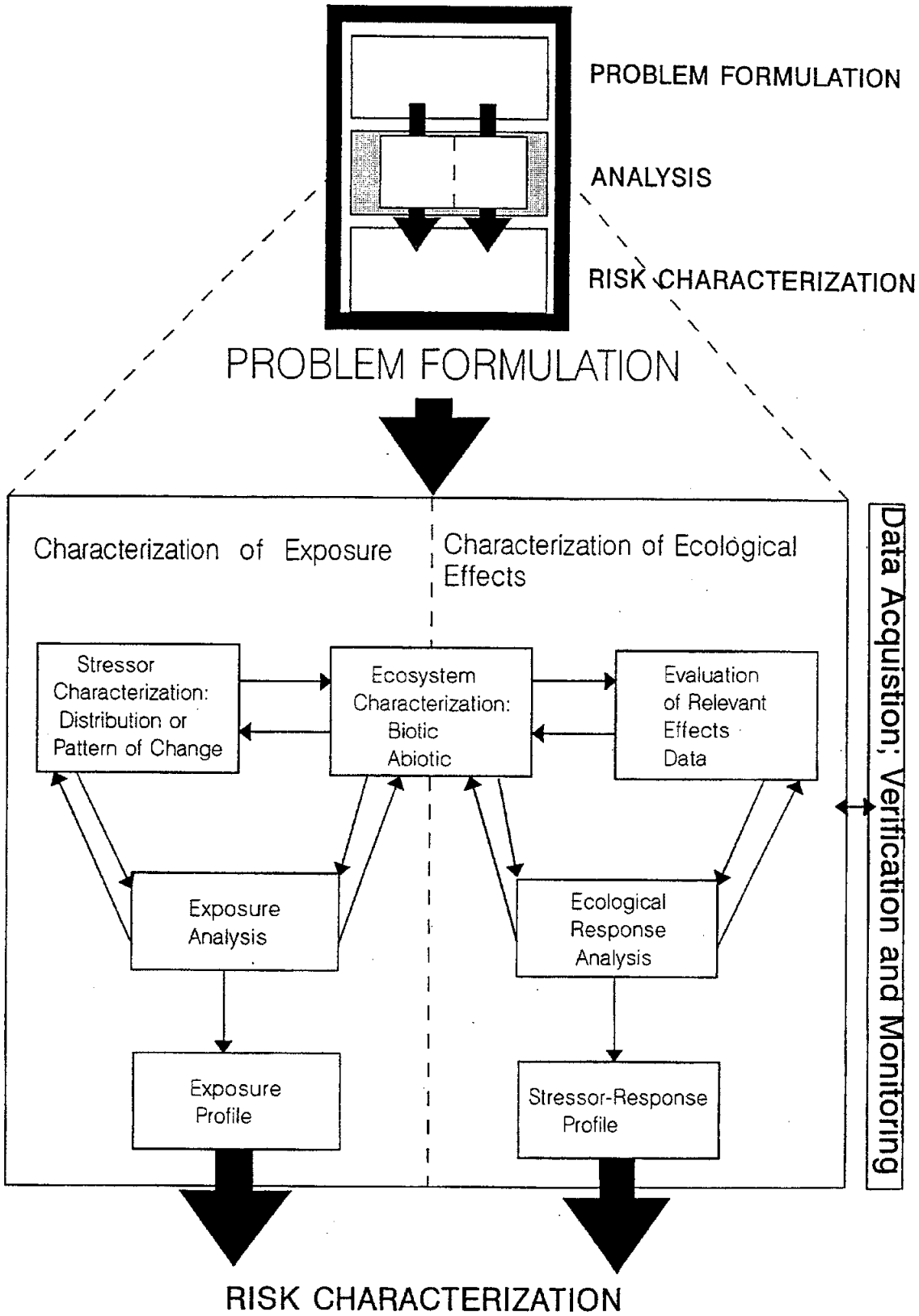

Figure 8. Analysis Phase

* Chemical Types Commonly Measured at High Reporting Limits

Polycyclic Aromatic Hydrocarbons (PAH),
Pesticides, PCBs, and some metals.

To correct this situation it is necessary to involve the risk assessment personnel early in the workplan stage. Their role should be to assure that all necessary parameters are being measured at appropriate reporting limits. Alternative analytical chemistry methods are available which allow reporting much lower detection limits than those reported using CLP standards. The risk assessment personnel should work with the analytical laboratory to determine appropriate analytical methodology. In addition, the sampling plan should be assessed to assure that proper numbers and types of samples are being taken. Biota samples will commonly be completely unsampled, and because the waste engineers tend to focus on "hot spots", by definition a biased sampling procedure, exposure will often be overestimated.

3.1 Exposure Characterization

3.1.1 Stressor Characterization

Characterization of exposure begins with determining what stressors are present at the site. Ecological risk assessment is complicated by the necessity of determining multiple stressors, often including stressors such as habitat and human actions like dredging a stream or water body.

This step determines the stressor's distribution over space and time at the study area. The primary stressor is evaluated as well as any secondary effects which have occurred due to impacts from the initial stress to the system. Background or preliminary information on the chemical-of-concern is important for the stressor characterization because such information points towards expected stressor-responses. For example, lipid-soluble organochlorine pesticides bioaccumulate fairly readily in aquatic ecosystems. Organic chemicals with low K_{ow} do not accumulate readily and direct toxicity, rather than tissue uptake, is the primary concern for exposure.

Characterization of exposure begins with determining where the contaminant is on the site, where, if and how the contaminant moves from the site, and what physical/chemical characteristics lead to its bioaccumulation, degradation, transport, etc. For many chemicals, historical files provide information on quantities produced, used, stored on-site, or sprayed (pesticides, solvent cleaners). Often, chemical characteristics of the contaminant, including rates of degradation (via photolysis, hydrolysis, microbial), adsorption, solubility in water or lipid may be obtained from literature sources, on-line

chemical databases (Table 3), Material Safety Data Sheets (for industrial chemicals), and technical reports. An excellent source for environmental degradation rate is Howard et al.[21], general fate and transport data can be found in the Lewis Publishers (Chelsea, Michigan) series titled "Handbook of Environmental Fate and Exposure Data for Organic Chemicals". This series, ultimately to have seven volumes, presently consists of Large Production and Priority Pollutants (Volume I), Solvents (Volume II), Pesticides (Volume III), and Solvents 2 (Volume IV). Data provided in these volumes include basic chemical and physical properties (boiling point, melting point, molecular weight, water solubility, octanol-water partition coefficient, vapor pressure, etc.) and a description of basic fate and exposure potential including sources, important transport processes, and reported concentrations in the environment. While there are many computer databases available, the most current and reliable database encountered so far for fate and transport data is produced by the Syracuse Research Corporation, Merrill Lane, Syracuse NY 13210. They maintain several databases including BIOLOG (Biodegradation database) and CHEMFATE. CHEMFATE can be used to search for many properties and characteristics ranging from soil adsorption constants to photolysis degradation rates. The above references refer to fate and transport of organic chemicals. There are several excellent references available regarding fate and transport of metals in the environment[22,23].

The information required for a Tier 1 exposure characterization would be obtained via the documents described above. Ecological assessments may be "effects-driven" or "stressor-driven." For example, the abundance of a sediment benthic community is often used as a measure of sediment "health". If the benthic community is found to be deficient, it is commonly used as an "effects-driven" assessment. Alternatively, known dump sites, with no apparent ecological effects are an example of a "stressor-driven" assessment. This implies that the initial focus may be on understanding how the measured effects were induced ("effects-driven") or on understanding the behavior of the chemical(s) of concern ("stressor-driven"). In characterizing exposure, the RA identifies measurement endpoints along each contaminant pathway where data collection or computer simulations and models are applied to evaluate contaminant fate and consequent ecological impacts. Data collected for these measurement endpoints help reduce uncertainty by validating or refuting whether predicted contaminant movement is actually occurring. In characterizing exposure, the RA identifies measurement endpoints along each contaminant pathway where data collection or computer simulations and models are applied to evaluate contaminant fate and consequent ecological impacts. Data collected for these measurement endpoints help reduce uncertainty by validating or refuting whether predicted contaminant movement is actually occurring.

The environmental fate and potential transport of contaminants is crucial to effective risk assessment because the bioaccessibility (whether organisms come in contact with toxicants) and bioavailability (whether contact leads to uptake) are controlled by these processes. For pesticides, degradation, volatization, binding, leaching, and aging determine ultimate exposure concentrations[24]. Metals availability is controlled largely by pH and oxidation-reduction relationships in environmental media[22,25]. The chemistry and distribution of the compounds of interest must be thoroughly understood for effective risk analysis. It is crucial for the risk assessment/risk management team to understand that the bulk concentration of chemical compounds as measured in typical laboratory extraction tests (such as those provided with Contract Laboratory Program quality assurance documentation under CERCLA) do not reflect the biologically active concentrations. In practice, binding and uptake processes depend on complex environmental processes which need to be accounted for in projecting risks.

The environmental fate and transport of mercury in anoxic (oxygen depleted) environments is shown in Figure 9. Mercury has been identified as a chemical of concern in many areas of the country, primarily due to its volatilization and transport within the atmosphere. For example, within the everglades of Florida mercury has been identified as a chemical of concern for many fish, raccoons, and cougars preying on the raccoons. Obviously, there are no point sources of mercury directly in the everglades, pointing to long range transport from outside the boundaries of the everglades. The fate and transport of mercury is complex, and involves bacteria who can methylate the ion and form a highly bioaccumulative methylmercury.

Similar fate and transport figures can be produced for other metals and organic chemicals. Environmental factors will influence chemical fate and transport dependent upon the type of chemical of concern. For example, lipid-soluble (high octanol-water partition coefficient, K_{ow}) organochlorine pesticides bioaccumulate readily in aquatic ecosystems. Alternatively, low K_{ow} chemicals do not readily bioaccumulate and direct toxicity, rather than tissue uptake, is the primary route of exposure.

Models in Tier 1 analyses serve as "screening analysis" to provide initial qualitative assessments of contaminant transport into the environment. They are designed to (1) identify each transport process controlling movement of various contaminants within and among media, (2) estimate the direction and rate of chemical movement from the site and, (3) identify areas to which contaminants have been or may be transported. Fugacity models[26,27], which calculate where a given chemical will tend to accumulate in the environment, are an example of this level of

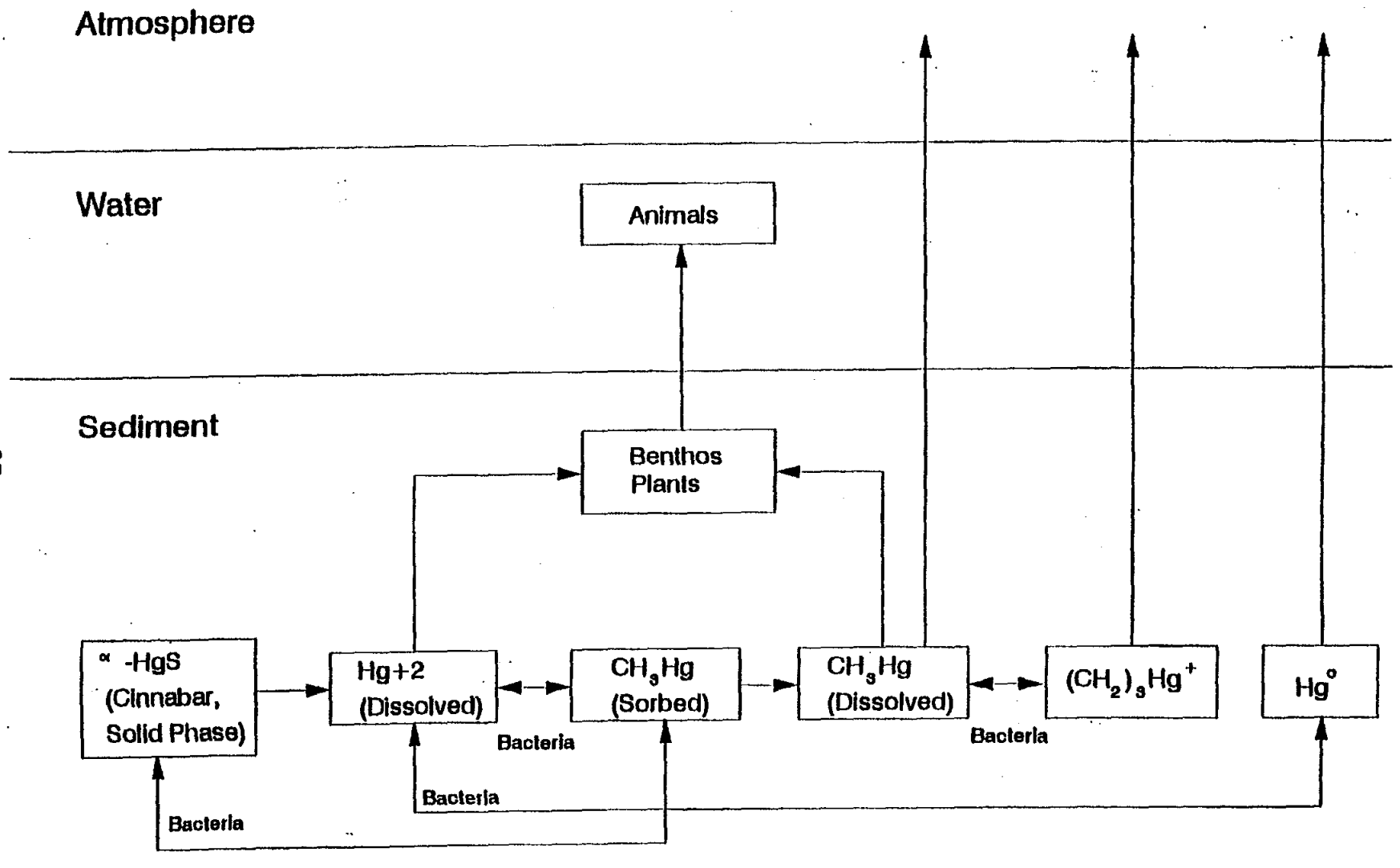

Figure 9 Environmental Fate and Transport of Mercury in Anoxic Aqueous Environments

detail. This level of modeling provides an initial organization and direction for subsequent in-depth analyses of contaminant transport. When a more in-depth analysis of environmental fate is desired, the RA should seek advice on which modeling procedure is most appropriate to the circumstances. In general, the more sophisticated models are data-, time- or resource-intensive. Table 4 is a ranking of relatively simple to complex models[28]. Criteria to consider when selecting in-depth environmental fate models are:

(1) capability of the model to account for important transport, transformation and transfer mechanisms;

(2) the fit of the model to site-specific and substance-specific parameters;

(3) data requirements of the model, in relation to the availability and reliability of site-specific data; and

(4) the form and content of the model output. That is, does the model output address relevant questions and provide data required for use as input to further analyses.

At the end of a Tier 1 study for the exposure characterization, the RA should have:

1) identified the major COCs,
2) listed physical and chemical parameters of the COCs,
3) collected environmental fate information from the literature,
4) compiled site-specific sampling data on COCs,
5) identified contaminants that may bioaccumulate,
6) identified data gaps.

As the exposure characterization progresses to tiers 2 and 3, contaminant pathways examined in Tier 1 of exposure characterization will continue to be evaluated through such options as data collection of previously unsampled measurements endpoints identified in the Tier 1 PF phase or a more intensive sampling over the same habitats to more closely characterize contaminant distribution. In Tiers 2 and 3 more intensive chemistry sampling may allow sampling of degradation products spread in a more diffuse manner throughout the site. Further data collection reduces the uncertainty of environmental fate and distribution estimates.

Monitoring data are useful for analyzing contaminant transport and fate. However, monitoring data may not allow discrimination of the contributions of contaminant loadings from point versus non-point sources. A combination of monitoring data with modeling techniques is necessary in Tiers 2 and 3 to conduct

Table 4. Progressive Levels of Aquatic Chemical Models

Level	Features	Data Needs	Answers
0	Dilution model, yields initial complete mix concentration	Effluent design flow, critical low flow in receiving water or allowable mixing radius/zone, upstream chemical concentration, effluent load or ambient standard-model solves for missing parameter	Worst case ambient concentration in the water column following mixing; additional calculations using K_{oc} yields information on the expected phase distribution (particulate or dissolved)
1a	Steady-state model, simple one-dimensional (1-D) segmentation, first order loss from the water column	River physiography, chemical concentration versus river mile and/or knowledge of first-order loss rates	More realistic estimate of concentration as a function of distance from the effluent, rough estimate of the chemical retained in the system
1b	Steady-state model, 1-D segmentation, partitioning to solids, net settling links water to sediment	Solids loads, solids versus river mile, solids characteristics, and partitioning coefficient	Chemical distribution in particulate and dissolved phases in the water column
1c	Steady-state model, 1-D segmentation, partitioning, full solids dynamics	Literature and site-specific analysis of resuspension and gross settling rates	Provides chemical levels in the sediment and the water compartments
1d	Steady-state model, 1-D segmentation, partitioning, separation of abiotic and biotic solids	Information on water column abiotic-biotic solids origin and transport rates	More accuracy, better differentiation of biotic component
2a	Time-variable model, 1-D segmentation, partitioning, full solids dynamics	Time variable loads and environmental conditions, better vertical solids transport rates	Response as a function of time and distance from the source(s)
2b	Steady-state model, 2-D segmentation, partitioning, full solids dynamics	Hydraulic transport or routing, more spatially distributed field data	Spatially distributed (2-D) results, better representation of certain systems, a broader range of questions addressable to correspond to locations of specific interest
2c	Time-variable model, 2-D segmentation, partitioning, full solids dynamics	Typically more highly resolved data (time and space)	Temporal and spatially related questions
3	More hydraulic (3-D), sorbent, chemical, or biological complexity	Additional problem-specific site data and potentially supporting research	More complex questions of source, chemical interaction, fate, transport, or effects

analyses of contaminant fate in sites for which Tier 1 results do not allow a sufficiently accurate determination of exposure and risk.

3.1.2 Ecosystem Characterization

In ecosystem characterization the abiotic and biotic parameters of the system of concern are evaluated. Their impact on the distribution and bioavailability of the stressors of concern are critical parts of the exposure assessment. Migration and resource use by biota and behavioral effects of the stressors on organisms are also considered.

To fully characterize exposure and develop an exposure profile for the site, the RA must recognize the ecosystem components and functions described as important in the conceptual model formulation.

Included in the ecosystem characterization are physical characteristics of the ecosystem, including topography, geology, and hydrology, climatic patterns of the area such as precipitation, insolation, temperature, humidity, and the flora and fauna of the sites. Understanding these components and their interrelationships, in conjunction with data on the contaminant distribution, allows the RA to evaluate whether the contaminants are confined to specific areas and remain *in situ*, or whether the contaminants have the potential to move through various ecosystem components.

Barnthouse et al.[29] presented modeling approaches to link water quality to reductions in "dose" under various scenarios of ecosystem productivity. One example of a modeling approach that illustrates how ecosystem trophic status modifies the bioavailability of toxicants and decreases the subsequent dose to biota was performed by McCarthy and Bartell[30]. Their model predicts the association of a contaminant with dissolved organic material (DOM) or particulate organic material (POM) significantly lessens the bioavailability of a toxicant and, thus, the potential dose experienced by the organisms. Importantly, this paper shows the necessity of estimating the true bioavailability of a contaminant in the environment.

Seasonal or habitat variances in bioavailability can be modeled (e.g., mapped onto expected environmental chemical concentrations for species of known life history, feeding, and habitat requirements) and are a cost-effective approach to the hazard characterization of complex chemicals. For a given concentration, species may be subject to exposure for a <u>relatively</u> longer period of their life-span if they are smaller or less likely to move beyond the boundaries of the contaminated area (examples are earthworms, burrowing invertebrates, or small

mammals). Further, if a chemical is susceptible to being bound by organics, burrowing (or thigmotactic) benthic invertebrates (or benthos-feeding fish) may be subjected to higher exposures than would otherwise be predicted. Volume 2 includes certain models available for evaluating transport, transformation and fate of contaminants in the environment (e.g., EXAMSII, LPMM). In addition, several models estimate biotic exposure or uptake of contaminants (e.g., FGETS).

If available data indicate little potential for movement, the assessment may move in the direction of evaluating the potential for uptake by flora and fauna in the immediate vicinity of contamination. Questions might focus on whether the material is being bound within the soil by specific soil constituents or within specific soil horizons, or taken up by plants or burrowing invertebrates. These initial lines of inquiry may lead to further questions about the potential for effects on plant distribution and floral composition. Questions stemming from the hypotheses formulated in the PF phase may include: Are soil microorganisms affected to the extent that soils become infertile or soil-plant interactions disrupted? Are processes of nutrient cycling disrupted? Answers may lead to other lines of inquiry, such as the potential for movement of contaminants into animal matrices.

3.1.3 Exposure Analysis

Once stressor characteristics and the ecosystem potentially at risk have been identified, potential pathways for contaminant(s) through the ecosystem must be identified. The spacial and temporal distribution of the stressors and the ecological characteristics of the system of concern are combined to evaluate exposure. The concentrations of the stressor are combined with assumptions about contact or uptake by biota to determine co-occurrence with measurement endpoints. However, concentration of a contaminant does not equate to exposure. Bioavailability and the environmental fate of the chemical must also be considered. The environmental fate and potential transport of contaminants is crucial to effective risk assessment, because the bioaccessibility (whether organisms come in contact with toxicants) and bioavailability (whether contact leads to uptake) are controlled by these processes. For pesticides, degradation, volatization, binding, leaching, and aging determine ultimate exposure concentrations[24]. Metals availability is controlled largely by pH and oxidation-reduction relationships in environmental media[25]. The chemistry and distribution of the compounds of interest must be thoroughly understood for effective risk analysis. It is crucial for the risk assessment/risk management team to understand that the bulk concentration of chemical compounds as measured in typical laboratory extraction tests (such as those provided with Contract Laboratory Program

quality assurance documentation under CERCLA) do not reflect the biologically active concentrations. In practice, binding and uptake processes depend on complex environmental processes which need to be accounted for in projecting risks.

The environmental fate of a contaminant will generate pathways that may be simple and straightforward or complex and highly branched. Pathways are generally defined by naturally occurring physical, chemical, and biological components of the ecosystem. As an example, consider the evapotranspiration potential, precipitation, soil type, slope, local vegetation, and ground squirrels (*Citellus* sp.) foraging on the vegetation in a given ecosystem. In this example, the movement of an organic contaminant might be a function of the seasonal food source sought by the rodent species. In other seasons, the ground squirrels are absent or dormant; hence, they would not be subject to exposure by the same pathway.

The origin of each contaminant pathway is typically from soil or water, at the site of contamination and the end of each pathway is a component of the ecosystem where adverse effects may occur (such as threatened or endangered species, a resident small mammal population, or fish species in a downstream lake or reservoir). Several assessment endpoints may exist at the end of a contaminant pathway because pathways will seldom be unidirectional or linear. Chemical pathways generally branch and proceed in multiple directions; for example, a contaminant may have the potential for moving from a contaminated site into an aquatic system, with no potential impacts (branches) en route to a pond. However, once the contaminant enters the pond, potential contaminant pathways may include uptake of the contaminant by aquatic vegetation, by aquatic organisms (e.g., mollusks, gastropods, aquatic insects), uptake by fish, or amphibians, or transport back to the terrestrial environment via birds or mammals that feed on aquatic organisms.

The number of contaminant pathways are determined by the characteristics of the contaminant and the complexity of the ecosystem. Contaminant pathways must by identified on each Army Superfund site; however, similarities in pathways will likely exist among many sites resulting from similar ecosystems. Greater definition (closer focus) of specific contaminant pathways will be a function of Tier 2 and Tier 3 chemical analyses. Ultimately, however, if a pathway is incomplete or does not exist at a particular site, no cause and effect relationship exists and there is no associated risk.

Several models are currently used to assess the fate and distribution of toxic chemicals in ecosystems and link distribution to exposure and effects assessment. Many of these are discussed in detail in Volume 2 of this document. Most exposure models tend to be conservative because they are based on

an assumption of equilibrium, and thus overestimate exposure. Thus model validation is very important when using any predictive model. For example, if one is modeling bioconcentration of chemicals into fish at a site, the results can be compared to measured concentrations of chemicals in fish at the site to validate the model. The text that follows is meant as an introduction of modeling efforts which have been successfully used to assess chemical fate, transport and exposure.

Estimation of contaminant bioaccumulation (the net accumulation of a chemical by an organism as a result of uptake from all routes of exposure) at the site through the food web is very important to address because, in many cases, it provides a link to human health risk assessment. For example, the octanol-water partition coefficient (K_{ow}) may be known or estimated for organic chemicals. Typically, log K_{ow} values less than 4.3[31] to 5.0[32] do not biomagnify in fish. Garten and Trabalka[33] reviewed terrestrial food-chain data and concluded that only organic chemicals with K_{ow} values greater than 3.5 significantly bioaccumulate in mammals or birds. Models such as FGETS (Food and Gill Exchange of Toxic Substances) and SARAH (Surface Water Back Calculation Procedure) can be used to predict bioaccumulation potential (see Volume 2).

An example of the use of fate, transport, and exposure models were used to predict risks to humans can be found in a Newark Bay study[34]. Dredged material from Newark Bay containing dioxin was proposed for disposal at a disposal site in the New York Bight. Models were used to predict human exposure via ingestion of fish by humans (Figure 10). Accumulation factors (AF) found in Pruell et al.[35] were used to directly model transfer of dioxin from sediment to benthic organisms associated with that sediment. In order to estimate the exposure of dioxin associated with the dredged material to other aquatic organisms, it was initially partitioned to sediment interstitial water. An equilibrium fugacity model developed by Mackay[26,27] was then used to predict sediment overlying concentrations of 2,3,7,8-TCDD (Dioxin). Thomann[36,37] developed a simple aquatic food chain model using contaminant body burdens of organisms in various trophic levels, thus quantifying bioaccumulation. This same model was expanded to include interaction of aquatic biota with sediment chemicals in Thomann et al.[38]. These models were used to predict concentrations of dioxin in lobster, flounder, and bluefish in a food web. Ultimately the risk to humans ingesting these fish was calculated.

Fordham and Reagan[39] developed a food web model to evaluate potential exposure pathways for a site (Figure 11). Data collection can be complex and many assumptions on exposure and uptake are made. The model estimates acceptable concentrations

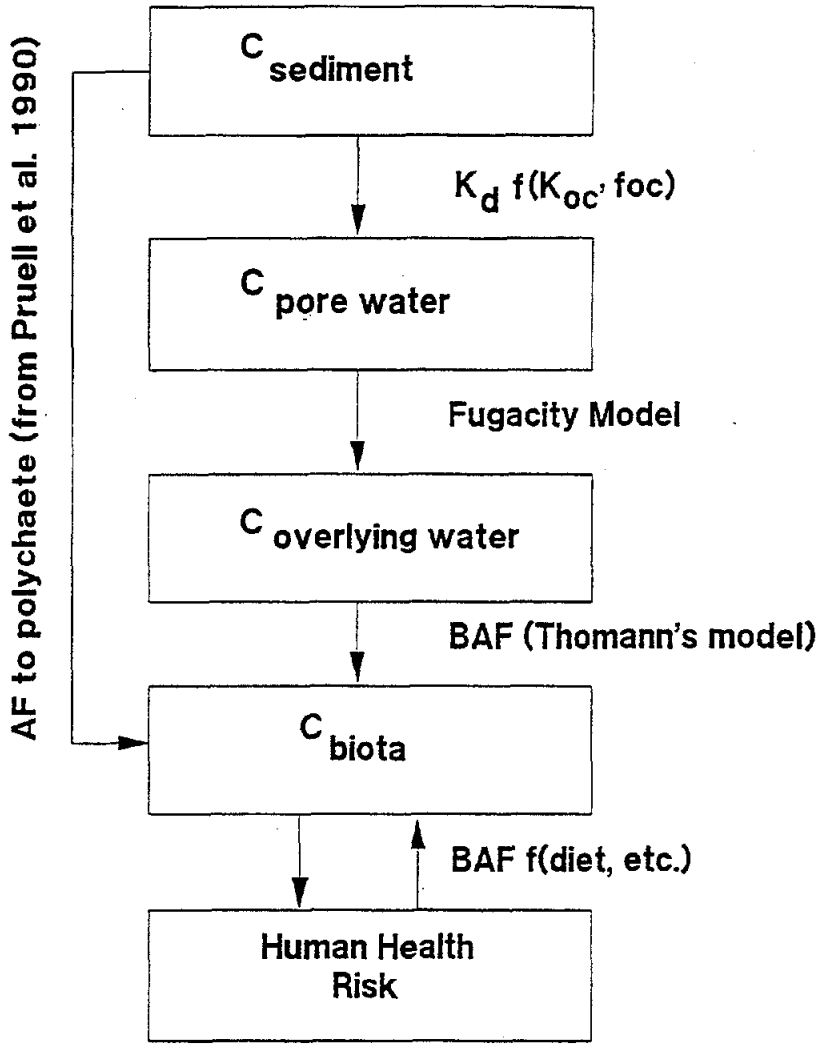

Figure 10 Schematic presentation of the approach used to assess risk of dioxin associated with sediments.

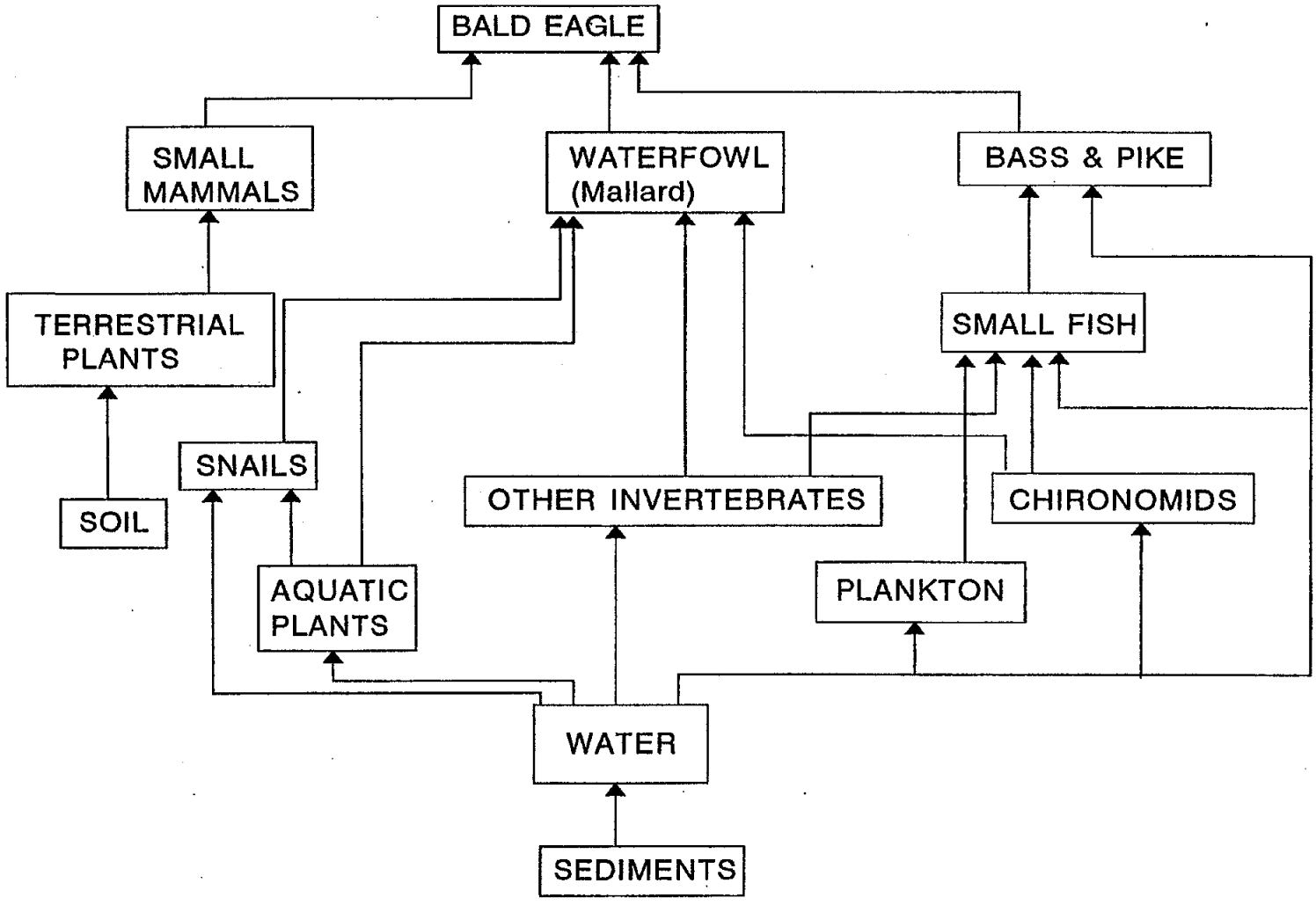

FIGURE 11. Pathways of contaminants from sediment and water to a target organism in a representative sink food web for aquatic ecosystems at Rocky Mountain Arsenal.[39]

in abiotic media for each exposure pathway. Further, it develops a site-specific food web by entering data from on-site sampling as well as literature sources. Finally, the model addresses bioaccumulation in multiple food chains that terminate in a high trophic level species (e.g., bald eagle). Uncertainty and data gaps need to be stated when using this method. Data from this type of study can be utilized in ecological risk assessments when evaluating risk to populations of biota exposed to site-related contaminants via different pathways.

3.1.4 Exposure Profile

The exposure profile presents the concentration of the stressor and its distribution over the area of study. Exposure over time can also be addressed so that the units match those presented in the stressor-response profile. The exposure profile evaluates pathways and determines exposure or dose to measurement endpoints. The extent to which ecosystem properties are analyzed depends upon the nature of the stressors and ecosystem components, bioavailability, and the resources available. Analyses should concentrate on those ecosystem components that are determined to be at greatest risk. Knowing the stressor characteristics can help to narrow the focus of the investigation on the components of the ecosystem that are potentially most susceptible.

The exposure profile for chemical stressors usually involves consideration of sources (e.g., explosives burning ground), environmental transport (e.g., rate of movement through soil column), partitioning of the chemical among various environmental media (e.g., soil particles vs. organic matter), chemical/biological transformation or speciation processes (e.g., photolysis, biodegradation), and identification of potential routes of exposure (e.g., ingestion, plant root absorption, etc.). Exposure profiles for non-chemical stressors such as soil compaction, or habitat alteration describe the ecological components exposed and the general temporal and spatial patterns of their co-occurrence with the stressor. Shaw and Diersing[19] described the extent and distributional pattern of compacted and disturbed soil in a field used for military training with tracked vehicles, the soil microflora, vegetation and wildlife occupying or using this training field. They compared the size and distribution of these populations with those in adjacent undisturbed fields.

Statistical techniques commonly used in the exposure profile are geostatistical techniques (kriging) to determine loci of contaminant residues in soil or water and multivariate techniques (cluster analyses, canonical correlation, principal components). Perland[40] presented an effective integration of chemical fate and transport information into an exposure profile of an ecological

risk assessment. In this case, groundwater was contaminated with benzene and barium in the vicinity of valuable wetlands habitat. Surface water exposure concentrations were projected based on measured groundwater data and information regarding local precipitation, soil chemistry, contaminant binding, pH, Eh, and volatization and dilution. It was concluded in the risk characterization that potential ecological risks were not associated with groundwater contamination and site remediation proceeded as dictated by non-ecological issues.

3.2 Characterization of Ecological Effects

3.2.1 General Overview

The determination of ecological effects at a site is a critical component of the ERA because data generated in this section may drive the decision making for the rest of the assessment. Assessment endpoints guide what data or measurement endpoints are required to assess impacts. To quantify ecological effects, data can range from sublethal or behavioral effects, to lethal effects, to population shifts, to community changes, habitat loss, ecosystem structural and/or functional changes, to biomagnification of chemicals through a food web (Volume 2). Subcellular biomarkers may be useful for identifying subtle effects. Data on threatened or endangered species offer special consideration because individuals, as well as populations, must be protected[41]. Evaluating ecological effects at a particular site is made more difficult because site-specific toxicity data or specific data on a species of concern are often lacking. Ecological surveys and Geographical Information Systems (GIS) are used to support a qualitative determination of ecological health, diversity, and habitat distribution and they can help to fill such data gaps.

Potential cause and effect relationships between a contaminant and the ecological measurement endpoint must be established. Hill's criteria[4] provide a listing of the primary questions that should be addressed (Table 5). The major criteria such as strength (a high magnitude of effect associated with exposure to the stressor), consistency (the association is repeatedly observed under different circumstances) and specificity (the effect is diagnostic of a stressor) need to be recognized and considered. We caution against establishing a cause - effect relationship based on simple observations (i.e., the contaminant is present in a forest soil and the forest is in decline, therefore the decline is caused by the contaminant). Many factors such as drought, insect infestation, disease, nutrient stress, management practices, etc. may be contributing to the decline.

Table 5. Hill's Criteria for evaluating causal associations[4].

1. **Strength:** A high magnitude of effect is associated with exposure to the stressor.
2. **Consistency:** The association is repeatedly observed under different circumstances.
3. **Specificity:** The effect is diagnostic of the stressor.
4. **Temporality:** The stressor precedes the effect in time.
5. **Presence of biological gradient:** A positive correlation between the stressor and the response.
6. **A plausible mechanism of action.**
7. **Coherence:** The hypothesis does not conflict with knowledge of natural history and biology.
8. **Experimental evidence.**
9. **Analogy:** Similar stressors cause similar responses.

note: Not all of these criteria must be satisfied, but each incrementally reinforces the argument for causality. Negative evidence does not rule out a causal association but may indicate incomplete knowledge of the relationship.

At most DoD hazardous waste sites, the initial environmental effects may have occurred years ago. Cause and effect evidence of contaminant toxicity may be difficult to determine because of adaptation of the community or system. Therefore, it is important to determine as much of the natural history and biology of the site as possible and to determine whether a continuing exposure pathway exists and whether it poses a threat to the currently-existing ecosystem. The ecological system in which the contaminants or stressors are present influence the impact they have on the biota. For instance, it is well-documented that physical and chemical changes in aquatic systems affect the toxicity and distribution of chemicals. An example is the inverse correlation between toxicity of heavy metals and increasing water hardness[42] and pH[43]. Terrestrial systems can act in a similar fashion with various soil parameters such as CEC or organic matter content, enhancing the ability of a soil to adsorb chemicals[44].

Thus physical, chemical, and biological components of the ecosystem need to be considered for their impact on the bioavailability and exposure of the contaminants at the site. Furthermore, if the contamination or stress occurred years ago, the ecosystem may have had time to recover to another state. The adapted state of the system needs to be evaluated to judge habitat change, and to determine whether the changes have reduced the "value" or productivity of the site. System resilience is also important in assessing the impact of the contaminant on the biota. Resilience, defined as the capacity of the system to return to a "pre-disturbed" state, has to be defined in terms of the important effects endpoints. For example, it may be the time it takes for a bird or small mammal population to re-establish itself (years to decades) or a soil invertebrate fauna to re-establish (months to years). Resilience is most often measured in lower trophic level animals or plants, simply because of the ability of the assessor to measure their ability to recover.

Selecting appropriate reference sites is difficult but very important to accurately evaluate the ecological effects in a risk assessment. The reference habitat should be similar in all aspects but for the contamination. For example, a terrestrial location with contaminated soil should have as a reference site one that has a similar soil type with similar vegetation and wildlife habitat. It may be useful to study soil survey maps obtained from the Soil Conservation Service, consult with the National Wildlife Federation about wildlife habitats, seek categories of "reference watershed" from the EPA EMAP program, or to link gradients of chemical contamination to observed effects or measured body burdens. Lacking such data, information from regional or state parks, undisturbed areas on the site (and known to not have been subject to previous contamination) may serve for use under Tier 1.

Various data on cause and effect of the contaminant(s) at the site then need to be formatted into a contaminant/response profile. Each measurement endpoint should, in theory, have its own profile. The profile may include NOEL's and LOEL's, LC_{50}'s, LD_{50}'s, EC_{50}'s or other quantitative measures, as well as the percentile of the population community or system affected versus exposure dose. In practice, these data can be hard to find and difficult to generate.

An example method of profiling toxicity and exposure assessment is provided by Toxicity Reference Values (TRV) (Figure 12). The TRV method uses available toxicity data on a specific COC to generate an estimated No Observed Adverse Effects Level (NOAEL) for a species of concern at the site with safety factors or uncertainty values included in the process. Laboratory-generated TRVs for a given time period (i.e., the lowest observed effect concentration, LOEC, for a 10-day exposure) may be linked to a specific exposure duration for the population in the field. Although there are sets of limiting assumptions required for the use of TRVs, they can provide an estimate of expected toxicity for given exposure periods.

Multi-contaminated sites offer unique problems. Often, many receptors are exposed to multiple stressors simultaneously. Ecological risk is much more difficult to discern at these sites. Individual as well as synergistic effects of the stressors must be estimated to accurately determine risk. Chemical mixtures influence toxicity in two ways. First, chemical mixtures can cause a toxic effect that is qualitatively or quantitatively different from any of the individual stressors acting alone. Second, the effects of one chemical may influence the kinetics of uptake, metabolism, and excretion of other chemicals. Examples include coating of fish gills by thick mucus when exposed to excessive aqueous concentrations of zinc and damage to nephridia that may be caused by cadmium-metallothionine complexes. The metabolic kinetics of a chemical may also be affected by other chemicals that induce or inhibit enzymes, or that simply reduce the physiological capacities of an organism[7].

Direct effects of stressors on variables such as mortality or growth need to be evaluated at higher levels of organization (population, community, or system) than the organismal (individual or species) level alone. These variables will typically be harder to measure, but usually will provide more pertinent information on the ecological effects caused by the stressors. A population shift, in and of itself however, does not imply a negative impact on the community. The relevance of

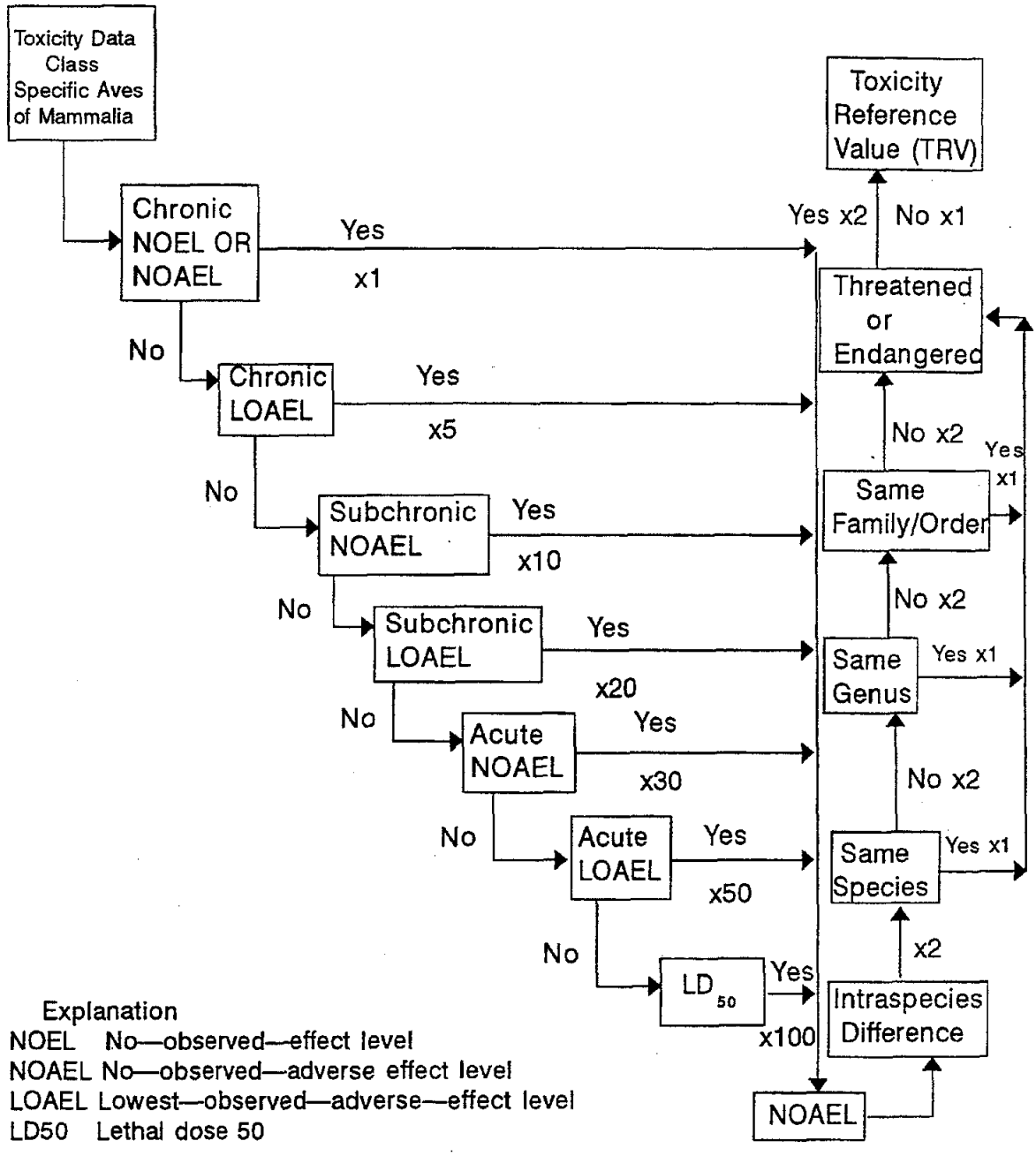

Figure 12. Methodology to derive toxicity reference values (TRV's) from class—specific toxicity data.

effects at the population level to the stressors of concern must then be determined.

Indirect effects must also must be considered and include impacts on habitat, effects on biota in the food web, changes in reproductive capacity, etc. The interaction of all indirect effects to each other and to direct effects should be obtained in order to accurately characterize risk. The simplest assumption is that indirect effects are additive, but more complex interactions are possible. The best understood of the nonadditive effects are thresholds[7]. For instance, populations of a certain species will not be supported once habitat area drops below a certain size; anoxia occurs once the organic input into a water body rises above a certain level, and extinction occurs when mortality rates rise above a certain level in a population. Identification and quantification of such thresholds is a critical component of cumulative affects assessment. Synergistic and antagonistic relationships are more difficult to delineate. Mixtures of chemicals may have more or less than additive effects. In the case of the California condor (*Gymnogyps californianus*), habitat degradation and toxic exposures had a joint effect (extinction in the wild) that was greater than would have been expected from simply adding the losses that either would have caused acting alone[7].

In ecological effects analyses, information collected on measurement endpoints must relate to appropriate assessment endpoints. Extrapolations may include those between species, between responses, from laboratory to field, or from field to field. For example, the responses of organisms (earthworms, plants, small mammals) exposed to soils in the laboratory could be extrapolated to similar populations in the field[45]. An example of a field-to-field extrapolation is provided by La Point et al.[46], in which the diversity of soil invertebrates in ten heavy-metal contaminated sites were compared. The more heavily contaminated sites had fewer insects, leading to the determination that management practices were influencing insect distribution. Assessment endpoints may also be predicted by analysis of indirect effects such as relating removal of long-leaf pine to reduced populations of the red-cockaded woodpecker, or by analysis of higher organizational levels, e.g., relating reduced individual fecundity to reduced population size. These extrapolations require professional judgment. The thought process must be clearly and carefully described to avoid confusion. Conservative assumptions are often used during Tiers 1 and 2. If and when the risk assessment proceeds beyond Tier 2, the data and information gathered to this point reduces uncertainty and fills data gaps to enable the risk assessor to use less conservative assumptions in Tier 3. The assumptions should be clearly stated so a reviewer or risk manager is aware of them. These assumptions should be restated in the Risk

Characterization phase so that reviewers are, once again, aware of the thought process.

3.2.2 Method of Characterizing Ecological Effects

* Tier 1

Methods used in Tier 1 should focus on available information, estimation methods, and literature searches. Available information includes past site reports, surveys or assessments, on-site record searches and Installation Assessments. Much of this information would be gathered under the RI. Wildlife and habitat information may be available from the installation, National Biological Survey, U.S. Fish and Wildlife Service, the State Natural Resources Dept, or other local resources (Table 6). Regional Biological and Technical Assistance Group (BTAG) of U.S. EPA (Table 7) and the U.S. Army BTAG (Table 8) should be able to provide further sources of contacts, information and technical assistance.

Critical focus needs to be placed on threatened or endangered species at the installation. A threatened or endangered species may dominate the concerns of ecological effects and drive the decision on risk characterization. The reason for this is because individuals of threatened or endangered species must be protected as assessment endpoints instead of general populations, communities or ecological systems.

At the end of a Tier 1 study for ecological effects of contaminants at the site, the risk assessor should have:

(1) the available toxicity data on the chemicals of concern (COC);

(2) any available ecological information and information on biological incidents e.g., fish kills, dead birds;

(3) identified threatened or endangered species at the site and estimated their homerange or migrational pattern;

(4) identified any contaminants that may bioaccumulate;

(5) identified habitat areas of concern and areas known to be adversely affected by contaminants; and

(6) identified data gaps.

This information is summarized in a contaminant/response profile for the COC. At this stage and level of effort, the degree of uncertainty may be high and data gaps will occur, but the risk

Table 6. Sources of Site Information

U.S. EPA Environmental Research Laboratories
U.S. Department of Agriculture (e.g., Southern Forest Experiment Station, New Orleans, LA)
U.S. Soil Conservation Service (e.g., County soil surveys, Natural resources inventories)
U.S. Fish and Wildlife Service
National Oceanic and Atmospheric Administration
State Parks and Wildlife Departments
Agricultural Experiment Stations (within University systems)
Sierra Club (e.g., Naturalist's guides)

Table 7. U.S. EPA Regional BTAG Coordinators/Contacts

EPA HEADQUARTERS
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ERT/EPA (MS-101)
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Steve Ells
(703) 603-8934

John Miller
(703) 603-9076

EPA/OWPE (5502G)mail
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REGION 5
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Table 8. Tri-Service Ecological Risk Assessment Working Group

		Army		
Asaki, Arthur	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Surface Water and Wastewater Program	Attn: MCHB-ME-WM Aberdeen Proving Ground, MD 21010-5422	Com 410-671-3816 DSN 584-3816 Fax 410-671-8104 aasaki@aeha1.apgea.army.mil	Aquatic ecological risk assessment Aquatic toxicology Sediment toxicology
Bouwkamp, Carl	US Army Center for Health Promotion and Preventive Medicine (CHPPM)	Attn: MCHB-ME-WM Aberdeen Proving Ground, MD 21010-5422	Com 410-671-8124 DSN 584-8124 Fax 410-671-8104 cbouwkam@aeha1.apgea.army.mil	Aquatic ecological risk assessment Aquatic toxicology Fish contamination
Bridges, Todd	US Army Corps of Engineers Waterways Experiment Station (WES)	ES-F 3909 Halls Ferry Road Vicksburg, MS 39108	Com 601-634-3626 Fax 601-634-3713 bridget@ex1.wes.army.mil	Sediment toxicology Aquatic ecological risk assessment
Checkai, Ron	US Army Edgewood Research, Development, and Engineering Center (ERDEC)	Attn: SCBRD-RTL (E3220) Aberdeen Proving Ground, MD 21010-5423	Com 410-671-2129 DSN 584-2129 Fax 410-612-7274 or 410-671-2081 rtchecka@cbdcom.apgea.army.mil	Soil chemistry Ecological risk assessment Bioavailability Ecotoxicology
Cline, Jody	US Army Medical Research Detachment	MCMR-UWW 2800 Q Street, Bldg 824 Wright-Patterson AFB, OH 45433-7947	Com 513-255-0607 DSN 785-0607 Fax 513-476-7599 jcline@raven.al.wpafb.af.mil	Freshwater ecology Aquatic ecosystems Industrial hygiene research
Guelta, Mark	US Army Edgewood Research, Development, and Engineering Center (ERDEC)	Attn: SCBRD-RTL (E3220) Aberdeen Proving Ground, MD 21010-5423	Com 410-671-2129 DSN 584-2129 Fax 410-671-2081 maguelta@cbdcom.apgea.army.mil	
Hayes, Wendy	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Surface Water and Wastewater Program	Attn: MCHB-ME-WM Aberdeen Proving Ground, MD 21010-5422	Com 410-671-3816 DSN 584-3816 Fax 410-671-8104 whayes@aeha1.apgea.army.mil	Aquatic toxicology
Johnson, Mark S.	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Health Effects Research Program	Attn: MCHB-ML-HE Aberdeen Proving Ground, MD 21010-5422	Com 410-671-3980 DSN 584-3980 Fax 410-612-6710 mjohnson@aeha1.apgea.army.mil	Terrestrial ecology Avian ecology Population ecology
Leach, Glenn	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Health Effects Research Program	Attn: MCHB-ML-HE Aberdeen Proving Ground, MD 21010-5422	Com 410-671-3980 DSN 584-3980 Fax 410-612-6710 gleach@aeha1.apgea.army.mil	General toxicology Risk assessment

Leach, Glenn	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Health Effects Research Program	Attn: MCHB-ML-HE Aberdeen Proving Ground, MD 21010-5422	Com 410-671-3980 DSN 584-3980 Fax 410-612-6710 gleach@aeha1.apgea.army.mil	General toxicology Risk assessment
Maly, Mary E.	US Army Environmental Center (AEC) Restoration, Program Management, and Oversight Division	Attn: SFIM-AEC-RPO Aberdeen Proving Ground, MD 21010-5401	Com 410-671-1523 DSN 584-1523 Fax 410-671-1548 memaley@aec1.apgea.army.mil	Project manager Army BTAG coordinator Environmental engineering
McAtee, Matthew	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Environmental Health Risk Assessment and Risk Communication Program	Attn: MCHB-DC-EHR 5158 Blackhawk Road Aberdeen Proving Ground, MD 21010-5422	Com 410-671-2953 DSN 584-8552 Fax 410-671-8170 matthew_mcatee@chppm-ccmail.apgea.army.mil	Risk assessment Environmental biology Community ecology
Muhly, Bob	US Army Environmental Center (AEC) Environmental Technology Division	Attn: SFIM-AEC-ETD Aberdeen Proving Ground, MD 21010-5401	Com 410-612-6839 DSN 584-6839 Fax 410-612-6836 rlmuhly@aec1.apgea.army.mil	Army BTAG coordinator Environmental planning General biology
Robert, Matt	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Health Risk Assessment Division	Attn: MCHB-ME-R Aberdeen Proving Ground, MD 21010-5422	Com 410-671-8119 DSN Fax	
Tannenbaum, Larry	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Environmental Health Risk Assessment and Risk Communication Program	Attn: MCHB-DC-EHR 5158 Blackhawk Road Aberdeen Proving Ground, MD 21010-5422	Com 410-671-5210 DSN 584-5210 Fax 410-671-8170 lawrence_tannenbaum@chppm-ccmail.apgea.army.mil	Human health and ecological risk integration Theoretical ecology BTAG
Walker, Steven J.	Uniform Services University of the Health Sciences Division of Occupational and Environmental Health	4301 Jones Building Road Bethesda, MD 20514-4799	Com 301-295-1975 DSN Fax 301-295-1974	
Walker, Terry L.	Hazardous, Toxic and Radioactive Waste Center of Expertise	USACE HTRW CX 12565 West Center Rd Omaha, NE 68144	Com 402-697-2591 Fax 402-697-2595 Terry.L.Walker@mrd01.usace.army.mil	Risk Assessor Human and ecological
Wentsel, Randy	US Army Edgewood Research, Development, and Engineering Center (ERDEC)	SCBRD-RTL Aberdeen Proving Ground, MD 21010-5423	Com 410-671-2036 DSN 584-2036 Fax 410-671-2081 rswentse@cbdcom.apgea.army.mil	Ecological risk Terrestrial toxicology Risk policy

Whaley, Janet	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Health Effects Research Program	Attn: MCHB-DL-HE Aberdeen Proving Ground, MD 21010-5422	Com 410-671-3980/5084 DSN 584-3980 Fax 410-612-6710 jwhaley@acha1.apgea.army.mil	Veterinary medicine Wildlife toxicology Fish health Risk assessment
Williams, Keith	US Army Center for Health Promotion and Preventive Medicine (CHPPM) Health Risk Assessment Division	Attn: MCHB-ME-R Aberdeen Proving Ground, MD 21010-5422	Com 410-671-5206 DSN 584-5206 Fax 410-671-5237 kjwillia@acha1.apgea.army.mil	Risk assessment methodologies Wildlife exposure assessment Soil ecology
Air Force				
Caldwell, Dan	Armstrong Laboratory Toxicology Division	AL/OET Wright-Patterson AFB, OH 45433	Com 513-255-0607 DSN 785-0607 Fax 513-476-7599 dcaldwell%raven@eagle.aamrl.wpafb.af.mil	
Hammer, Don	Armstrong Laboratory Toxicology Division	Armstrong Laboratory Toxicology Division	Com 210-536-6136 DSN 240-6131 Fax 210-536-2315 don.hammer@guardian.brooks.af.mil	Hydrology Ecology Geology
Hewins, Stanley	Air Force Center for Environmental Excellence (AFCEE)	HQ/AFCEE/ERC 8001 Arnold Drive Brooks AFB, TX 78235-5357	Com 210-536-4755 DSN 240-4755 Fax 210-536-5989 shewins@afceeb1.brooks.af.mil	Veterinary medicine Environmental toxicology Human risk assessment
Larcom, Barbara	Armstrong Laboratory Toxicology Division	AL/OET Wright-Patterson AFB, OH 45433	Com 513-255-5150 DSN 785-5150 Fax 513-255-1474 blarcom@raven.al.wpafb.af.mil	Veterinary medicine General toxicology Ecological risk assessment
Long, Cornell	Armstrong Laboratory Environmental Science Branch	Armstrong Laboratory Toxicology Division	Com 210-536-6121 DSN 536-6121 Fax 210-536-2315 guy.long@ guardian.brooks.af.mil	Organic, analytical, environmental chemistry Environmental science
Maccabe, Andy	Armstrong Laboratory Environmental Science Branch	AL/OEMH 2402 E Drive Brooks AFB, TX 78235-5114	Com 210-536-6136 DSN 240-6113 Fax 210-536-2315 andrew.maccabe@guardian.brooks.af.mil	Veterinary medicine Public health Risk assessment

MacMahon, Kathleen	Armstrong Laboratory Toxicology Division	AL/OET Wright-Patterson AFB, OH 45433	Com 513-255-0607 DSN 785-0607 Fax 513-476-7599 kmacmahon%raven@eagle.aamrl.wpafb.af.mil	Environmental toxicology "Killém and countém"
Porter, Ron	Armstrong Laboratory Environmental Science Branch	AL/OEMH 2402 E Drive Brooks AFB, TX 78235-5114	Com 210-536-6127 DSN 536-6127 Fax 210-536-2315 ronald.porter@ guardian.brooks.af.mil	Environmental tox Food chain tox Ecological risk assessment
Strickland, Judy	Air Force Center for Environmental Excellence (AFCEE)	HQ/AFCEE/ERC 8001 Arnold Drive Brooks AFB, TX 78235-5357	Com 210-536-5230 DSN 240-5230 Fax 210-536-5989 jstrickl@afceeb1.brooks.af.mil	Risk assessment Environmental toxicology
Navy				
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Burleson, John		Marine Corps Base Quantico, VA	Com 703-784-4030	
Corbett, Janet	Southwest Division Naval Facilities Engineering Command		Com 619-532-1446 DSN 522-1446	
Douglas, Barbara	Northern Division Naval Facilities Engineering Command	10 Industrial Highway Mailstop #82, Code 1831 Lester, PA 19113-2090	Com 610-595-0567 Ext 183 DSN 443-0567 Fax 610-595-0555 bdouglas@efdnorth.navfac.navy.mil	
Eng, Sherry		1510 Gilbert St. Norfolk, VA 23511-2699	Com 804-322-4787 Fax 804-322-4805 engsr@efdlant.navfac.navy.mil	
Fisher, William S.	Southwest Division Naval Facilities Engineering Comman	1220 Pacific Hwy Code 231WF San Diego, CA 92132	Wildlife/Terrestrial Impacts	
Hahn, Simeon	Northern Division Naval Facilities Engineering Command	10 Industrial Highway Mailstop #82, Code 1831 Lester, PA 19113-2090	Com 610-595-0567 Ext 190 DSN 443-0567 Fax 610-595-0555 sphahn@efdnorth.navfac.navy.mil	Entomology Policy Regulatory implementation

Johnson, Robert J.	Marine Environmental Support Office-East Detachment Naval Command Control and Ocean Surveillance Center	RDTE Div Code 3621 27 Tarzwell Drive Narragansett, RI 02882-1154	Com 401-782-3128 Fax 401-782-3030 rjohnston@narvax.nar.epa.gov	Ecological Risk Assessment Case Studies DON/EPA Research Coordinator
Kincaid, Stephen	Northern Division Naval Facilities Engineering Command	10 Industrial Highway Mailstop #82, Code 1831 Lester, PA 19113-2090	Com 610-595-0567 Ext 170 DSN 443-0567 Fax 610-595-0555 skincaid@efdnorth.navfac.navy.mil	
McDaniel, Paul H.	Naval Facilities Engineering Service Center Environmental Restoration Division	560 Center Drive Port Hueneme, CA 93043-4328	Com 805-982-2640 DSN 551-2640 Fax 805-982-4304 pmcdani@nfesc.navy.mil	Innovative technology applications
Merting, Connie	South Division Naval Facilities Engineering Command		Com 803-743-0386 DSN 563-0386	
Yamamoto, Jeff	Pacific Division Naval Facilities Engineering Command	Bldg 258 Makalapa Pearl Harbor, HI 96860-7300	Com 808-474-5416 Fax 808-474-4519	BRAC Environmental Coordinator NAF Midway Island

assessor must use professional judgement to summarize this information with appropriate uncertainty included.

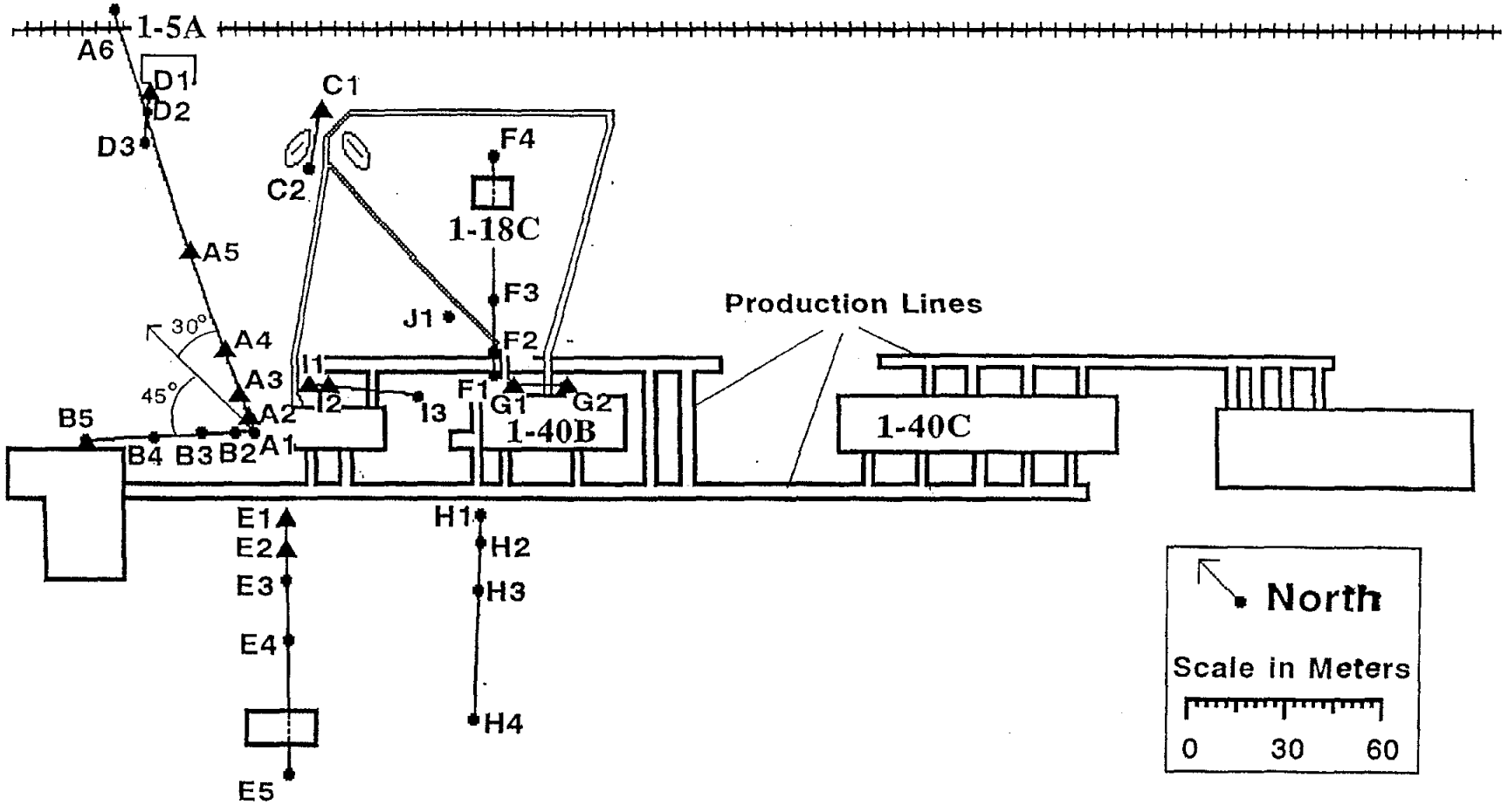
* Tier 2

The purpose of Tier 2 is to build on information gathered in Tier 1 by addressing data gaps to reduce uncertainty. Ecological effects data need to focus on the main COCs and reduce uncertainty when addressing their impacts on threatened or endangered species, habitat, or important populations. Measurement endpoints used in Tier 1 may become more complex or sophisticated in Tier 2. An example would be the use of literature toxicity data in Tier 1 verses specific laboratory toxicity studies in Tier 2.

Pathways where COCs could biomagnify in the food web to affect threatened or endangered species are addressed in this tier. Simple estimation methods of contaminant biomagnification for Tier 1 need to be upgraded in Tier 2 to reduce uncertainty or to fill data gaps. An example of a simplified approach to measuring biomagnification is a food-chain laboratory microcosm[47], in which lower trophic level organisms are exposed to contaminated water or sediments and subsequently fed to top predators to develop estimates of biomagnification. Estimation methods based on K_{ow} values and other physical and chemical parameters of the COCs should provide a technically sound estimate of the ability of the COCs to biomagnify. If the COC has been estimated by models or by use of K_{ow} values to biomagnify in the food web, then field or laboratory tissue studies will provide confirmation of model estimates.

Laboratory toxicity studies using site specific soil or sediment may also serve to reduce uncertainty and data gaps identified in Tier 1. Soil or sediment tests for sites contaminated with multiple COCs provide useful specific data on toxicity of mixtures of COCs. The results from laboratory toxicity studies, used as Tier 2 measurement endpoints, should provide information to better define areas at the site where the soil, water or sediment are toxic or nontoxic. An example of how toxicity testing can help delineate between toxic and non-toxic areas at a site was a study of soils conducted at Joliet Army Ammunition Plant, Joliet, IL.

In the Joliet study, six sites were identified by a remedial investigation as potentially having high concentrations of explosives and heavy metals[48]. Soil sampling was performed along transects through areas suspected of having high contamination at each site. Subsequent toxicity testing and chemical analyses identified the two most toxic sites, defined the shape and extent of the toxic areas within each site (Figures 13 and 14) and



Area L7 Group 1

Transects and sampling locations

Figure 13. Joliet Army Ammunition Plant Group 1 load, assemble, and pack area showing transects and locations with non-toxic ● or toxic ▲ response to at least one toxicity test.[45]

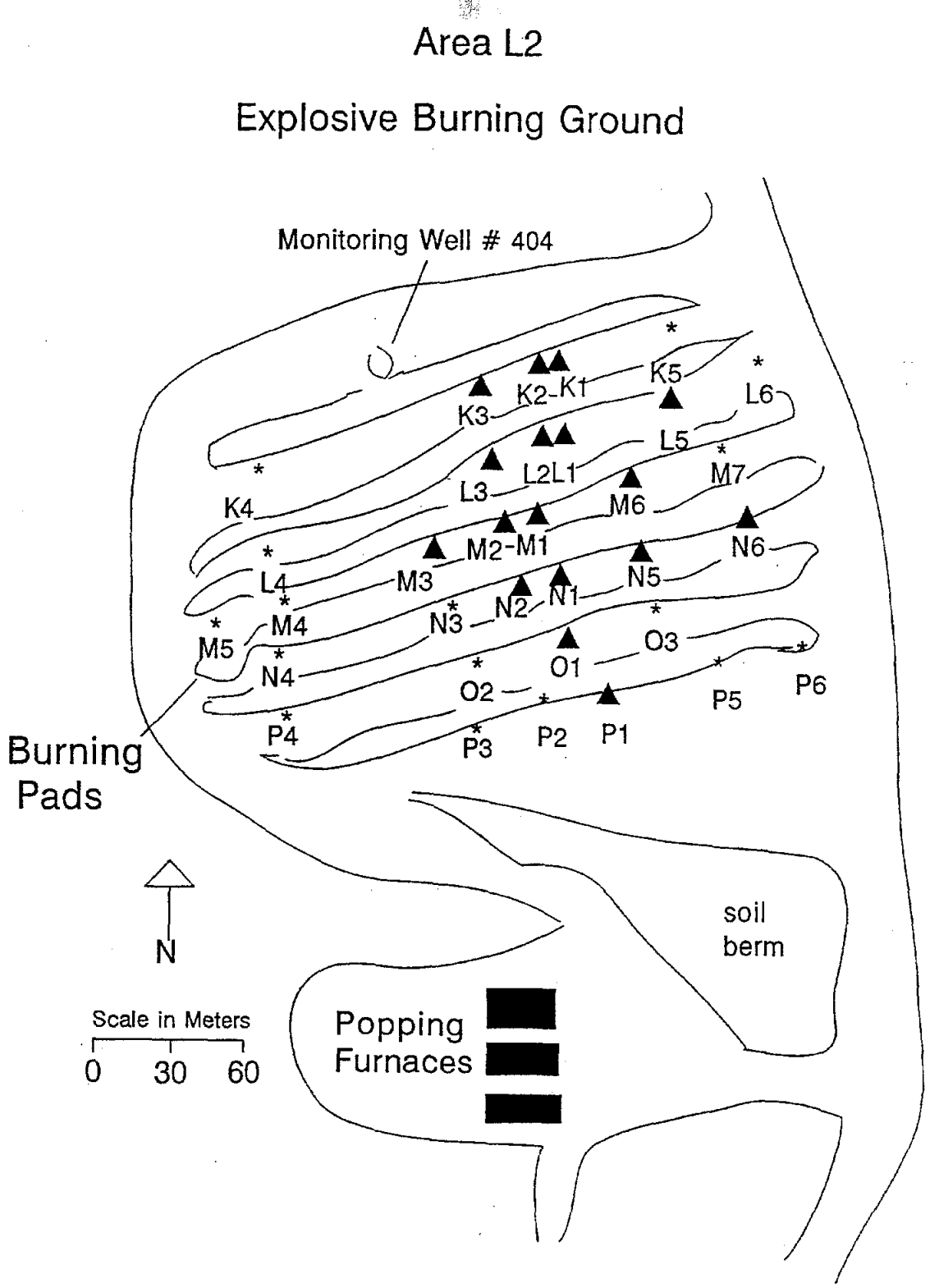


Figure 14. Joliet Army Ammunition Plant Area L2 explosive burning ground showing transects and soil sampling locations with non-toxic • or toxic * response to at least one toxicity test.[45]

against concentration values of explosives. TNT was determined to have the greatest R^2 (coefficient of determination) value of the eight compounds detected. Lowest observed effects concentrations (LOEC) of TNT were then extrapolated from these data.

In the preceding study, relatively inexpensive, short term (≤14-day) toxicity tests provided information to risk managers that will save time and money in the long run. For instance, remediation can be concentrated on the two sites that pose the greatest ecological risk. Within sites, risk managers can use these results, together with results from studies of other components of the ecosystem, to decide on the extent and type of remediation. Managers may also use these results to decide if further, more extensive testing is necessary in areas where soil concentrations are on the borderline of causing toxic effects. Furthermore, this study incorporated a series of bioassays to investigate effects at different levels of biological organization. This approach is more effective than using bioassays at the same organizational level because response to a stressor may vary among organisms at different levels of organization.

It is important to note that ANOVA results, LOEC's, and R^2 values in this study are site-specific and highly dependent upon soil characteristics and concentrations of other soil contaminants. As cited previously, toxicity of many chemicals, and soil explosives in particular are highly dependant on pH, organic matter, CEC and other characteristics of the site soil. Therefore, soil characteristics should be considered before extrapolating toxicity data between sites and between studies.

The Joliet case study is example of the use of toxicity testing established gradients of concentrations of explosives in site soils[45]. Plant (two species), earthworm, and Microtox™ bioassays were used to assess soil toxicity. Highly toxic, moderately toxic, or not significantly toxic soils were determined based on statistical significance compared to control soils. These categories were used to define the shape of toxic areas at each site. Soil samples with significant toxicity, according to at least one test, and representative samples displaying no toxicity, were analyzed for explosives at each site. The explosives, trinitrotoluene (TNT), cyclotrimethylenetrinitramine (RDX), and their degradation products were identified via HPLC analyses. End points of toxicity tests were then regressed to assess risk in Tier 2 of an ERA. An extensive compilation of ecological effects methods is presented in Volume 2. The reader is referred to Volume 2 for measurement endpoints to support specific goals of the ERA.

Field studies conducted in Tier 2 will be focused to address data gaps identified in Tier 1 and the overall assessment endpoints for the ERA. Ecological assessment may be necessary if the installation or other agencies do not have the information on biota present at the site. GIS can be used to identify habitat and land use patterns at the installation. Biotic surveys can determine species diversity, predominant populations, and identify population shifts.

Results from the Tier 2 ecological effects studies will further support cause and effect relationships between the COCs and the biota, community or ecological system. Uncertainty will have been reduced and most data gaps addressed. Various measurement endpoints will be "mapped" onto site locations to generate contaminant response profiles of species tested at the sites. These will, as in Tier 1, be related to the assessment endpoint(s) identified in the initial phase of the ERA.

* Tier 3

Tier 3 should involve larger levels of effort reflecting increased levels of concern to reduce uncertainty and address ecological effects data gaps in the ERA. Investigations in Tier 3 are not meant to deal with the highly toxic or hazardous areas within a site. The highly toxic sites could, and should, be identified in Tier 1 as areas where significant ecological effects occur and significant risk is probable. In Tier 1 or 2, these areas would be recommended for remediation. In Tier 3, there is no need to analyze the specific toxicity of contaminants or conduct more in-depth ecological studies on the highly toxic sites, if it is clear from Tiers 1 and 2 that they will be remediated. Tier 3 should focus on the "gray" areas, where it is still uncertain if significant ecological effects occur.
By the end of the Tier 2 investigation, sites should have been identified that are clearly affected by COCs, as well as sites where no effects occur following COC exposure. Further laboratory and field toxicity tests may be required to establish NOEL concentrations. These refined measurement endpoints are designed to reduce uncertainty and address data gaps not covered in Tiers 1 and 2. In Tier 3, collecting field data to determine tissue concentration in wildlife should be conducted to confirm the presence and extent of bioaccumulation, bioconcentration, and/or biomagnification that was suspected from results of Tier 2 studies. Additionally, if chronic physiological effects are suspected, they should be performed in Tier 3, particularly if evidence for such effects is obtained in previous tiers. However, these types of studies are often time consuming and expensive. Work should proceed only if all parties agree that the studies are essential to adequately complete the risk assessment and enough funds and resources are available to do quality experimentation.

A study by McBee et al.[49] is a good example of focused field research appropriate for a Tier 3 study to examine subtle, chronic ecological effects. In this study, the existence of environmental mutagenesis was determined by examining standard metaphase chromosome preparations from resident small mammals (*Peromyscus leucopus*, *Sigmodon hispidus*) trapped over a two-year period at a site polluted with petrochemical waste products, heavy metals, and PCBs. Significant differences in levels of chromosomal aberrations were found between animals collected at the contaminated site and those captured at two uncontaminated sites, even though acute toxicity was not apparent. Levels of chromosomal aberrations were not significantly different between the control sites. Potential longer-term, chronic effects suggested by the cytogenetic analyses, however, clearly indicated responses relevant to site assessments evaluating adverse ecological effects, and reinforced the importance of reference sites when correlative analyses are considered in the assessment of biological effects in the field.

Food web sampling is more complex but offers more complete information on contaminant pathways through the food web. Fordham and Reagan[39] (Figure 11) developed a food web model to evaluate potential exposure pathways for a site. The model estimates acceptable concentrations in abiotic media for each exposure pathway. Further, it develops a site-specific food web by entering data from on-site sampling as well as literature sources. Finally, the model addresses bioaccumulation in multiple food chains that terminate in a high trophic level species (e.g., bald eagle). Data from this type of study can be utilized in ecological risk assessments when evaluating risk to populations of biota exposed to site-related contaminants via different pathways.

When conducting any field study, various problems must be anticipated. The data collected will be more variable than laboratory studies. Analytical detection limits for tissue, soil, or water need to be known before data are collected. Detection limits in tissue need to be low enough so a no effect level can be related back to soil or water concentrations. Estimates from Tier 2 should be used to provide a guide for setting detection limits in Tier 3.

Co-locating tissue samples with soil or water samples at the site of collection may be necessary to accurately assess the toxicity of the COCs. The spatial relationship of data points collected during a field survey will be important for relating tissue concentration to exposure[15]. Maps have been used extensively to study and display spatial patterns. Many cartographic and GIS techniques are available for displaying spatially varying quantitative data. For example, if the variable of interest (e.g., distribution of TNT) is spatially continuous, it can be

conceptualized as a surface in three dimensions. The surface can be displayed as contour lines, isopleths, or as perspective plots. Alternatively, if the variable is discontinuous, the magnitude of an observation at a point can be represented by a symbol size or color. Synopses of these methods with references are found in Volume 2 of this publication.

Additional data needed to assess wildlife impacts include: home range, feeding area, and migratory patterns of the biota of concern at the site. This information can be provided by USFWS, site specific sources (i.e., state fish and wildlife department, military installation records, etc.) or the open literature. Identification of critical habitat to species of concern should be conducted. These data, together with spatial and temporal patterns of the COCs within the site help characterize the extent of ecological effects. Contaminant effects on local habitats, if extensive enough, can be related to cumulative impact on the watershed in which the site or sites are contained. These data may be used later to mitigate impacts through the additional critical habitat areas to the site. Mitigation options need to be viewed in light of minimizing further damage or risk to the resource. For example, if a habitat has been shown to be critical for a top avian predator (e.g., old-growth tree snags for osprey), it would not be suitable to suggest grading and incinerating of the vegetation from the site, unless similar habitat were set aside elsewhere as a mitigation option. Additional laboratory studies may focus on establishing no effect levels for the COCs. These studies should include tissue analyses so toxicity responses can be related to COC concentrations in tissues. These data are valuable for determining no effect levels of COCs in soils, water, or sediment. Other Tier 3 studies may be driven by regulatory or local concerns that may arise only after previous studies have been performed.

3.2.3 Linking Exposure and Stressor-Response Profiles

During the final stages of the Analysis phase, ecological effects and exposure are characterized concurrently. Data on fate and effects are objectively evaluated for their utility in ascribing cause and effect of the stressor. The degree to which organisms are adversely affected beyond those due to "normal" physical or biological stressors must be quantified. To this end, collected data are often subject to statistical methods to describe the inherent mean tendency and distribution of the population parameters (behavior, growth, reproduction, mortality, etc.). Among the statistical techniques commonly applied to such situations are geostatistical techniques (kriging) to determine loci of contaminant residues in soil or water, multivariate techniques (cluster analyses, canonical correlation, principal components) and univariate approaches to measure the organismal or population responses (e.g., differences in mean body burden of

chemical in an exposed set of organisms; differences in reproductive success of exposed small mammals).

The paths by which contaminants move from the point of origin through the biota and ecosystem may be simple and straightforward or complex and highly branched. Contaminant pathways will generally be defined by naturally-occurring physical, chemical, and biological components of the ecosystem (e.g., soil, vegetation growing on those soils, and microtine rodents foraging on the vegetation). The necessity of moving to Tier 2 or 3 under an ERA will largely depend on the complexity of the pathways, as determined by stressor-response and ecological analysis portion of the Analysis Phase.

On typical CERCLA sites, most contaminant pathways branch and proceed in multiple directions; for example, contaminants may have the potential for moving from the point of contamination into an aquatic system, with no potential impacts (branches) en route. An example of such a scenario is provided by groundwater movement of soluble nitrogenous compounds or pesticides, emerging via seepage into a stream or pond. Once the contaminant enters the water body, potential contaminant pathways may include uptake of the contaminant by aquatic vegetation, aquatic organisms (e.g., mollusks, gastropods, aquatic insects), fish or amphibians, or transport of the contaminant to birds or mammals feeding on aquatic organisms. Within each tier, contaminant pathways must by identified on each Superfund site. However, similarities in pathways will likely exist among many sites because of similarities of habitats and organisms in similar ecosystems (e.g., grasslands, deciduous forest, bottomland hardwood, etc.). It should be re-emphasized that the number of contaminant pathways are determined by the characteristics of the contaminant and the complexity of the ecosystem. Under situations of high complexity and/or diversity, when the magnitude, frequency or duration of the stressor varies in unpredictable ways, the estimates of ecological response(s) and exposure scenarios may require effort and cost beyond Tier 1.

A summary of the Analysis phase is provided by a stressor-response profile. In developing such, the RA identifies measurement endpoints along each contaminant pathway where data collection or computer simulations and models are applied to evaluate contaminant fate or assess potential impacts. This can be conducted early in Tiers 1 or 2. Data collected for these measurement endpoints will help validate or refute whether predicted movement or effects on assessment endpoints are actually occurring. As testing progresses to higher tier levels, these same contaminant pathways will continue to be evaluated through such options as data collection at previously unsampled measurements endpoints identified in the Tier 1 PF phase, or by more intensive data collection at previously sampled measurement endpoints to reduce the uncertainty of analyses. The Tier 1 or

Tier 2 identification of contaminant pathways (and modeling efforts) thus unify the investigative efforts of the ecological risk assessment through all levels of the tier structure.

Under Tier 1, the RA analyzes a suite of previously-compiled data and evaluates site-specific characteristics collected in the PF phase of the analysis (Figure 3). The RA might consider which contaminants were present and estimate the extent of contamination. Under Tier 1, information on chemical/physical properties of the contaminants would be examined in the context of tabled or otherwise compiled physical, biological, chemical and climatological characteristics of the ecosystem. How the contaminants interact with the physical and biological components of the ecosystem will be predictable, within certain constraints. In any case, using reports, maps and some preliminary sample collections would allow the RA to estimate the likelihood of the contaminants remaining *in situ* or moving off-site or through the ecosystem. In the final components of the Analysis phase, information should have been collected to link contaminant exposure to biotic response of critical species and/or habitats. The linkage is made by measuring the response in toxicity, biomagnification, reduction in population density, or other critical measurement endpoints to exposure. Hence, model development is critical at this juncture to understand how the COCs are accumulated by the biota and what a given tissue concentration means to the organisms[18]. In Tier 1, the models used may be as in Thomann[36] or relating concentrations in the soil to toxicity and species presence (as in Apparent Effects Threshold[50]). Under Tiers 2 and 3, model predictions are approximated empirically using on-site or laboratory exposures of naive organisms to measure uptake and consequent effects.

It should be stressed that in Tier 1 analyses, highly conservative risk measures should be developed from the assessments. As more information is collated under Tier 2 and 3 investigations, the need for "application factors" of safety should diminish. This means that, as effects are measured directly or via the use of surrogate organisms exposed on-site, the need for wide confidence limits around the estimates of effect lessens. The measures of risk become more direct.

3.2.4 Examples of Linking Biotic Responses and Exposure

Three examples linking exposure to biotic response are provided to describe situations in which exposure is related to biotic effects. The first involves birds subject to agricultural pesticides used on crops in the midwest. The second is an example of mammals located on a terrestrial grassland site. The third example describes assessment in an aquatic system.

* Avian example integrating exposure and stressor-response profile.

Birds are often used in evaluating wildlife exposure to, and trophic transport of, environmental contaminants[11]. Birds have a high metabolic rate and, therefore, consume large amounts of food relative to their body weight. This may lead to elevated bioaccumulation or biomagnification, even with contaminants less persistent in the environment. The avian respiratory system, characterized by lungs with air sacs, is highly efficient, moving large amounts of air through the lungs. This physiological characteristic may yield avian species highly susceptible to exposure and accumulation of particulate or vaporized air-borne contaminants. Additionally, many species of birds prey heavily on larval or adult insects during the breeding season[8]. Most of these insects spend all or a portion of their life cycle on or in soil or thatch where they are highly likely to come in contact with environmental contaminants. Other bird species prey upon flying adult insects as they emerge from aquatic and benthic larval forms, and are thus exposed to contaminants in water and sediments. Birds are often numerous in natural and disturbed habitats and can provide an adequate sample size to satisfy quantitative analyses.

Procedures for sampling exposure and response to contaminants in birds can be designed for each level of effort and costs relative to Tier 1, 2, or 3 studies as defined in this document.

A Tier 1 effort may involve avian censusing techniques to determine relative frequency and abundance of bird species on the study area. Habitat use and activity data collected during the census, graphically displayed, will quickly identify which species are most likely to be exposed to the contaminant(s) of concern. Once susceptible species are identified, efforts to assess exposure may be concentrated on these species. In certain situations, susceptible species may have been extirpated from the site.

At the Tier 2 level, the RA can attempt to answer more complex questions. In such cases, reference sites are necessary to determine reference (e.g., "control") estimates of contaminant uptake. Contaminant levels may be determined by collecting individuals and conducting residue analyses. A limited sampling of food items of targeted species may provide insight into the nature of exposure route. If the species included for study are cavity nesting birds, nest boxes can be erected on the study site to increase that species' presence and activity level on the site and increase access for sampling[51]. In some cases, one species may naturally nest in abundance on the study site, providing adequate sampling opportunity.

Sampling across several environmental matrices, such as soil, water, invertebrates, and adult and nestling birds can quantify contaminant availability to the species under investigation at different trophic levels. Monitoring contaminant intake in nestling birds quantifies exposure; then, measuring endpoints such as enzyme response (i.e., cholinesterase in the case of organophosphorus or carbamate insecticides), immune system response, growth and survival, quantifies effects at the measured exposure levels.

Tier 3 levels of funding and personnel would allow thorough assessment of exposure and effects along several food chains, each of which having a different bird species as the top predator. Exposure duration may play a significant role in the degree of effects observed in higher trophic levels. This is particularly true for the more environmentally persistent contaminants. Therefore, selecting a food chain with a long-lived, resident, predacious bird (e.g., bald eagle; Figure 9) at the top would likely provide an assessment of the worst case exposure scenario.

Certain birds of prey such as barn owls (*Tyto* sp.), screech owls (*Otus asio*) and barred owls (*Strix varia*) utilize nest boxes, thus providing easy access to nestlings. By selecting several top predators, each representing a different food chain, adequate data can be gathered to predict risk to a broader array of species. European starlings (*Sturnus vulgaris*), tree swallows (*Iridopocne bicolor*), and barn owls, for example, represent diverse food chains that would provide exposure and effects data applicable to numerous other species.

In a Tier 3 study, long term monitoring of adult birds using tarsus banding or radio telemetry provides valuable data on survival and demographics relative to exposure and accumulation of environmental contaminants. In some cases, multiple captures and non-lethal sampling of blood or fecal urates over extended periods of time provide temporal patterns of exposure. For example, repeated blood samples from an individual bird provides insight into exposure to certain heavy metals or to exposure to anticholinergic compounds. The more information determinable in diverse food chains about routes of exposure, bioaccumulation, biomagnification, and organism response to exposure, the more accurately the RA can predict risk for various avian species.

* Example of integrating exposure and stressor-response profile for small mammals on a hazardous waste site.

Initially, maps of the site provide estimates of "hot spots," on which small mammal distributions are mapped. Species lists of mammals and birds were collected from local resource managers. In situations in which small mammals are known to be abundant on a site, the collection and study of small mammals provides an

excellent "model" with which to relate exposure characterization and ecological effects. For example, deer mice (*Peromyscus* sp.) or cotton rats (*Sigmodon hispidus*) are often widely distributed over terrestrial sites, are easily live-captured, and respond to contaminants[49,52,53]. Small mammals have relatively small home ranges, ensuring that they are exposed to on-site contaminants. Depending on the local species, rodents, shrews (Insectivora) and mustelids (e.g., badgers) represent different trophic levels, feeding on a variety of food sources, from grasses and seeds to meat. Hence, using such local populations, observed individual or population responses can be readily attributed to contaminants at a particular site.

Under Tier 1, the estimates of effect would stem from, initially, estimates of contaminant concentration in the soil and developing a quotient of soil concentration to body burden. In addition, published information on effects of given concentrations for other small mammals (e.g., laboratory mice) would provide estimates of expected effects for given body burdens. However, variance in the diversity and concentration of contaminants at hazardous waste sites and in "reference" sites may make it desirable to empirically determine exposure using individual mammals with a known, uncontaminated history. This procedure moves to efforts and cost related to Tiers 2 and 3.

Under Tiers 2 or 3, the use of clean, "sentinel" animals introduced onto the site(s) allow quantification of contaminant accumulation and any consequent biological effects. The use of such organisms also experimentally controls for differences in intra-specific variability. Finally, linking the use of biomarkers to population dynamics in introduced organisms allows a conservative estimate of how successful remediation efforts are to minimize biological effects subsequent to site clean-up. If sufficient justification for exposure is determined and justification for closely assessing exposure the organisms experience "removed" some distance from the highly hazardous areas.

Tier 3 calls for measuring endpoints of controlled-exposure small mammals. Such endpoints include monitoring metabolic enzyme activity, such as hepatic microsomal ethoxyresorufin-O-deethylase[54], immunological endpoints[53] and reproduction[55]. Such biomarkers of exposure may be linked to population presence and abundance, the final measure of continued population survival at a site.

* Impacts of multiple contaminants

An example of a study that sought to determine the ecological effects and potential risk of multiple contaminants to multiple receptors was the Commencement Bay study[56]. This study investigated the extent of sediment contamination and adverse biological effects in a heavily industrialized area at the southern end of the main basin of Puget Sound. The tide flats area comprises seven waterways and associated shoreline with water depths less than 60 feet. Chemicals of concern included eight metals and 18 organic compounds. Exposure was evaluated by measuring concentrations of chemicals in sediments. A model was used to predict natural recovery. Effects were evaluated by determining benthic abundance, occurrence of liver abnormalities in fish, and various measures of sediment toxicity.

Risks to the fish and invertebrates in Commencement Bay were characterized by comparing conditions at contaminated sites to benchmark or reference locations, applying apparent effects threshold (AET) values for chemical concentrations in sediments. An AET was defined as the concentration in sediments above which statistically significant biological effects (relative to reference sediments) would always be expected. This study included several notable examples for a successful ERA: 1) multiple chemical measurements and biological endpoints were used; 2) the combination of field-collected sediment bioassays and AET's helped to differentiate between effects associated with different contaminants; and 3) by expressing all chemical and biological measures as elevations relative to a reference site, comparisons among these measures and demonstrations of concordance were straightforward.

The Commencement Bay ERA has certain limitations, including: 1) the ecological assessment was neither predictive nor probabilistic, although not originally conceived as a risk assessment; 2) the empirical significance of some endpoints was not explained, particularly with respect to individual site characteristics; 3) the definition of AET as the highest concentration at which no effect is observed (rather than the lowest concentration at which any effect is observed) is the least protective of possible definitions for effects thresholds. This method assumes a consistently increasing biological response at increasing concentrations of chemical. Unmeasured chemicals, physical conditions, species interactions, and other community-level processes may alter the dose-response relationship.

The Commencement Bay study was a multi-year, multimillion dollar effort to explain the ecological effects of many stressors on biota within an ecosystem. Other case studies offer smaller-scale, less expensive, but equally effective methods to examine individual and synergistic effects caused by multiple stressors[16].

3.2.5 Example of Ecological Risk Assessment at a U.S. Air Force Site

Massachusetts Military Reserve (MMR) / Otis Air National Guard Base on Cape Cod, MA, has several groundwater plumes contaminated with organic and inorganic contaminants from leachate caused by spills of fuels and solvents and from a landfill.[57] Preliminary Ecological Risk Assessments were performed on seven plume areas to assess the potential for groundwater contaminants to impact surface water and sediment receptors. These ERAs were based on data gathered from monitoring wells during remedial investigations (RIs). ERAs were also performed on two ponds, Ashumet Pond and Johns' Pond, that would potentially receive plume contaminants in the future.

Monitoring wells were placed at strategic locations within each plume and sampled several times during the remedial investigations. However, most wells were concentrated near the original source of contamination or near the leading edge of each plume. Also, organic compounds were sampled more frequently than were inorganic compounds. COCs were identified based on Ambient Water Quality Criteria (AWQC) for aquatic and marine organisms. Ecosystems potentially at risk were surface water bodies and sediments in ponds, rivers and estuaries. Hazard Quotients were calculated based on the AWQCs. No adjustment was made to the risk estimations to account for percentage of loading of contaminants relative to the flux of total water entering surface water bodies. Nor were adjustments made to account for degradation of contaminants over time or solubility of inorganics relative to water chemistry. Therefore, risk estimates were extremely conservative.

The Risk identified some heavy metals as COCs in most of the sites. Organics such as trichloroethylene (TCE), parachloroethylene (PCE), ethylene dibromide (EDB), xylenes and benzene were of concern in a few areas in some plumes. In most cases, the organic compounds were well below reference levels for toxicity to ecological receptors.

A Plume Containment Plan was formulated to remediate the plumes. This plan involves pumping and treating groundwater to remove contaminants followed by re-injection into the ground. A team of experts was assembled to assess the ecological risk of the plume contaminants based on the original RIs and Preliminary Risk Assessments as well as data obtained from more recent sampling. Review of the previous RIs and Ecological Risk Assessments by the team of experts led to adjustment of the Hazard Quotients based on the latest reference values for ecological receptors. For example, a food chain model designed to predict uptake of manganese and copper in Osprey were originally calculated using reference values determined for monkey and swine, respectively.

monkey and swine, respectively. Hazard Quotients were re-calculated using new reference values that were determined for Osprey. Risk was also re-evaluated using contaminant concentrations found in wells sampled subsequent to the RIs. In addition, some studies performed in the RIs were found to be of poor quality and, hence, not suitable for use in risk assessments.

Reviewers constructed weight-of-evidence tables to evaluate measurement endpoints, data quality objectives, strength of relationship between measurement and assessment endpoints, study design, potential for risk, magnitude of risk, and uncertainties associated with the risk based on the RIs. Risk was then re-evaluated using contaminant concentrations taken from the latest sampling, new Hazard Quotients, and estimates of contaminant flux into ground water bodies over time. Data gaps were identified based on the uncertainty associated with each plume and water body. Recommendations for future studies were then made based on these data gaps. The TRET recommended that several of the Tier One studies, such as surveys of fish papillomas and levels of heavy metals in fish and mussels be done again since statistical design of the original studies was inadequate to accurately assess risk.

Conclusions drawn by the TRET, which exposed weaknesses in the original Risk Assessments, underscored the significance of knowing the quality of data generated and the importance of using up-to-date data before characterizing risk and making recommendations for remediation or future studies. The lesson learned in this case study is that risk assessors should not extract numbers from previous studies to estimate risk without first assessing the quality of those numbers.

3.2.6 Example of Ecological Risk Assessment at a U.S. Navy Site

Phase I of an Estuarine Ecological Risk Assessment was performed for Portsmouth Naval Shipyard, Kittery, Maine[58]. This cooperative effort was performed by the Navy, USEPA, and University scientists. The approach followed the USEPA Region 1 guidance and the USEPA Framework to assess ecological risk from past disposal practices of the Portsmouth Naval Shipyard on the Great Bay Estuary.

A network of stations was established in depositional areas where the greatest likelihood of contamination would occur to determine the temporal distribution of contaminants and to assess ecological effects. Other sites were established near the shipyard to provide information on the extent of contamination from the shipyard, identify other sources of contamination in the estuary, and establish background reference levels of contaminants of concern (COC). This network was established for

the problem formulation phase which included: identification of the stressor characteristics, the ecosystems potentially at risk, ecological effects, selection of assessment and measurement endpoints, and formulation of initial first-tier and second-tier conceptual models.

A number of studies were performed to address the above-mentioned parameters including texture of sediments, sediment toxicity, characterization of water-column conditions, water toxicity, fecal-borne microbial contamination, hydrodynamics, eelgrass analysis, fucoid analysis, flounder and lobster analysis, mussel analysis, infaunal invertebrate analysis, analysis of field samples of marine sediments, tissues, water, and analysis of organic chemical markers to distinguish shipyard contamination from contamination caused by other sources in the estuary.

Results of field and laboratory investigations indicated limited toxicological impact and absence of severe environmental contamination. Elevated levels of Hg, Pb,Cr, and Ni were found in mussel tissue. The authors suggested that chronic exposure to these levels could possibly cause long-term impacts. However, contamination likely originated from many sources, some of which may not be from the shipyard. Data gaps were identified, including initial assessment of the health of the salt grass communities, and additional information about the trophic transfer of contaminants. These studies were recommended for Phase II investigations. The Conceptual Model was revisited and modified based on the results of sampling and toxicity testing.

This study represents a logical, step-wise procedural example of a Tier I Problem Formulation. Statistically sound studies were based on well thought out estimates of the nature of potential contaminants and there spatial and temporal patterns. Contaminant loading into the estuary from sources other than the shipyard were considered. Although all sources of contamination were not found in Phase I of the program, the initial studies determined COCs, characterized exposure and effects and identified data gaps to be considered in Phase II of the program.

4. RISK CHARACTERIZATION

4.1 General Overview

Risk characterization is the critical process in an ERA. In the risk characterization, information on exposure, exposure-effects relationships, and defined or presumed target populations (whether from direct sampling efforts or from estimates derived from reports and literature) is integrated to attribute the likelihood, severity, and characteristics of adverse effects to environmental stressors present at the site (Figure 15). It is these parameters which determine the ecological significance of risk, and therefore the appropriate level of risk management response.

It is important to understand that "risk" is an integrative concept, not a single, directly measurable value. Risk is estimated by calculation from information on exposure and contaminant fate. However, risk assessment findings and conclusions may be verified and confirmed by measurement. Direct measures of impact and effect may be important in developing the weight of evidence which supports the attribution of risk to different sources of stress.

The framework document[4], outlined in Section 1, emphasizes the possible interaction of alternate sources of stress and the necessity to identify contaminant-related effects in this context. Draft guidance[3] provides a conceptual foundation for implementing this evaluation. The various components of a weight-of-evidence evaluation should be developed in advance of conducting the analyses, and the relative importance of each should be determined *a priori*. This procedure helps prevent biased conclusions by employing previously agreed-upon input information in deriving risk estimates. In many cases it will be up to the risk manager to understand the administrative record for project plan approvals and act accordingly, because experience has shown that when preconceived notions of risk are not supported by site-specific evidence, risk assessors may come to disagreement or indeed attempt to stretch the assessment process by undertaking further, unplanned and possibly unnecessary studies.

Risk calculations must always be related to assessment endpoint(s) via measurement endpoints. It is this relationship that supports the utility of risk assessment for risk management. It is crucial that assessment and measurement endpoints be understood in the context of the range of ecological stressors present at a site, and that the ERA be conducted to effectively attribute effects (if any) to site-related contaminants. Ecological risk assessment is one of a number of sources of information that must be considered in evaluating the possible

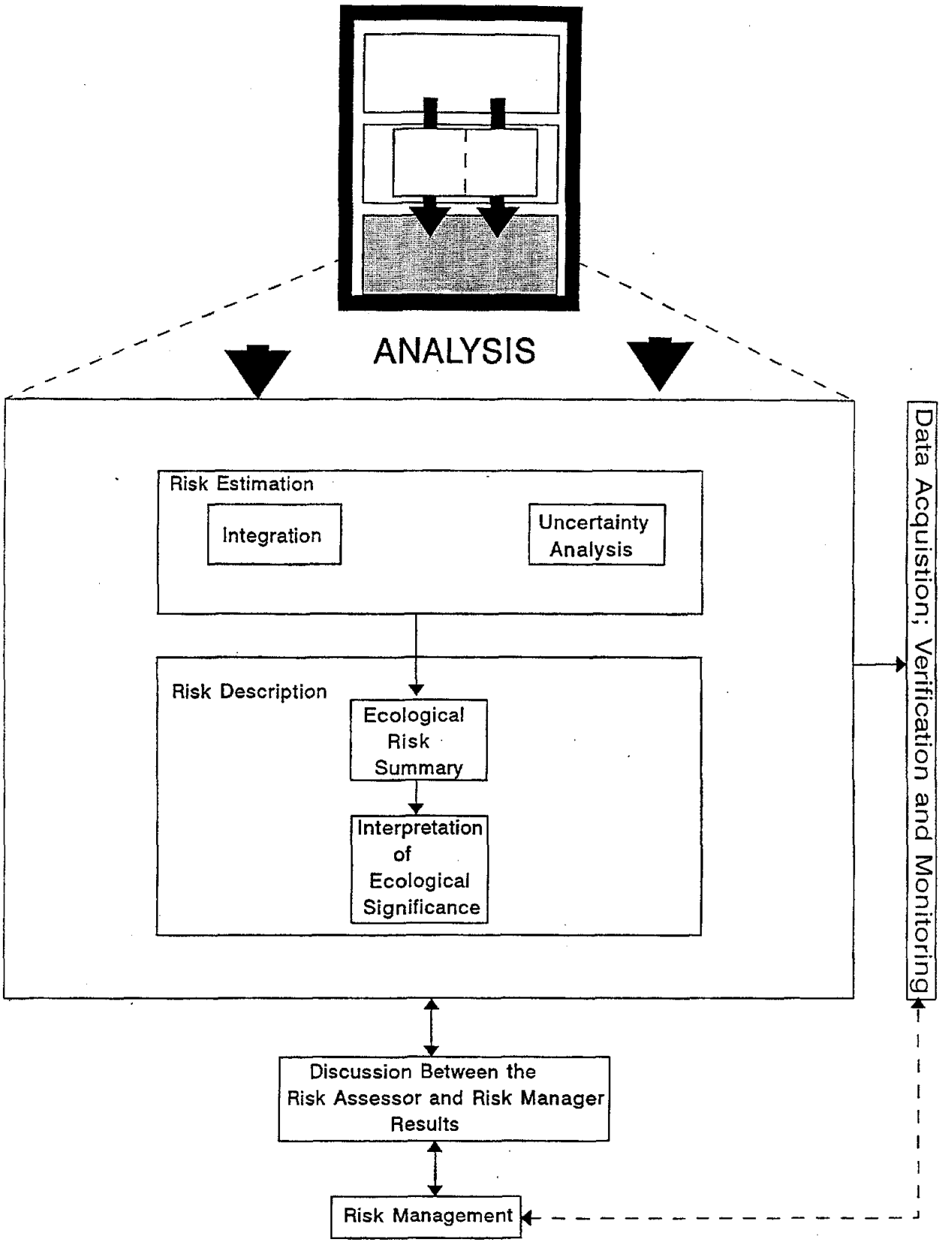


Figure 15. Risk Characterization Phase

remediation of a contaminated site. For ecological assessment to play a proper role in this process, ecological risk characterization must be as accurate and scientifically sound as possible[59] in keeping with the objectives of the assessment. These objectives are identified during the problem formulation phase. Risk assessment objectives in the tiered approach are related to specific decision points which can be useful in determining possible need for further data gathering, evaluation effort, or management actions. Decision points are fundamental to successful implementation of a tiered ERA[3].

4.2 Decision Points

The tiered approach to ecological assessment provides an effective framework for risk estimation. The key to successful implementation of the phased approach at the risk characterization stage is the *a priori* provision of decision points for the risk assessment. Review Draft Guidance[3] identifies a series of administrative decision points relating to the review and approval of certain documents. In practice, the risk assessment/risk management team needs to identify technical decision points at which the possible requirements for further investigation, uncertainty evaluation, or risk management consideration are characterized. It is important that such decision points be built in to project planning, to avoid the truncation of the process by time and effort constraints which fail to account realistically for the needs of the assessment process.

Action-oriented decision points will vary with site conditions and assessment objectives, and thus cannot be detailed generically. However, certain categories of decision points can be identified based on habitats present and the overall role of risk assessment in the site management and weight-of-evidence evaluation processes. This section provides brief examples of decision points appropriate for different habitats and levels of assessment. For any particular site, the risk assessment/risk management team should develop in advance detailed decision points on which to base technical progress.

<u>Terrestrial Habitats.</u> Tier 1 investigations in terrestrial habitats will identify areas of heavily contaminated soils. Tier 2 and 3 investigations will focus on the margins of the heavily contaminated zones, and quantify risks associated with contaminant transport and chronic exposure.
Presence of elevated concentrations of organic toxicants or metals relative to "reference" conditions is a primary decision threshold determining the need for further investigation. The need for quantitative assessment beyond Tier 1 can be ascertained by simple, point estimate of exposure vs. known effects concentrations. In general, simple point estimates of risk are

most valuable as indicators of need for further evaluation, and not for defining risk management. Decisions to move to Tier 3 level of investigation should be based on the nature of contamination (bioaccumulative organic compounds, for example) and the complexity of site conditions. For example, presence of endangered or threatened species in areas of elevated contamination suggest the need for advanced analyses.

Risk management decisions in terrestrial habitats should incorporate realistic estimates of exposure based on bioaccessibility and bioavailability of toxicants (Section 3.1). Hypothetical risks based on highly conservative assumptions should not, in general, define active remediation.

Three categories of biota are often the focus for decision making in terrestrial habitats. Vegetation is often not demonstrably impacted (except by herbicide discharge) unless contaminant concentrations are very high. However, vegetation can be a key exposure route through uptake to the consumer food web. Soil fauna, because of local nature of exposure and intimate contact with the primary medium, may provide excellent decision points, and some promising techniques for assessing contaminant effects on soil fauna communities are being developed[60]. Vertebrate organisms are often exposed primarily through the food web. Probabilistic risk estimates based on all exposure routes (see discussion of Conceptual Models in Section 2) provide the decision making thresholds for these receptors.

Aquatic Habitats. Tier 1 investigations in aquatic habitats may focus on point estimates of exposure compared with effects levels such as the available EPA Ambient Water Quality Criteria. Such comparisons should not be made simplistically, however. The published criteria for some metals are weighted relative to water hardness, and this should be accounted for in making decisions on this basis. In addition, the criteria may be modified on a site specific basis to account for resident species (with a recalculation based on supporting toxicity data) or based on site specific toxicological testing. The latter should be considered Tier 2 and 3 studies, respectively, with the decision to undertake such investigations dependent on the level of risk inferred from simple point estimates.

Beyond criteria comparisons, aquatic food web models and probabilistic exposure estimates should be applied when Tier 2 and 3 studies are warranted by potential contaminant-related effects. Effects may be verified by community structure measurements of water column and benthic biota, and perhaps direct toxicity testing. These techniques have the disadvantage, however, of integrating all sources of impact and exposure. They should only be employed when the potential site risks are

sufficient to support the level of technical effort necessary to apportion impacts.

Monitoring and Assessment Validation. In all habitats and under all risk management scenarios, post-assessment monitoring or assessment validation data collection may be important. In general, monitoring is useful in situations where residual contamination will be present after the remedial alternative is implemented. The decision to undertake post-cleanup monitoring is best based on: 1) the relative uncertainty of the risk assessment (more uncertain assessments, especially those based on single point estimates, may need a greater investment in monitoring); and 2) projected exposure reductions associated with the remediation. Properly designed monitoring programs serve simultaneously to assure the efficacy of the cleanup and to validate the risk assessment and its application, i.e., determine the accuracy of the original estimate of risk[61].

The most elaborate and expensive monitoring and validation programs will be used where Tier 1 and 2 assessments have been employed to support cleanup decisions. Tier 3 assessments will generally include intensive field investigations to validate risk assessment parameters. The low uncertainty associated with this greater investigation effort may be reflected in reduced monitoring requirements.

4.3 Risk Estimation

The fundamental tools of risk estimation are the simple hazard quotient and probabilistic risk estimates. Each has its uses, and each supports certain decision points for a particular site.

4.3.1 Hazard Quotient

The simple hazard quotient is a tool primarily useful in the Tier 1 and some Tier 2 levels of investigation. Simple hazard quotients are point estimates relating presumed exposure concentrations to known or extrapolated effects levels of toxicants. Conceptually, the hazard quotient is represented as:

$$HQ = \frac{EEC}{TEC}$$

where EEC is the expected exposure point concentration and TEC is the appropriate toxicological endpoint concentration. As a basis for risk assessment, separate hazard quotients are calculated for each contaminant/receptor pair. It may be possible to derive hazard indices by combining hazard quotients for different compounds for a single receptor taxon. Such indices are generally constructed by simple addition, and the result is very poorly supported by existing toxicological data. Assessment uncertainty is greatly increased by combining hazard quotients. Where necessary, such combinations should only be made of compounds likely to have similar modes of action. For example, some organochlorine pesticides which each act to suppress brain enzyme activity, or some metals which each act to damage kidney cells might be combined for risk assessment. It would be inappropriate and ineffective to construct a hazard index which combined hazard quotients of, for example, trichloroethane, PCB Arochlor 1248, and arsenic. Each of these compounds has a different mode of action, and their effect in combination is not additive or even directly related, particularly at the chronic dose level usually observed in relation to hazardous sites.

Uncertainties surrounding point estimates arise from extrapolation of the available toxicity data bases and inference regarding exposures. Because the hazard quotient is a point estimate only, the estimate itself must account for uncertainty in application to the field situation. As illustrated in Figure 12, the process of extrapolating toxicity data for point estimates sometimes incorporates divisors which compensate for possible uncertainties but which could lead to inflated and unrealistic hazard estimates. Similarly, inflated exposure assumptions could be employed to compensate for presumed uncertainty. Despite these drawbacks, the quotient method is a useful and appropriate tool for Tier 1 and certain Tier 2 investigations. The risk assessor must, however, be vigilant in deriving realistic, site-specific quotients rather than simply applying generic, overly conservative values[62].

LD_{50} estimates, ambient water quality criteria, and reproductive effects thresholds are examples of single number effect and exposure profiles. The LD_{50} is that level of exposure dose that is lethal to 50% of the population exposed. The ratio, or quotient, of the exposure value to the effect value provides the relative estimate of risk. Under any tier, the quotient method may be employed to estimate the possibility of an adverse effect from single sources[63]. In general, ratios of EEC to TEC greater than 1.0 are considered to indicate a potential risk. Because the quotient method yields only a point estimate, effects probabilities cannot be easily specified. To account for this, safety factors are sometimes considered in interpreting findings. For example, Menzie et al.[20] interpreted HQs between 1 and 10 as having "some small potential" for adverse effects, HQs between 10

and 100 as having "significant potential", and HQs greater than 100 as indicating "expected" adverse effects. However, it is important to note that no statistical analysis supports this interpretation, and indeed none is possible within the context of a single site investigation.

For more quantitative assessment, lower (F_L) and upper (F_U) safety factor(s) may be included in the basic HQ equation so that if the ratio is less than the lower-bound factor ($EEC/TEC<F_L$), the release is considered potentially "safe". If the quotients exceed some upper-bound factor(s) ($EEC/TEC>F_U$), exposure concentrations are considered "unsafe". Quotients between F_L and F_U indicate uncertainty about safety and imply the need for further assessment. In many cases, such boundary limits cannot be specified, and a single factor (F) is used (i.e., if EEC/TEC<F, the release is considered safe; otherwise, it is not). The quotient is deterministic, in that it establishes a number without an associated variance.

A practical example of a Tier 1 application of the Quotient Method is an evaluation of DDT residues at a Superfund site. Because DDT is known to accumulate in earthworms, and because American robins feed almost exclusively on earthworms in the spring, the robin would be a good population on which to base a bird safety assessment using the Quotient Method. Assume we determined from the literature the DDT 6-month LC_{50} for robins is 5 ppm and a conservative upper allowable exposure level for the site (F_U) was established at 50% of the LC_{50}. If the mean residue level in earthworms on site was 3.7 ppm, the quotient equation would be EEC (3.7 ppm)/TEC (5 ppm) = 0.74 > F_U (0.5). Therefore, the site contamination level is greater than the acceptable safety criteria. In this case, the decision is made to remediate the site and no further study on the site is required. If, however, there are not adequate data in the literature regarding the TEC, there is tremendous uncertainty about what level of exposure may be considered safe, or there are numerous species for which risk estimation is needed, the Quotient Method may still be applicable but would be elevated to a Tier 2 or 3 effort.

One example of the use of Quotient method in Tier 2 of a RA was conducted by Charters, *et al.*[64] at a PCB and lead contaminated wetland. They evaluated three pathways of exposure and established measurement and assessment endpoints for each. The measurement endpoints were toxicity values or body contaminant burdens; assessment endpoints were population maintenance (continuance of viable populations). Exposure estimates incorporated field and laboratory measurements and information derived from available scientific literature. In keeping with the objectives of a Tier 2 level of effort, risk estimates were focused on sensitive receptors and suggested the need for further

action (quantitative Tier 3 site evaluation and remedial actions).

Another effective application of the simple quotient method in a Tier 2 assessment is described in Boucher[65]. In this case, protective criteria for representative receptor organisms were derived based on extrapolated toxic hazards and site-specific exposure levels. Exposure concentrations were verified with field data, and point estimates were incorporated in a weight-of-evidence evaluation of cleanup alternatives. Some of the uncertainties inherent in the point estimate approach were accounted for by the use of site-specific measurement data on concentrations in environmental media and biotic tissues. Others were accounted for by employing realistic, technically sound estimates for toxicity and exposure parameters.

4.3.2 Probabilistic Risk Estimates

Probabilistic risk estimates provide a technically sound basis for evaluating possible contaminant hazards in the "gray zone" beyond heavily contaminated areas and for cases where remedial activities would be costly and highly destructive. Probabilistic approaches allow much more precise quantitation of risks and the nature and location of contaminants driving risks. In general, probabilistic estimates are most useful in Tier 2 and 3 investigations, where the level of site complexity and decision making importance warrant more accurate and precise risk evaluation.

Probabilistic approaches require more investment of resources in the assessment, but provide a substantial return on this investment by more clearly and effectively guiding risk management engineering. Probabilistic risk estimates are based on ranges of input values manipulated mathematically to yield an ecologically realistic picture of potential site related exposure and exposure related effects. Statistical distributions of input data are derived from available scientific information, and risk quantitation is calculated for various combinations of these distributions. Risk quantitation by this approach avoids the highly conservative uncertainty divisors which are often applied to assure the protective nature of risk estimates based on single point estimates. Probabilistic assessment also offers the risk manager objective specification of the level of protection provided by cleanup scenarios which may require understanding of the trade offs inherent in environmental destruction associated with active remediation vs. the benefit of contaminant removal or exposure reduction.

A detailed description of a comprehensive approach to probabilistic risk estimation is provided in Bartell et al.[1]. The fundamental components of a probabilistic assessment are:

- identify contaminants of primary concern;
- develop statistical distributions of concentration-dependent effects of contaminants on representative receptor organisms;
- develop statistical distributions of site-specific exposure of receptor organisms to contaminants;
- combine effects and exposure distributions to yield probabilistic estimates of effect.

Because the distributions account for data-driven uncertainties, elaborate and conservative uncertainty factors are not applied. The distributional nature of the estimates allows the risk assessor to provide the risk manager with clear statements of risk probability. Thus, for example, should risk management objectives include "protecting 95% of species present in a body of water from adverse effects of cadmium", the distributions of exposure and toxicological effect allow the risk assessor to determine, in light of site specific bioaccessibility and bioavailability, realistic and protective concentration objectives.

Analysis of distributions of exposure and effects, rather than using single values, makes probabilistic risk estimates possible. Risk is quantified by an expression of the overlap between the two distributions, with greater overlap indicating greater risk (Figure 16). Figure 16 presents a simplistic view of the overlap between exposure and effect, relating to risk. In reality, exposure varies temporally and spatially. The heuristic model presented in Figure 16 can be expanded in other dimensions (time and space), with an integration of the multi-dimensional curves, to arrive at a more realistic estimate of the risk. We are unaware of such an approach being taken to date. One method which has been applied to multidimensional risk evaluation is fuzzy modeling[66]. Such an approach could be used to fully incorporate spatial and temporal considerations in risk quantitation.

An example of this method, Analysis of Extrapolation Error (AEE), is described in Suter[7]. The AEE approach uses the variability in and relationship between responses of particular species to a range of contaminants to predict effects of unstudied contaminant-receptor pairs. For example, the distribution of effects of varying concentrations of various contaminants may be known for fish species A and B, while the contaminant of interest may only be known for species A. Relative sensitivity to other contaminants predict, with quantifiable uncertainty, the response of species B to the untested contaminant of interest. When data are available to support AEE, the approach has substantial value.

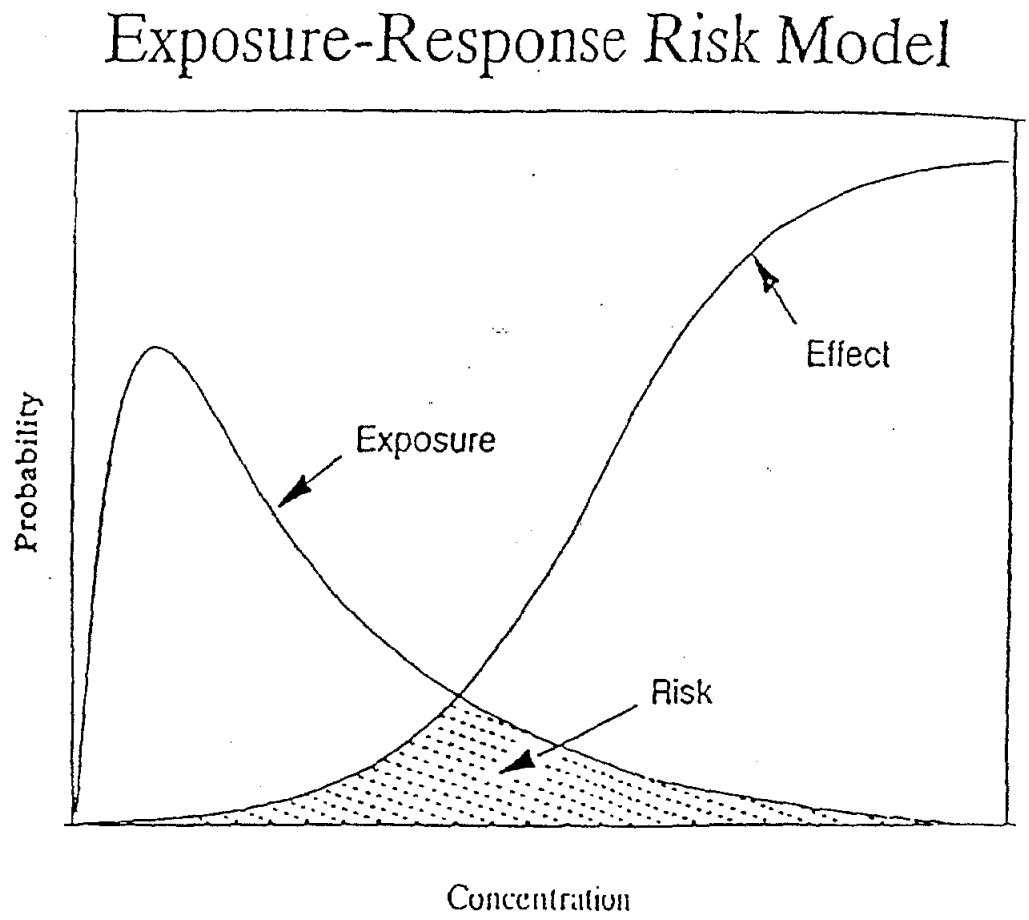


Figure 16. Exposure-Response Risk Model.

As Suter[7] states:

> The main advantage of the AEE method is that it clearly distinguishes, quantifies, and displays both the extrapolations that must be made from the toxicity data and relate it to the assessment endpoints and the uncertainties associated with the process of extrapolation. In contrast, the quotient method with factors treats uncertainties and correlations as equivalent and does not systematically account for either one.

However, AEE only addresses the response component of risk. The exposure component must often be measured or modeled directly in Tier 2 and 3 assessments, accounting as necessary for contaminant bioaccessibility and bioavailability (Section 3).

Probabilistic approaches to risk assessment have been applied for investigations at hazardous waste sites. For example, Cardwell et al.[67] employed effects and exposure distributions to estimate risk probabilities associated with metals contamination of river ecosystems (Figure 17). In this approach it is relatively simple to visualize the proportion of species in the community potentially at risk of chronic or acute contaminant effects. In this case, test species (measurement endpoints) were assumed to represent the balanced, indigenous community in the rivers (assessment endpoints). Because the presentation is essentially a cumulative probability density function (CPDF) of the toxicity data obtained, it is critically important that the assumption of representativeness is realized to the greatest extent possible. If the species and endpoints used in the presentation are not representative of the community potentially at risk, the CPDFs generated will not accurately reflect potential risks in the environment. For example, if the CPDF is constructed from data for *Daphnia* and *Hyalella*, two invertebrate species, but is used as a reference for fish, the results may be far too uncertain to use. The concept of balance is also critically important when using this form of presentation. If the data upon which the CPDF is based are not balanced with respect to numbers and types of test species and endpoints (e.g., 20 *Daphnia* values and only 2 for fathead minnow values), the resulting CPDF will be biased toward the one test species and again, comparisons will be very uncertain. If CPDFs are constructed from data which accurately represent the composition and balance of the community potentially at risk, the technique presented by Cardwell et al.[67] can contribute a valuable additional layer to the presentation of uncertainty.

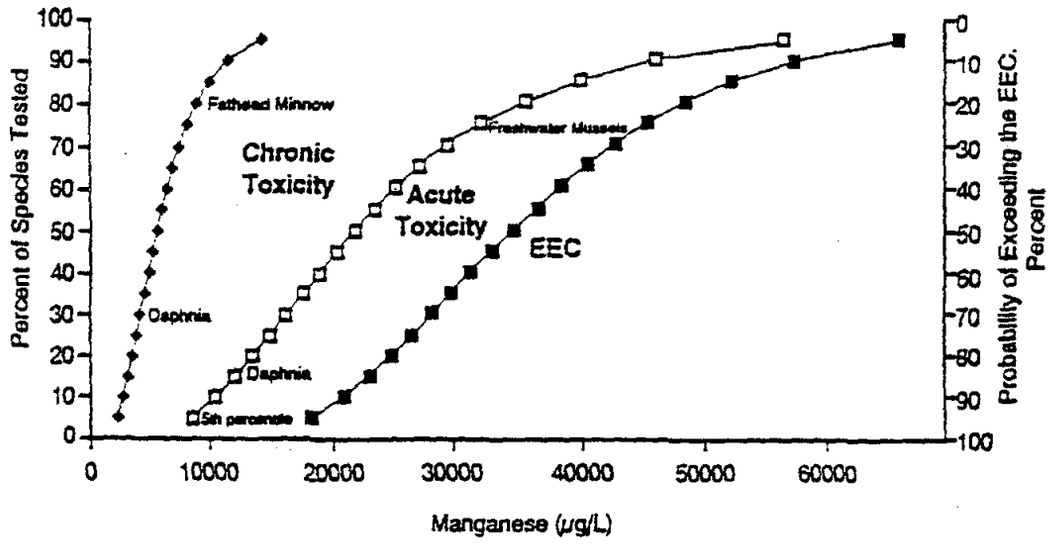


Figure 17. Comparison of Expected Environmental Concentrations of dissolved manganese with concentrations causing acute and chronic toxicity (from Cardwell et al. 1993).

4.4 Simulation and Exposure Modeling

Simulation and exposure modeling may be useful in any investigation tier. For Tier 1, simple exposure models incorporating estimated bioaccumulation factors and initial engineering investigation data on the nature and extent of contamination in environmental media can be used to "screen" sites or areas for further investigation. For Tiers 2 and 3, modeling, usually with integral probabilistic components, is often crucial to the overall weight of evidence evaluation.

It is desirable in risk characterization to obtain probabilistic estimates of risk for a species or group of species. Simulation models can provide such estimates by integrating exposure and stressor-response profiles. These profiles may include information on the frequency, timing, and duration of the exposure in addition to the variables which characterize the stressor-response.

There are two basic types of simulation models used in ecological risk assessments: 1) single-species population models and 2) multi-species models. Single species population models are used to predict direct effects on single populations, using measurement endpoints at the individual organism level. Multi-species models include various components of the ecosystem, such as food-web relationships (i.e., predator-prey, competition), plant succession, etc. Multi-species models evaluate both direct and indirect effects. An example of an indirect effect predicted through modeling is the potential for a change in avian behavior that would tip the balance of interspecific competition for nest sites or behavior that reduces some aspect of parental care. The influence such responses may have on population status may be either very obvious or subtle and only substantiated by empirical results or complex models. When the population response is less complex, such as reduced fledgling success in a bird species, it may be advantageous to use simpler, single-species population models to predict the probability of a given response level. When selecting a model, it is important to thoroughly consider the appropriateness of the model for the particular application.

Information needed to develop an estimation of risk may come from field studies, existing literature, or a combination of the two. In some cases risk estimation need not require a full-scale field study conducted over several years or seasons. As stated in the examples under the Analysis phase, above, the risk characterization may proceed using key sentinel species, with known life-history requirements (feeding, reproduction, habitat). Use of such surrogate species, which may be free-ranging wild individuals or individuals introduced to the site, may be far less costly than full-scale field surveys. When naturally occurring individuals or introduced individuals are exposed on the site for a defined time, the body burdens,

biochemical responses, and/or alterations in behavior may be correlated to distributions of the contaminants. Such an assessment would provide the variety of measures (measurement endpoints) and allow estimates of variance within each set. In this manner, site-specific probabilities could be associated with each of the expected adverse effects.

The Rocky Mountain Arsenal Environmental Risk Assessment is used as a case history example in "A Review of Ecological Assessment Case Studies from a Risk Assessment Perspective"[16]. This case study presents an example of a food chain-based model developed to predict effects on animal species on the site. The model is developed and tested using data from Tier 3 level field studies of exposure and effects in sentinel species.

For probabilistic estimates of risk, there are a wide variety of available models useful in any of the tiers (Volume 2, Appendix A). Several models focus on how the environment modifies the contaminant bioavailability (e.g., FGETS model, Volume 2). Modeling approaches presently exist to link water quality to reductions in "dose" under various scenarios of ecosystem productivity[29]. One example of a modeling approach that illustrates how ecosystem trophic status modifies the bioavailability of toxicants and decreases the subsequent dose to biota was performed by McCarthy and Bartell[68]. Their model predicts the association of a contaminant with dissolved organic material (DOM) or particulate organic material (POM), which significantly lessens the bioavailability of a toxicant, and thus, the potential dose experienced by the organisms. Importantly, this paper shows the necessity of estimating the true bioavailability of a contaminant in the environment.

Model projections which include seasonal or habitat variances in bioavailability (e.g., mapped onto expected environmental chemical concentrations for species of known life history, feeding, and habitat requirements) are a cost-effective approach to the hazard characterization of complex chemicals. For a given concentration, species may be subject to exposure for a <u>relatively</u> longer period of their life-span if they are smaller or less likely to move beyond the boundaries of the contaminated area (examples are earthworms, burrowing invertebrates, or small mammals). Further, if a chemical is susceptible to being bound by organics, burrowing (or thigmotactic) benthic invertebrates (or benthos-feeding fish) may be subjected to higher exposures than would otherwise be predicted. Volume 2 includes certain models available for evaluating transport, transformation and fate of contaminants in the environment (e.g., EXAMSII, LPMM). In addition, several models estimate biotic exposure or uptake of contaminants (e.g., FGETS).

Environmental and ecological monitoring data may be evaluated

using a Geographical Information System (GIS) as part of a Tier 3 effort to gain a higher level of understanding of potential contaminant-associated problems and approaches to effective risk management. Coupling modeling and GIS is particularly effective when geographic distributions of contaminants and the integration of these contaminants and wildlife activities on the study site are important parts of the risk analysis and characterization. For example, animal home-range analyses can be incorporated to GIS software and home-range use can be correlated with geographic distributions of contaminants to estimate potential for exposure. From this information, risk management alternatives can be evaluated on a "what if" basis by having remediation engineers identify contaminant parcels most amenable to control. The risk assessment benefit of such projected risk management efforts can then be evaluated directly through the GIS. Such an approach is being explored for remediation at Rocky Mountain Arsenal[69]. In this case, the site-wide risk reduction associated with local "hotspot" removal is clearly demonstrated by linking exposure models to GIS for immediate evaluation of the benefits of various remediation scenarios. This is illustrated in Figure 18 which contains "risk surfaces" for burrowing owls exposed to dieldrin via diet at Rocky Mountain Arsenal. The upper surface is prior to remediation and clearly shows the dieldrin "hot spot" (HQ=434). The bottom surface is a post-remediation projection with no HQ greater than 1.0.

Using GIS in the risk assessment process is also a highly effective way to produce graphics and visual aids to demonstrate and explain (to military and regulatory personnel, and to the public) the critical environmental relationships that influence ecological risk.

4.5 Uncertainty Analysis

Risk estimation infers a degree of uncertainty. The estimation is derived from comparison of organism exposure to organism response to the stressor(s) under investigation. The stressor-response profiles used in this process may involve a single value response such as an LD_{50}, or a suite of responses such as immune system function responses combined with contaminant blood levels. The degree of uncertainty around the estimate is related to the precision of the stressor-response profiles used. When the response evaluated is death, or death of 50% of the population (LD_{50}), the uncertainty of an adverse effect will be greater than if the response level of concern is a measured level of sublethal immune system response. The more conservative response variables are more likely to err on the safety side of the equation, and result in lower uncertainty of the negative effects under consideration. Within each tier, there will be assumptions and uncertainties involved in characterizing the ecological risk. By the very nature of the lower effort and cost at the lower tiers,

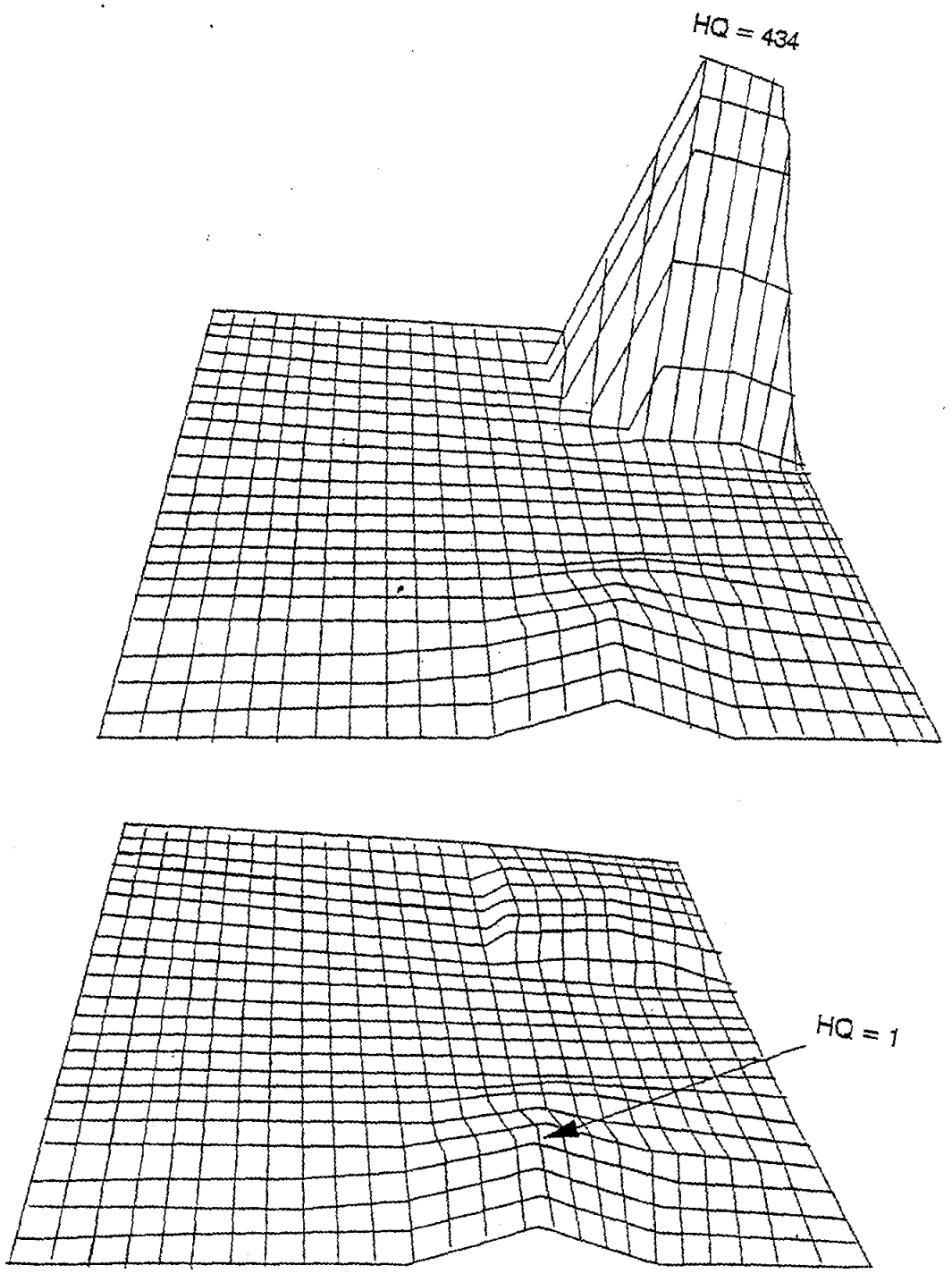


Figure 18. GIS-based "risk surfaces" for dieldrin at Rocky Mountain Arsenal.

risk characterization will have larger uncertainties. The benefit of more focused effort in the higher tiers becomes primarily one of incorporating more site-specific information, thus reducing the need for simplifying assumptions, and therefore reducing the level of associated uncertainty.

Uncertainty analysis is thus an important part of the Risk Characterization phase and occurs as a function of questions and variances from all phases of an ERA. The objective of uncertainty analysis is to identify and quantify, to the highest degree possible, the cumulative uncertainty surrounding the estimates of risk. Products of the uncertainty analysis are an evaluation of the effects of uncertainties on the overall assessment and on the risk management process. For example, if risk assessment uncertainty is high, and conservative assumptions were used to suggest a major cleanup effort, additional investigation to reduce uncertainly might be warranted. However, if conservative or realistic risk estimates yield an objective, credible risk management program, the level of uncertainty is clearly appropriate to the assessment goals.

Sources and effects of uncertainty overlap throughout the risk assessment. The reader can find in-depth discussions of the subject in the references listed in the Risk Assessment Framework[70,71,72]. Some major sources of uncertainty include:

1) formulation of the conceptual model: are the correct working hypotheses established?

2) incomplete information and data: if the correct data are not collected, little can be said of the exposure or response.

3) natural variability: variance in spatial, temporal distributions of the COC, biotic and abiotic stressors, and population at risk.

4) procedural or design error: unless data quality assurance plan is formulated, it is likely that errors and greater uncertainty will increase from incorrect or inappropriate analyses.

4.5.1 Conceptual Model Formulation

Flaws in the conceptual model may be the most pervasive source of uncertainty, and the most difficult to identify, quantify or reduce. The conceptual model, which is the product of the problem formulation phase, provides the basis for the analysis phase and the development of the exposure and stressor-response profiles. If incorrect assumptions are made during the

conceptual model development regarding the potential effects of a stressor, the influence of environmental variables, the interaction of wildlife species with the stressor, or the sensitivity of organisms to the stressor, the final risk assessment will be flawed. Once the conceptual model is correctly developed during the course of the ERA, care should be taken not to incorporate factors that erroneously increase uncertainty, lead to incorrect conclusions, or limit management decisions. Awareness and avoidance of factors that unduly increase uncertainty are critical at all phases of the assessment.

4.5.2 Incomplete Information and Data

The risk assessor will invariably encounter situations where information or data are incomplete. In some cases the assessment may be halted until further information is obtained or further study completed to fill in data gaps. However, there will be cases when the resources, technology, or fundamental ecological knowledge needed to close such gaps are not available. In these cases, the risk assessor must rely on professional judgement and cautious use of assumptions. When judgement and assumptions are inserted into the assessment, they must be clearly identified as such throughout the various phases of the assessment, and thoroughly explained and evaluated during the Risk Characterization phase.

4.5.3 Natural Variability

Natural variability (stochasticity) is an ever-present condition that influences the distribution, availability and influence of stressors in the environment. It equally biases our perception and interpretation of these factors. Variability inherent in the physical environment (moisture, nutrients, organic material, temperature, etc.) causes variability in biological components of the environment (animal health, size, sensitivity, exposure level, etc.). Although the uncertainty caused by variability may be complex, it can be acknowledged and described, but not reduced[72]. When sufficient databases exist, stochasticity can be quantitatively estimated and analyzed via such methods as Monte Carlo simulation and statistical uncertainty analyses[73,74].

4.5.4 Procedural and Design Error

Errors in measurement and sampling can be reduced through adherence to a good quality control program or Good Laboratory Practices Guidelines. Raw data review and data entry verification procedures are invaluable in reducing the introduction of human errors. Errors in study design are best avoided by assuring a strong peer review of protocols. Errors and uncertainty in the development of simulation models can be

addressed through sensitivity analysis and field verification or model validation.

4.6 Risk Description: Ecological Risk Summary and Interpretation of the Significance.

The EPA Framework[4] describes two elements of ecological risk description: 1) a summary of the risk estimation results to describe the confidence level in the risk estimates; and 2) interpretation of ecological significance, identified in the Framework Document as the magnitude of the risks relative to the assessment endpoints. This approach has been carried into the Review Draft Superfund Guidance[3] as a weight-of-evidence foundation for ecological risk assessment. A weight-of-evidence approach incorporates the judgement of how variable are estimates of contaminant distribution, exposure and biotic uptake potential, and the probability of adverse effects of residual contamination and possible remedial activities.

4.6.1 Ecological Risk Summary

The ecological risk summary succinctly reports results of the risk estimation phase and discusses the uncertainty of previous phases of the assessment. This involves an overview of measured endpoints (or estimates) of exposure and response at the individual or population level, bioaccumulation potential, integration of single or distributional exposure and stressor-response profiles, and/or model predictions. This overview must also include a discussion of the uncertainty inherent in each phase of the assessment. Whenever possible, the conclusion of the risk estimation should be expressed as a quantitative expression (there is a 30% probability of 25% mortality in American robins). Another example consists of a study on the effects of molybdenum mine tailings on marine fish and invertebrates[16]. The scientists calculated the risk to aquatic organisms by developing a probability of exceeding a water quality criterion level for copper (over a 55 year period) and -conservatively- assuming 100% mortality if organisms were exposed to concentrations higher than the criterion. Hence, the probability of greater-than-criterion levels for copper in water and sediments becomes the probability of effect. The conservatism of this approach could be made less, with greater accuracy, if more data were collected from the field or laboratory exposures were developed using native organisms. However, the example does provide a case where the effects are cast in probabilistic terms.

However, ecological risk assessments completed to date usually express the risk estimation in qualitative format with terms such as "high likelihood", "moderate", "low likelihood" of a given negative impact (e.g., avian mortality). Uncertainty also will

be expressed in quantitative or qualitative terms. In the discussion of uncertainty, it is important to include evaluation of the relative contributions of the uncertainties from different aspects of the assessment to the final estimate of risk.

4.6.2 Weight of Evidence and Ecological Significance

Weight of evidence for projecting risks and impacts is a conceptual approach which dictates that all sources of information be considered in making risk management decisions. Because the weight of evidence links the risk assessment to the risk management process, it is imperative that the risk assessor provide clear characterization of uncertainty in each component of the weight of evidence and the meaning of each component for ecological impacts.

The evaluation of the ecological significance of risk is a process at the very edge of the capability of ecological science. Biological populations are very dynamic and population measures and models are relatively simple compared to the underlying ecological complexity. Yet it is at the population level that ecological significance must be evaluated (except for endangered or threatened species, which are often evaluated at the individual level). Suter[7] provides an example of an approach to quantifying population level effects of toxicological risks. Yet this exercise cannot be validated, and is only tested by additional modeling[75].

An instructive example of the difficulty of projecting the significance of risk estimates is provided in Barnthouse et al.[76]. While this paper discusses the impacts and importance of power plant withdrawals on finfish communities, the principles developed apply to contaminated site assessments. Barnthouse et al. evaluated more than ten year's worth of effort to extrapolate the effects of cooling water withdrawal on fish populations in the Hudson River. Such withdrawals are inevitably associated with the loss of individuals. At issue was the relative ecological importance of such losses. In practice, despite highly certain estimates of the loss rates, estimates of importance at the population and ecosystem levels were so uncertain as to be useless.

Whenever possible, the assessment should clearly distinguish between impacts to individuals, or even portions of populations, and those impacts that affect whole populations. For example, Hinckley and Porter[77] demonstrated at a midwestern NPL site some individual impacts to white-footed mice from lead. However, impacts to the population as a whole were minimal. In contrast, although many fewer red-tailed hawks were impacted, a much greater proportion of their population was involved.

Thus, the current state of ecological science is not conducive to elucidating "ecological significance" of estimated risks. The most productive approach for most sites, in keeping with the conclusions of Barnthouse et al.[76], is to document for risk managers the potential impacts of contamination and remediation and make site specific decisions on risk reduction. It may be appropriate at large, complex sites to undertake attempts to quantify ecological significance as a component of Tier 3 evaluation, but such efforts should be tempered by sound risk management judgement.

Weight of evidence in an ERA is supported by the quality and sufficiency of data. Quality assurance programs are paramount in <u>any</u> ERA and provide confidence in precision, reproducibility, etc. Sufficiency of the data is addressed relative to the effort involved. Tier 1 information provides primarily corroborative information, such as lists of known chemicals (and, hence, toxicity and physico-chemical characteristics), suspected distribution on the site, and limited data on direct measures of exposure and effects. Models applied at this tier may require several default assumptions for parameters.

In Tiers 2 and 3, the information on exposure and ecological effects provide a higher degree of correlation between the stressor and consequent effects. For example, a better resolution of contaminant effects of metals in a wetland on waterfowl may be obtained for migratory avian species when the timing and distribution of the migratory species is matched to times when their food base (burrowing insects) lead to exposure to the contaminant. To discern how much of the exposure stems from on-site, relative to exposure elsewhere takes time and effort not available under Tier 1. Ultimately, to reduce uncertainty in analyses, one must understand the situation in greater detail. Hence, it may be necessary to conduct follow-on studies to corroborate initial judgement calls.

When a population responds to a contaminant, its response may range from biochemical or physiological responses at the cellular level to behavioral changes or (ultimately) death and the reduction of population numbers. The significance of the responses need to be addressed in an ERA relative to the ecological context. Organismal responses (physiological, behavioral) may be transient enough, relative to the exposure duration or life history characteristics of the species, that they have little or no influence on the assessment endpoint. However, it may be that such "lower level" responses provide sufficient questions as to sub-lethal effects that another problem formulation may be called for. As an example, such a situation might exist within a site with multiple contaminant point sources, such as certain hazardous waste sites with a history of uncontrolled dumping of multiple, complex wastes. The

tiered assessment may focus on chemicals known to have been dumped at the site; however, some animals may be exposed to an unknown or unrecognized source. Biomarkers of exposure (cf., cytochrome P450 induction, porphyrin profiles; Volume 2, Appendix B) would indicate that exposure has occurred and that the potential for adverse affects on the population may warrant further investigation of the nature and extent of risk.

The interpretation of ecological effects also needs to take into account the spatial and temporal nature of the stressor and population exposed. Risk stemming from a wide area of diffuse contamination will be more difficult to summarize than areas with defined "hot spots" of contamination. Further, if the area and duration of exposure are long enough relative to the generation time of the species, then one may expect sublethal toxicity be expressed. For certain species, a small area of contamination may lead to local population extermination if the stress is high. This might occur if a species requires a very specific habitat (e.g., wood ducks in wetlands). Should the habitat be altered even a little, the effect on the species could be catastrophic.

In addition to local, catastrophic effects, stressor responses identified throughout the risk assessment process may have ecological significance of a broader, more diffuse nature. For example, it may be determined that the response of nestling birds to a contaminant consumed in their food is 25% mortality. However, a follow-on evaluation of nestling fledgling rates and post-fledgling survival indicates there is an increase in overall fledgling and survival. For these results, the explanation is that nestling survival is density-dependent and the loss of an average of one nestling per nest resulted in more parental attention and more food for the remaining nestlings. Thus remaining nestlings were of greater body weight at fledgling and this equated to greater overall post-fledgling survival compared to non-dosed nestlings. In a case such as this, we may conclude that while there was a significant effect to individuals, the effect on population was positive, not negative. Therefore, there was little or no ecological significance.

The interpretation of ecological significance places risk estimates in the context of the types and extent of anticipated effects. Interpretation of these factors relies heavily on professional judgement. The significance of effects may be evaluated in context of several variables:

1) the nature and magnitude of effects,
2) the spatial and temporal patterns of effects,
3) the duration of effects, and
4) the potential for the system or species to recover from the effects.

All the above factors help to place expected risks into broader

ecological perspectives. Interpretation of significance may take into consideration other ecological components not specifically addressed in the risk assessment. For example, the risk assessment may have addressed reduction in a population of breeding voles (a species of small mouse-like mammals) thought to be due to a stressor. The reduction in vole numbers may not be discernable following the reproductive season, when autumn vole populations are no different on the impact site than on reference sites. The significance of the toxic effect to the vole population may prove to be small. However, as part of the interpretation of the significance of the spring decline in adult voles, the risk manager may make the connection with a separate report that northern harrier production in the area has declined and question whether this is related to the decreased availability of voles, the harrier's staple diet.

A final strength of the tiered approach to risk assessment is related to resolving the question "how does one go about measuring when clean is clean enough?" The tiered approach provides some guidance: for example, if surrogate organisms are used as part of a Tier 3 evaluation of exposure (i.e., nest boxes), this assessment process could be left intact, or repeated, as an on-site biomonitoring assessment following mitigation. If mitigation truly reduced bioavailability, the exposure in the surrogate species should measurably decline. If biochemical markers of exposure indicate no exposure, then the contaminant (even if at detectable levels in soil) is not being taken up by the organisms. Hence, a measure of the success of clean up efforts becomes available.

The summary decisions and projections of risk within the Risk Description phase concludes the risk assessment process and provides the basis for communication between the risk assessor and the risk manager, ultimately responsible for making the appropriate regulatory decisions.

4.7 Risk Management

Environmental cleanup actions have technical and social foundations. At many sites, various stakeholders and stakeholder groups have divergent interests and concerns. Remedial activities are truly effective when stakeholder interests are satisfied. For example, the site assessment team might agree that low, but elevated, concentrations of a particular contaminant could remain in place without adverse effects. Owners of adjacent properties, concerned about real estate values, might be more concerned about *de minimis* residual contamination. Or the risk management team might determine that destructive remediation of a wetland is warranted by contaminant levels, while local recreational boaters might desire simple monitoring.

Clearly, there is a trade off in risk management, between destructive remediation (all currently available technologies destroy the habitat in place) and residual contamination. While it is desirable to make decisions on a "risk averse" basis, it is not always clear what is "riskier": site remediation or site contamination. Risk assessment uncertainty (described below) plays a crucial role in this decision threshold, because the risk of remedy associated with site cleanup is highly certain, and must be balanced against the weight of evidence for contaminant-related risks.

The trade off between risks due to existing contaminants and those due to remediation was illustrated at a midwestern site by Hinckley and Porter[77]. These authors demonstrated that removal of lead from a wetland entailed its destruction, while only providing minimal reduction in hazard quotients for mice and raptors.

Once the decision has been made to undertake site cleanup, the nature and extent of remedial activities must be determined. With the exception of highly contaminated "hotspots", these definitions are best supported by Tier 2 and 3 evaluations with decision criteria developed in advance.

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Attachment 4

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METHODS AND TOOLS FOR ESTIMATION OF THE EXPOSURE OF TERRESTRIAL WILDLIFE TO CONTAMINANTS

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EXECUTIVE SUMMARY

A critical component in ecological risk assessment is the evaluation of exposure experienced by endpoint receptors. Exposure can be defined as the coincidence in both space and time of a receptor and a stressor, such that the receptor and stressor come into contact and interact. Without sufficient exposure of the receptor to the contaminants, there is no ecological risk.

Unlike some other endpoints considered in ecological risk assessments, terrestrial wildlife are significantly exposed to contaminants in multiple media. They may drink or swim in contaminated water, ingest contaminated food and soil, and breathe contaminated air. Exposure models for terrestrial wildlife must therefore include multiple media. In addition, because most wildlife are mobile, moving among and within habitats, exposure is not restricted to a single location. They may integrate contamination from several spatially discrete sources. As a consequence, the accurate estimation of wildlife exposure requires the consideration of habitat requirements and spatial movements.

This report presents methods for estimating exposure of terrestrial wildlife to both chemical (Sect. 2.1) and radionuclide (Sect. 2.2) contaminants. Approaches for probabilistic exposure estimation (Sect. 2.3) and extrapolation from individual-level exposures to population-level effects (Sect. 2.4) are reviewed. Finally, methods and models to estimate contaminant concentrations in selected food types consumed by wildlife (Sect. 3.2) and life history parameters (Sect. 3.3) needed to accurately estimate exposure are presented.

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1. INTRODUCTION

Exposure can be defined as the coincidence in both space and time of a receptor and a stressor such that the receptor and stressor come into contact and interact (Risk Assessment Forum 1992). In the context of ecological risk assessment, receptors include all endpoint species or communities identified for a site [see Suter (1989) and Suter et al. (1995) for discussions of ecological endpoints for waste sites]. In the context of hazardous waste site assessments, stressors are chemical contaminants and the contact and interaction are represented by the uptake of the contaminant by the receptor. Without sufficient exposure of the receptor to the contaminants, there is no ecological risk.

Unlike some other endpoint assemblages, terrestrial wildlife are significantly exposed to contaminants in multiple media. They may drink or swim in contaminated water, ingest contaminated food and soil, and breathe contaminated air. Exposure models for terrestrial wildlife must therefore include multiple media. In addition, because most wildlife are mobile, moving among and within habitats, exposure is not restricted to a single location. They may integrate contamination from several spatially discrete sources. As a consequence, the accurate estimation of wildlife exposure requires the consideration of habitat requirements and spatial movements.

The purpose of this report is to present generalized methods for the estimation of exposure of terrestrial wildlife, focusing primarily on methods and models for birds and mammals. Reptiles and amphibians are not considered because few data exist with which to assess exposure to these organisms. In addition, because toxicological data are scarce for both classes, evaluation of the significance of exposure estimates is problematic. The general exposure estimation procedure developed for birds and mammals, however, is applicable to reptiles and amphibians (EPA 1993).

Methods are presented for estimating exposure to both chemical (Sect. 2.1) and radionuclide (Sect. 2.2) contaminants. Approaches for probabilistic exposure estimation (Sect. 2.3) and extrapolation from individual-level exposures to population-level effects (Sect. 2.4) are reviewed. In addition to exposure models, methods and models to estimate contaminant concentrations in selected food types consumed by wildlife (Sect. 3.2) and life history parameters (Sect. 3.3) needed to accurately estimate exposure are presented.

2. METHODS FOR ESTIMATION OF EXPOSURE

Contaminants to which terrestrial wildlife may be exposed may be grouped into two broad classes: chemical (e.g., heavy metals, organics) and radionuclide. Because the mode of action differs greatly between these two general classes of contaminants, methods for estimation of exposure also differ. Methods for estimation of exposure to both chemical and radionuclide contaminants are presented below.

2.1 ESTIMATION OF EXPOSURE TO CHEMICAL CONTAMINANTS

As terrestrial wildlife move through the environment, they may be exposed to contamination via three pathways: oral, dermal, and inhalational. Oral exposure occurs through the consumption of contaminated food, water, or soil. Dermal exposure occurs when contaminants are absorbed directly through the skin. Inhalational exposure occurs when volatile compounds or fine particulates are respired into the lungs. The total exposure experienced by an individual is the sum of exposure from all three pathways or

$$E_{total} = E_{oral} + E_{dermal} + E_{inhal}, \tag{1}$$

where

E_{total}	=	total exposure from all pathways,
E_{oral}	=	oral exposure,
E_{dermal}	=	dermal exposure,
E_{inhal}	=	exposure through inhalation.

Dermal exposure is assumed to be negligible for birds and mammals on most U.S. Department of Energy (DOE) hazardous waste sites. While methods are available to assess dermal exposure to humans (EPA 1992), data necessary to estimate dermal exposure are generally not available for wildlife (EPA 1993). Additionally, many contaminants found at DOE facilities (e.g., metals and radionuclides) are unlikely to be absorbed through skin (Camner et al. 1979; Watters et al. 1980). Feathers and fur of birds and mammals further reduce the likelihood of significant dermal exposure by limiting the contact of skin with contaminated media. Therefore, dermal exposure is expected to be negligible relative to other routes in most cases. If contaminants that have a high affinity for dermal uptake are present (e.g., organic solvents and pesticides) and an exposure scenario for an endpoint species is likely to result in significant dermal exposure (e.g., burrowing mammals or swimming amphibians), dermal exposure may be estimated using the model for terrestrial wildlife presented by Hope (1995).

Inhalation of contaminants is also assumed to be negligible at most DOE facilities. This is for two reasons. First, because most contaminated sites are either capped or vegetated, exposure of contaminated surface soils to winds and resulting aerial suspension of contaminated dust particulates are minimized. Second, most volatile organic compounds (VOCs), the contaminants most likely to present a risk through inhalation exposure, rapidly volatilize from soil and surface water to air, where they are rapidly diluted and dispersed. Paterson et al. (1990) suggest that organic compounds with soil half-lives of <10 days are generally lost from soil before significant exposure can occur. As a consequence, significant exposure to VOCs through inhalation is unlikely. In situations where inhalation exposure of endpoint species is believed to be occurring or is expected to occur, models for vapor or particulate inhalation (Hope 1995) may be employed. In these cases, EPA (1993) recommends consulting an inhalation toxicologist.

Because contaminant exposure experienced by wildlife through both the dermal and inhalation pathways is negligible, the majority of exposure must be attributed to the oral exposure pathway. Equation 1 can therefore be simplified to

$$E_{total} \approx E_{oral} \, . \tag{2}$$

2.1.1 Estimation of Oral Exposure

Oral exposure experienced by wildlife may come from multiple sources. They may consume contaminated food (either plant or animal), drink contaminated water, or ingest soil. Soil ingestion may be incidental while foraging or grooming or purposeful to meet nutrient needs. The total oral exposure experienced by an individual is the sum of the exposures attributable to each source and may be described as

$$E_{oral} \approx E_{food} + E_{water} + E_{soil} \, , \tag{3}$$

where

E_{food}	=	exposure from food consumption,
E_{water}	=	exposure from water consumption,
E_{soil}	=	exposure from soil consumption.

For exposure estimates to be useful in the assessment of risk to wildlife, they must be expressed in terms of a body weight-normalized daily dose or milligrams of contaminant per kilograms body weight per day (mg/kg/d). Exposure estimates expressed in this manner may then be compared to toxicological benchmarks for wildlife, such as those derived by Sample et al. (1996a), or to doses reported in the toxicological literature. Models for the estimation of exposure from oral ingestion have been reported in the literature (EPA 1993, Sample and Suter 1994, Hope 1995, Pastorok et al. 1996, Freshman and Menzie 1996) and are generally of the form

$$E_j = \sum_{i=1}^{m} (I_i \; x \; C_{ij}) \, , \tag{4}$$

where

E_j	=	total oral exposure to contaminant (j) (mg/kg/d),
m	=	total number of ingested media (e.g., food, water, or soil),
I_i	=	ingestion rate for medium (i) (kg/kg body weight/d or L/kg body weight/d),
C_{ij}	=	concentration contaminant (j) in medium (i) (mg/kg or mg/L).

Very few wildlife consume diets that consist exclusively of one food type. To meet nutrient needs for growth, maintenance, and reproduction, most wildlife consume varying amounts of multiple food types. Because it is unlikely that all food types consumed will contain the same contaminant concentrations, dietary diversity is of one of the most important exposure modifying factors.

To account for differences in contaminant concentrations of different food types, exposure estimates should be weighted by the relative proportion of daily food consumption attributable to each food type and the contaminant concentration in each food type. In addition, wildlife may drink from different water sources

and consume soils that differ in contaminant concentrations. These differences must also be accounted for. This may be done by modifying Eq. 4 as follows

$$E_j = \sum_{i=1}^{m} \sum_{k=1}^{n} p_{ik}(I_i \ x \ C_{ijk}) \ , \tag{5}$$

where

n	=	number of types of medium (i) consumed (unitless),
p_{ik}	=	proportion of type (k) of medium (i) consumed (unitless),
C_{ijk}	=	concentration of contaminant (j) in type (k) of medium (i) (mg/kg or mg/L).

If the site is spatially heterogeneous with respect to either contamination or wildlife use, the model must be modified to include spatial factors. The most important spatial consideration is the movement of wildlife. Animals travel varying distances, on a daily to seasonal basis, to find food, water, and shelter. The area encompassed by these travels is defined as the home range (we use the term here to include territories). If the site being assessed is larger than the home range of an endpoint species and provides the habitat needs of the species, then the previously listed models are adequate. However, endpoint species often have home ranges that are larger than contaminated sites, or the contaminated site may not supply all of a species' habitat requirements. In those cases, the wildlife exposure model must be modified.

If the contaminated site has similar habitat quality to the surrounding area but is smaller than the home range, use of the contaminated site is simply a function of its area. That is, one can assume that for wildlife that use the entire contaminated area, exposure is proportional to the ratio of the size of the contaminated site to home range size. Eq. 5 can be modified as follows as follows:

$$E_j = \frac{A}{HR}[\sum_{i=1}^{m} \sum_{k=1}^{n} p_{ik}(I_i \ x \ C_{ijk})] \ , \tag{6}$$

where

A	=	area (ha) contaminated,
HR	=	home range size (ha) of endpoint species.

Note that *A* is the area contaminated, not the entire area that has been designated a hazardous waste site (e.g., an operable unit). Because boundaries are often drawn conservatively, they may contain a considerable uncontaminated area.

The previous equation (6) implies that all of the habitat within a contaminated area is suitable and that use of all portions of the contaminated area is equally likely. Because many waste sites are industrial or highly modified in nature, it is unlikely that all areas within their bounds will provide habitat suitable for endpoint species. If it is assumed that use of a waste site will be proportional to the amount of suitable habitat available on the site, Eq. 6 may be modified to read

$$E_j = P_h(\frac{A}{HR}[\sum_{i=1}^{m} \sum_{k=1}^{n} p_{ik}(I_i \ x \ C_{ijk})]) \ , \tag{7}$$

where

P_h = proportion of suitable habitat in the contaminated area.

One complication is the spatial heterogeneity of contaminants on waste sites. These models (Eqs. 4-7) are based on the assumption that either contaminants are evenly distributed on the site, or wildlife forage randomly with respect to contamination on the portion of the site that constitutes habitat so that they are exposed to mean concentrations. However, if contaminant levels are related to habitat quality, that assumption would not hold. For example, contaminant concentrations might be greatest near the center of a site, but the habitat quality might be highest near the edges. In such cases, it might be necessary to model the proportional contribution of each area with a distinct combination of contaminant level and habitat quality

$$E_j = \sum_{l=1}^{o} \left(\frac{A_l}{HR} \left[\sum_{i=1}^{m} \sum_{k=1}^{n} p_{ik} (I_i \; x \; C_{ijkl}) \right] \right) , \tag{8}$$

where

o = number of distinct contaminated habitat areas,

A_l = area (ha) of a distinct contaminated habitat area,

C_{ijkl} = concentration of contaminant (j) in type (k) of medium (i) from the lth area (mg/kg or mg/L).

As can be seen, if the distribution of contamination and habitat quality is complex, this approach to exposure estimation rapidly becomes ungainly. In such cases, it is advisable to implement the exposure in a Geographic Information System (GIS). Using a GIS, maps displaying the spatial distribution of habitat types may be overlaid with maps of contaminant distribution to accurately determine the degree to which habitat is contaminated. Furthermore, if information on the distribution or movements of wildlife (generated by radiotelemetry or censuses) are available, these data may be combined with the habitat and contaminant data to provide a more accurate visualization of exposure. Examples of the application of GIS to wildlife exposure and risk assessments can be found in Clifford et al. (1995), Banton et al. (1996), Henriques and Dixon (1996) and Sample et al. (1996b).

2.1.2 Exposure-Modifying Factors

Factors other than those described in these models modify contaminant exposure experienced by wildlife endpoint species. These factors include age, sex, season, and behavior patterns.

The models above imply that the endpoint species have uniform body size, metabolism, diet, home ranges, and habitat requirements. However, these properties may differ between juveniles and adults and between males and females. For example, because they are actively growing, metabolism (and therefore food consumption) is generally greater for juveniles of most endpoint species. Diet composition may also differ dramatically between juveniles and adults of the same species. Similarly, the food requirements of females during reproduction are greater than that for males for many endpoint species. These factors may serve to make certain age classes or a particular sex experience greater contaminant exposure than other segments of the population. Because of their greater exposure, contamination may present a greater risk to these segments of the population. If it is known that a particular lifestage or sex is sensitive to contamination, that lifestage should be emphasized in the exposure assessment.

Behavior may modify exposure by increasing or decreasing the likelihood of contact with contaminated media. Wildlife behaviors are frequently seasonal in nature. Some foods may be available and consumed only at certain times of the year. Similarly, some habitats and certain parts of the home range may

be used only in certain seasons. In addition, many species hibernate or migrate; by leaving the area or restricting their activity to certain times of year, their potential exposure may be dramatically reduced. All of these factors should be considered when evaluating contaminant exposure experienced by wildlife, and exposure models should be adjusted accordingly. The simplest approach to modifying the exposure estimates to take into account some of these exposure-modifying factors is to generate multiple exposure estimates. For example, if diet differs by season or by sex, calculate exposure estimates for each sex or season. Comparison of exposure estimates generated for differing exposure scenarios will aid in identifying the segments of population at greatest risk or times of year when risk is greatest.

2.2 ESTIMATION OF EXPOSURE TO RADIONUCLIDES

Estimation of exposure and effects from radionuclides is both qualitatively and quantitatively different from estimation of exposure to chemical contaminants. Exposures to radionuclides may be internal or external, and effects are caused by energetic particles or rays released as part of the decay of atoms. Decay energies of particles or rays emitted by each radionuclide must be accounted for. Unlike chemical exposures where effects of chemicals are generally evaluated individually, the internal and external doses from all radionuclides present must be summed to arrive at the appropriate exposure dose for a given organism. In addition, a number of radionuclides have daughter products that must also be included in the exposure calculations.

Internal exposures result from ingestion of contaminated food, soil, or water or inhalation of contaminated soil or dust (Templeton et al. 1971, IAEA 1976, Blaylock and Trabalka 1978, Woodhead 1984). External exposures result from direct exposure to radiation from the soil and may occur either above or below ground (or a combination of both), depending on the habits of the receptor (e.g., fossorial vs nonfossorial). Evaluation of the resulting radiation doses received by biota requires quantitative information on the radionuclides to which they are exposed. In all cases, the radiation source must be known in terms of the quantity of each specific radionuclide (pCi/g) and the corresponding energy released per disintegration (MeV/dis). Conversions for units of dose and activity generally reported in the literature are presented in Table 1.

Table 1. Comparison of units of activity and absorbed dose of ionizing radiation under the international and conventional systems of measure

Measure	International system	Conventional system	Relationship
Activity	Becquerel (Bq) = one nuclear disintegration/s	Curie (Ci) = 3.7×10^{10} nuclear disintegrations/s	1 Bq = 2.7×10^{-11} Ci 1 Ci = 3.7×10^{10} Bq
Absorbed dose	Gray (Gy) = 1 Joule/kg	rad = 0.01 Joule/kg	1 Gy = 100 rad 1 rad = 0.01 Gy

Models for estimating radiation dose rates (mrad/d) for plants, earthworms, and terrestrial wildlife species are based on methodology from Blaylock et al. (1993) and Baker and Soldat (1992). The general methodology and the equations specific to each exposure route used in estimation of dose rates for biota are described below. In practice, doses from alpha (α), beta (β), and gamma (γ) emissions (only β and γ for external exposures of earthworms and plants and only γ for external exposures of wildlife receptors) should be calculated for each radionuclide of concern, including the dose rates from all short-lived daughter products for the radionuclides. Doses from each radionuclide (plus daughters) should then be summed over all

exposure routes and all radionuclides to arrive at the overall estimate of the dose received for each receptor. Alpha particles have low penetration energy and are not considered for external exposures. Beta particles are unlikely to penetrate the epidermis of larger organisms, so they are only considered in external exposures to plants and earthworms.

2.2.1 External Exposures: Direct Radiation from Soil

The equation for estimating aboveground external dose rates (mrad/d) for terrestrial receptors exposed to contaminated soil uses dose coefficients published by Eckerman and Ryman (1993). These dose coefficients relate the doses to organs and tissues in the body to concentrations of radionuclides in soil and are available for soil contaminated to depths of 1, 5, and 15 cm or soil assumed to be contaminated to an infinite depth. A dose rate reduction factor is used to account for the fraction of time the receptor spends aboveground. This factor is necessary because a different model is used to estimate below-ground exposures to soil radionuclides. The fraction of time spent above or below ground by each receptor species should be estimated based on knowledge of the species' life history and behavior patterns. Dose coefficients assume that the source region is a smooth plane (Eckerman and Ryman 1993), but this is rarely the case in a terrestrial habitat. A representative average dose reduction factor for ground roughness is 0.7, although recommended values range from essentially unity for paved areas to about 0.5 for a deeply plowed field (Eckerman and Ryman 1993). For relatively small mammals (e.g., mice, voles, and shrews) that are effectively much closer than 1 m to the source, an elevation correction factor (ECF) of 2 should be applied to account for the increased dose expected at ground level relative to the effective height of a standard human used to derive the dose coefficients. For large animals the ECF may be set at 1. If desired, more complex modeling may be conducted to arrive at ECFs for organisms of any given effective height above the ground. For plants it may be assumed that the dose represents that to the reproductive part of the plant with an effective height similar to that of the standard human. An ECF of 2 may be appropriate for evaluating low-growing plant species. The equation for aboveground dose from external exposures for a plant or wildlife receptor is

$$D_{abovegrd} = F_{above} \; F_{ruf} \; \sum C_{soil,i} \; DF_{grd,i} \; CFb \; ECF \; , \qquad (9)$$

where

$D_{above\ grd}$	=	external dose rate to receptor from aboveground exposures to contaminated soil (mrad/d),
F_{above}	=	dose rate reduction factor accounting for the fraction of time the receptor spends aboveground (unitless),
F_{ruf}	=	dose rate reduction factor accounting for ground roughness (unitless) [Representative average of 0.7 (Eckerman and Ryman 1993) is reasonable default],
$C_{soil,i}$	=	activity of radionuclide *i* in surface soil (pCi/g),
$Df_{grd,i}$	=	dose coefficient for radionuclide *i* in soil contaminated to given depth (Eckerman and Ryman 1993) (Sv/s per Bq/m^3),
CFb	=	conversion factor to change Sv/s per Bq/m^3 to mrad g/pCi d (Equals 5.12 x 10^{14}),
ECF	=	elevation correction factor to adjust dose coefficients to value representative of effective height of animal aboveground.

Dose from alpha radiation is not a concern for external sources, as alpha radiation lacks penetrating power. The effective dose coefficients from Eckerman and Ryman (1993) incorporate both high-energy β and γ emissions. Radionuclide-specific parameters for selected radionuclides are provided in Table 2. These include dose coefficients assuming soil contaminated to a depth of 15 cm. Coefficients for soil contaminated to depths of 1, 5, and 15 cm and to an infinite depth are available in Eckerman and Ryman (1993).

Below-ground exposures are calculated assuming immersion in a continuous soil medium. Dose coefficients are unavailable for the immersion scenario, so exposures can be modeled as dose to soil adjusted for absorption by a small volume of tissue. The exposure fraction reflects the fraction of time the receptor spends below ground. Receptors that do not go below ground (e.g., nonfossorial wildlife: deer, hawks, turkey, etc.) do not receive a dose via this exposure route. Only γ radiations with energies greater than 0.01 MeV were evaluated for wildlife receptors as those with lower energies are unlikely to penetrate skin. Both β and γ radiations were evaluated for earthworms. The equation for below-ground external exposures of earthworms and wildlife receptors is

$$D_{belowgrd} = 1.05 \quad F_{below} \sum C_{soil,i} \quad \epsilon_i \quad CFa \ , \tag{10}$$

where

$D_{below\ grd}$	=	external dose rate to earthworm or wildlife receptor in burrow from contaminated soil (mrad/d),
F_{below}	=	dose rate reduction factor accounting for the fraction of time the receptor spends below ground (unitless),
$C_{soil,i}$	=	activity of radionuclide *i* in surface soil (pCi/g),
ϵ_i	=	energy for γ emissions by nuclide *i* (MeV/nt),
1.05	=	conversion factor to account for immersion in soil vs water (estimated value; Keith Eckerman, Health Sciences Research Division, Oak Ridge National Laboratory, personal communication, June 1996),
CFa	=	conversion factor to go from MeV/nt to g mrad/pCi d. (5.12×10^{-2}).

Note that the conversion factor of 1.05 used to account for the difference between immersion in soil vs water was meant for small volumes of tissue. While it can be roughly applied to large animals, it may be more appropriate to consult a health physicist and conduct more complex calculations of dose from below-ground exposures for large animals expected to spend significant time below ground.

Table 2. Average energy of decay and absorbed fractions for select radionuclides

Radionuclide	Average energy of decay[a]			Absorbed fractions (gamma)[b]					$DF_{grd0.15}$ (Sv m3/s Bq)[c]
	alpha	beta	gamma	A	B	C	D	E	
Actinium-228		0.475	0.971	0.01	0.0127	0.04	0.06	0.14	2.76e-17
Americium-241	5.479	0.052	0.033	0.04	0.05	0.12	0.16	0.3	1.23e-18
Antimony-126		0.283	2.834	0.01	0.01	0.03	0.04	0.11	8.13e-17
Antimony-126m		0.591	1.548	0.085	0.0123	0.03	0.05	0.12	4.44e-17
Astatine-218	6.697	0.04	0.007	0.63	0.79	0.94	0.94	0.94	3.13e-20
Barium-137m		0.065	0.597	0.011	0.015	0.04	0.06	0.15	1.71e-17
Beryllium-7			0.049	0.012	0.017	0.06	0.09	0.2	1.40e-18
Bismuth-210		0.389							1.86e-20
Bismuth-211	6.55	0.01	0.047	0.027	0.04	0.11	0.15	0.29	1.28e-18
Bismuth-212	2.174	0.472	0.186	0.01	0.011	0.04	0.06	0.14	5.36e-18
Bismuth-214		0.659	1.508	0.085	0.0123	0.03	0.05	0.12	4.36e-17
Cadmium-109		0.083	0.026	0.09	0.126	0.16	0.21	0.36	7.88e-20
Calcium-45		0.077							3.35e-22
Carbon-14		0.049							7.20e-23
Cesium-134		0.164	1.555	0.085	0.0123	0.03	0.05	0.12	4.47e-17
Cesium-137		0.187							3.94e-21
Cobalt-57		0.019	0.125	0.01	0.012	0.04	0.06	0.15	2.66e-18
Cobalt-60		0.097	2.504	0.01	0.01	0.03	0.04	0.11	7.25e-17
Curium-242	6.102	0.01	0.002	0.63	0.79	0.94	0.94	0.94	9.07e-22
Curium-243	5.797	0.138	0.134	0.01	0.0105	0.04	0.06	0.15	3.02e-18
Curium-244	5.795	0.009	0.002	0.63	0.79	0.94	0.94	0.94	6.74e-22
Europium-152		0.139	1.155	0.085	0.0123	0.03	0.05	0.12	3.75e-17
Europium-154		0.292	1.242	0.085	0.0123	0.03	0.05	0.12	4.11e-17
Europium-155		0.063	0.061	0.012	0.017	0.06	0.09	0.2	9.75e-19
Iodine-129		0.064	0.025	0.09	0.126	0.16	0.21	0.36	6.93e-20
Lead-212		0.176	0.148	0.01	0.011	0.04	0.06	0.15	3.62e-18
Lead-214		0.293	0.25	0.01	0.01	0.04	0.06	0.14	6.70e-18
Neptunium-237	4.769	0.07	0.035	0.027	0.04	0.11	0.15	0.29	4.16e-19
Plutonium-238	5.487	0.011	0.002	0.63	0.79	0.94	0.94	0.94	8.07e-22

Table 2. (continued)

Radionuclide	Average energy of decay[a]			Absorbed fractions (gamma)[b]					$DF_{gd0.15}$ (Sv m3/s Bq)[c]
	alpha	beta	gamma	A	B	C	D	E	
Plutonium-239	5.148	0.007							1.52e-21
Plutonium-239/240	5.148	0.007	0.002	0.63	0.79	0.94	0.94	0.94	1.52e-21
Plutonium-240	5.156	0.011	0.002	0.63	0.79	0.94	0.94	0.94	7.84e-22
Polonium-210		0.038	0.005	0.63	0.79	0.94	0.94	0.94	2.45e-22
Polonium-211	7.442		0.008	0.63	0.79	0.94	0.94	0.94	2.24e-19
Polonium-212	8.785								3.62e-18
Polonium-214	7.687								2.40e-21
Polonium-216	6.779								4.87e-22
Polonium-218	6.001								2.63e-22
Potassium-40		0.523	0.156	0.01	0.0115	0.04	0.06	0.14	4.57e-18
Protactinium-233		0.196	0.204	0.01	0.01	0.04	0.06	0.14	5.16e-18
Protactinium-234		0.494	1.919	0.085	0.0123	0.03	0.05	0.12	5.38e-17
Protactinium-234m		0.822	0.012	0.55	0.63	0.93	0.93	0.93	4.20e-19
Radium-223	5.667	0.076	0.134	0.01	0.0105	0.04	0.06	0.15	3.10e-18
Radium-224	5.674	0.002	0.01	0.63	0.79	0.29	0.35	0.52	2.62e-19
Radium-226	4.774	0.004	0.007	0.63	0.79	0.94	0.94	0.94	1.65e-19
Radium-228		0.017							0.00e+00
Radon-220	6.288								1.10e-20
Radon-222	5.489								1.14e-20
Sodium-22		0.194	2.193	0.085	0.0123	0.03	0.05	0.12	6.31e-17
Strontium-90		0.196							3.72e-21
Technetium-99		0.101							6.70e-22
Thallium-207		0.493	0.002	0.63	0.79	0.94	0.94	0.94	9.48e-20
Thallium-208		0.598	3.375	0.01	0.01	0.03	0.04	0.11	9.68e-17
Thorium-228	5.4	0.021	0.003	0.63	0.79	0.94	0.94	0.94	4.17e-20
Thorium-230	4.671	0.015	0.002	0.63	0.79	0.94	0.94	0.94	6.39e-21
Thorium-231		0.165	0.026	0.09	0.126	0.16	0.21	0.36	1.94e-19
Thorium-232	3.996	0.012	0.001	0.63	0.79	0.94	0.94	0.94	2.78e-21

Table 2. (continued)

Radionuclide	Average energy of decay[a]			Absorbed fractions (gamma)[b]					$DF_{grd\,0\,15}$ (Sv m3/s Bq)[c]
	alpha	beta	gamma	A	B	C	D	E	
Thorium-234		0.06	0.009	0.63	0.79	0.94	0.94	0.94	1.29e-19
Tin-126		0.172	0.057	0.012	0.017	0.06	0.09	0.2	7.90e-19
Tritium		0.006							0
Uranium-232	5.302	0.017	0.002	0.63	0.79	0.94	0.94	0.94	4.83e-21
Uranium-233	4.817	0.006	0.001	0.63	0.79	0.94	0.94	0.94	7.24e-21
Uranium-233/234	4.817	0.006	0.001	0.63	0.79	0.94	0.94	0.94	7.24e-21
Uranium-234	4.758	0.013	0.002	0.63	0.79	0.94	0.94	0.94	2.14e-21
Uranium-235	4.396	0.049	0.156	0.01	0.0115	0.04	0.06	0.14	3.75e-18
Uranium-235/236	4.396	0.049	0.156	0.01	0.0115	0.04	0.06	0.14	3.75e-18
Uranium-236	4.396	0.049	0.156	0.01	0.0115	0.04	0.06	0.14	3.75e-18
Uranium-238	4.187	0.01	0.001	0.63	0.79	0.94	0.94	0.94	5.52e-22
Yttrium-90		0.935							1.20e-19
Zirconium-89		0.101	1.165	0.085	0.0123	0.03	0.05	0.12	3.85e-17

[a] Values were obtained from ICRP (1983).

[b] Absorbed fractions for worms, plants, and mouse were derived from data in Blaylock et al. (1993).
Absorbed fraction for other receptors were derived following methodology of Cristy and Eckerman (1987).
Absorbed fractions for beta radiation were 100% for all radionuclides listed.
A = Plants and soil invertebrates. Derived from large insect values presented in Blaylock et al. (1993).
B = Small mammals and birds <<1 kg (e.g., pine vole). Derived from small fish values in Blaylock et al. (1993).
C = Small- to medium-sized mammals and birds (e.g., mink). Derived from values for ~0.76kg human infant after Cristy and Eckerman (1987).
D = Medium-sized mammals and birds (e.g., red fox). Derived from values for ~2.5kg 1-year old human after Cristy and Eckerman (1987).
E = Large mammals (e.g., white-tailed deer). Derived from values for ~28kg human after Cristy and Eckerman (1987).

[c] DF_{grd} is the dose coefficient for soil contaminated to a depth of 15 cm (Eckerman and Ryman 1993).

2.2.2 Internal Exposures: Ingestion

Wildlife receptors may receive internal radiation doses after ingesting contaminated prey, soil, or water or after inhaling contaminated dust. Blaylock et al. (1993) provide an equation for estimating the internal dose to fish contaminated with radionuclides. This equation can be modified to address consumers eating a variety of prey types, ingesting soil, and drinking water, as well as plants and invertebrates taking up contaminants directly from the soil

$$D_{ing} = \sum QF \quad C_{tissue} \quad \epsilon_i \quad CFa \quad AF \ , \tag{11}$$

where

D_{ing}	=	internal dose rate received after ingestion of contaminated prey and soil (mrad/d),
QF	=	quality factor to account for the greater biological effectiveness of α particles (20 for α; 1 for β and γ emissions; unitless),
C_{tissue}	=	activity (pCi/g) of radionuclide *i* in tissue of organism,
ϵ_i	=	energy for α, β, or γ emissions by nuclide *i* (MeV/nt),
CFa	=	conversion factor to go from MeV/nt to g mrad/pCi d (5.12×10^{-2}),
AF	=	absorption factor (unitless).

Radionuclide activity in tissue may be determined a number of ways, depending on data availability. Measured data should be used, if available. In the absence of measured data, soil-to-tissue uptake factors may be used. Uptake factors for selected radionuclides in plants, soil invertebrates, and small mammals are presented in Table 3; additional discussion of uptake factors is presented in Sect. 3.2.

Absorbed energy fractions for α radiations are assumed to equal one for all receptors. While absorption fractions for β radiations are assumed to be one for wildlife receptors, β absorption fractions for plants and earthworms are assumed to equal those for large insects from Blaylock et al. (1993) (assuming small reproductive parts of greatest concern). This is because β radiations are unlikely to have sufficient energy to pass through the wildlife tissues; however, some fraction may have sufficient energy to pass through smaller organisms such as earthworms and plants. Absorption fractions for γ radiations for plants and earthworms were also assumed to be equivalent to those for large insects presented in Blaylock et al. (1993). Absorption fractions for γ radiations derived for infant, 1-yr old, and adult humans using the methodology described in Cristy and Eckerman (1987) were used for wildlife receptors of similar sizes. Table 2 presents absorption factors used for several receptor-radionuclide combinations.

Energies (α, β, and γ) for selected radionuclides were obtained from Eckerman and Ryman (1993) and are provided in Table 2. Because different types of radiation differ in their relative biological effectiveness per unit of absorbed dose, a quality factor derived from data on humans is normally applied (NCRP 1987). The quality factor is determined by the linear energy transfer of radiation, and linear energy transfer for α particles is substantially higher than that for β or γ emissions. A quality factor of 1 should be used for β and γ radiation and 20 for α radiation (Blaylock et al. 1993).

Table 3. Radionuclide-specific soil-tissue uptake factors for plants, soil invertebrates, and small mammals and bioaccumulation factors for birds and mammals

Radionuclide	UF_{plants} [a]				UF_{invert} [a]	BAF_{bird} [b]	BAF_{mamm} [b]	UF_{mamm} [a]
	All plants	Grass	Herb. plants	Tree/shrubs				
228Ac	8.75e-04 c	8.75e-04 d	8.75e-04 d	8.75e-04 d	1.25e-03 e	1.25e-03 f	1.25e-03 c,g	
241Am						4.20e-03 g,j	2.00e-03 j	
212Bi	8.75e-03 c,g	8.75e-03 d	8.75e-03 d	8.75e-03 d	2.00e-02 e	2.00e-02 f	2.00e-02 c	
214Bi	8.75e-03 c,g	8.75e-03 d	8.75e-03 d	8.75e-03 d	2.00e-02 e	2.00e-02 f	2.00e-02 c	
45Ca						2.80e-02 g,j	1.00e-01 j	
244Cm						1.00e-03 f	1.00e-03 k	
57Co						1.40e+00 g,j	5.00e-03 g,j	
60Co						1.40e+00 g,j	5.00e-03 g,j	
134Cs	1.27e-03 l	1.27e-03 l	1.27e-03 l	1.27e-03 l		7.00e+00 g,j	2.56e+00 l	1.62e-02 l
137Cs	1.27e-03 d	1.27e-03 m	1.27e-03 d	1.27e-03 d		7.00e+00 g,j	2.56e+00 m	1.62e-02 m
152Eu	1.05e-02 d	1.05e-02 d	1.05e-02 g,n	1.05e-02 d	1.00e-01 e	1.00e-01 f	1.00e-01 k	
154Eu	1.05e-02 d	1.05e-02 d	1.05e-02 g,n	1.05e-02 d	1.00e-01 e	1.00e-01 f	1.00e-01 k	
155Eu	1.05e-02 d	1.05e-02 d	1.05e-02 g,n	1.05e-02 d	1.00e-01 e	1.00e-01 f	1.00e-01 k	
129I	3.40e-04 d	3.40e-04 g,j	3.40e-04 d	3.40e-04 d	2.00e+00 e	7.00e-03 g,j	2.00e+00 g,j	
40K						1.00e+00 f	1.00e+00 j	
22Na						4.00e+00 f	4.00e+00 j	
237Np	9.00e-03 d	9.00e-03 d	9.00e-03 o	9.00e-03 d	9.00e-03 e	3.84e-03 f	3.84e-03 g,n	
234mPa	6.25e-04 c,g	6.25e-04 d	6.25e-04 d	6.25e-04 d	5.00e-02 e	5.00e-02 f	5.00e-02 c	
210Pb						2.00e-02 f	2.00e-02 j	
212Pb						2.00e-02 f	2.00e-02 j	
214Pb						2.00e-02 f	2.00e-02 j	
238Pu	3.00e-04 l	6.00e-05 l	3.00e-04 l	6.00e-05 l		2.10e-03 g,j	5.00e-04 g,j	
239Pu	3.00e-04 d	6.00e-05 p	3.00e-04 p	6.00e-05 p	9.12e-03 q	2.10e-03 g,j	5.00e-04 g,j	
239/240Pu						2.10e-03 g,j	5.00e-04 g,j	
223Ra	7.50e-02 d	7.50e-02 d	7.50e-02 l,r	7.50e-02 d	7.50e-02 e	4.50e-02 f	4.50e-02 g,j	
224Ra	7.50e-02 d	7.50e-02 d	7.50e-02 l,r	7.50e-02 d	7.50e-02 e	4.50e-02 f	4.50e-02 g,j	
226Ra	7.50e-02 d	7.50e-02 d	7.50e-02 r	7.50e-02 d	7.50e-02 e	4.50e-02 f	4.50e-02 g,j	
228Ra	7.50e-02 d	7.50e-02 d	7.50e-02 l,r	7.50e-02 d	7.50e-02 e	4.50e-02 f	4.50e-02 g,j	
90Sr	4.95e-01 d	1.60e-01 s	4.95e-01 s	4.95e-01 d		5.60e-02 g,j	4.00e-01 g,j	
228Th	9.00e-04 d	4.00e-04 l,p	9.00e-04 g,r	9.00e-04 g,r	5.00e-03 e	5.00e-03 f	5.00e-03 k	3.20e-05 l

Table 3. (continued)

Radionuclide	UF_{plants} [a]				UF_{invert} [a]	BAF_{bird} [b]	BAF_{mamm} [b]	UF_{mamm} [a]
	All plants	Grass	Herb. plants	Tree/shrubs				
230Th	9.00e-04 d	4.00e-04 l,p	9.00e-04 g,r	9.00e-04 g,r	5.00e-03 e	5.00e-03 f	5.00e-03 k	3.20e-05 l
232Th	9.00e-04 d	4.00e-04 p	9.00e-04 g,r	9.00e-04 g,r	5.00e-03 e	5.00e-03 f	5.00e-03 k	3.20e-05 p
234Th	9.00e-04 d	4.00e-04 l,p	9.00e-04 g,r	9.00e-04 g,r	5.00e-03 e	5.00e-03 f	5.00e-03 k	3.20e-05 l
208Tl	1.00e-03 c,g	1.00e-03 d	1.00e-03 d	1.00e-03 d	2.00e+00 e	2.00e+00 f	2.00e+00 c	
232U	1.97e+00 d	9.00e-04 l	3.75e-03 l,r	1.97e+00 l,t		7.00e-01 g,j	1.50e-02 j	3.20e-04 l
233U	1.97e+00 d	9.00e-04 l	3.75e-03 l,r	1.97e+00 l,t		7.00e-01 g,j	1.50e-02 j	3.20e-04 p
234U	1.59e+00 d	9.00e-04 l	3.75e-03 l,r	1.59e+00 t		7.00e-01 g,j	1.50e-02 j	3.20e-04 l
235U	1.97e+00 d	9.00e-04 l	3.75e-03 l,r	1.97e+00 l,t		7.00e-01 g,j	1.50e-02 j	3.20e-04 l
235/236U	1.97e+00 d	9.00e-04 l	3.75e-03 l,r	1.97e+00 l,t		7.00e-01 g,j	1.50e-02 j	3.20e-04 l
238U	1.97e+00 d	9.00e-04 p	3.75e-03 r	1.97e+00 t		7.00e-01 g,j	1.50e-02 j	3.20e-04 p

[a] Soil-tissue uptake factors (UF) for plants, soil invertebrates, and small mammals were obtained from available literature. When necessary, values originally reported on a dry-weight basis were converted to a wet-weight basis based on tissue water content.

[b] Bird and mammal bioaccumulation factors (BAFs, ratio of tissue activity to activity in food) were obtained from available literature. Values originally reported as biotransfer factors (d/kg) were converted to BAFs by multiplying d/kg by the ingestion rate of the test species. When necessary, values originally reported on a dry-weight basis were converted to a wet-weight basis based on tissue water content.

[c] Baes et al. (1984).

[d] Assumed the same as other plant types.

[e] Uptake factor for earthworms was unavailable. Used the larger of the plant and mammal values.

[f] Assumed mammal BAF because of lack of bird-specific values.

[g] Elemental form of the analyte was used for isotope.

[j] IAEA (1994).

[k] NCRP (1989).

[l] Assumed uptake same as reported for other isotope of the radionuclide (i.e., ^{137}Cs values used for ^{134}Cs).

[m] Garten (1980a).

[n] Trabalka and Garten (1983).

[o] Garten et al. (1986).

[p] Garten et al. (1987).

[q] Garten and Dahlman (1978).

[r] Bondietti et al. (1979).

[s] Garten and Lomax (1987).

[t] Garten (1980b).

2.2.3 Internal Exposures: Inhalation

Wildlife species using burrows may receive an additional internal dose from inhalation of dust originating from contaminated soil. Intake of radionuclide *i* by inhalation is estimated as (DOE 1995b)

$$D_{inh} = QF \quad F_{below} \sum C_{soil,i} \quad A \quad \frac{1}{AD} \quad \epsilon_i \; CFa \quad AF \; , \tag{12}$$

where

D_{inh}	=	internal dose rate from inhalation of contaminated soil (mrad/d),
F_{below}	=	dose reduction factor for fraction of time receptor spends below ground (unitless),
A	=	mass of respirable dust per volume of air breathed (0.1 g/m^3; DOE 1995b),
AD	=	air density (1200 g/m^3; Eckerman and Ryman 1993),
ϵ_i	=	α, β, or γ radiation energies for radionuclide *i* (MeV),
CFa	=	conversion factor to go from MeV to mrad g/pCi/d (5.12 x 10^{-2}),
AF	=	absorption factor (unitless).

Healy (1980) suggests that 0.0001 g/m^3 would be a conservative value when addressing human exposures to dust. Because burrowing animals are likely to spend a greater portion of their time in a confined space (burrow) than humans and are physically closer to the soil surface, an air mass loading of 0.1 g/m^3 is suggested as a conservative estimate of the mass of respirable dust (A) to which these animals may be exposed.

Total internal exposures are obtained by adding ingestion and inhalation dose rates over all radionuclides, including all short-lived daughter products.

2.2.4 Effects Levels for Radionuclides

The discharge of radioactive waste into the environment results in long-term, low-dose exposure to organisms. In most cases, acute mortality can be discounted. Any potential increase in morbidity and mortality that might result from the exposure to chronic irradiation above background is unlikely to be detected because of natural fluctuations in the size of populations.

The International Atomic Energy Agency (IAEA) recommends limiting the dose for terrestrial organisms to 100 mrad/d (IAEA 1992). Studies evaluating reproductive success and survival were used to determine the dose limit. Species-specific effects data were not available, so 100 mrad/d was selected as the threshold dose for all representative wildlife receptors. A dose rate of this magnitude is unlikely to cause observable changes in terrestrial animal populations (IAEA 1992). Higher dose rates may result in impaired reproduction or reduced survivorship. A dose rate of 1 rad/d is generally considered protective of plant and invertebrate populations (IAEA 1992, Barnthouse 1995) based on studies of productivity and community characteristics. This dose rate or less is unlikely to cause observable changes in terrestrial plant populations (IAEA 1992). Higher dose rates may result in reduced productivity or changes in species composition within communities. Therefore, 1 rad/d was selected as the threshold dose for effects on plant and invertebrate populations. Invertebrates tend to be less radiosensitive than plants or vertebrates, and indirect responses to radiation-induced vegetation changes (e.g., habitat alteration) appear more critical than direct effects (e.g., mortality, etc.) from radiation (IAEA 1992).

2.2.5 Uncertainties in Radiological Risk Assessment

A number of areas of uncertainty exist in the estimation of exposure and risks to terrestrial biota from exposure to radionuclides. The methodology outlined above is likely to overestimate dose rates that endpoints may receive. Whereas some of the information needed to implement the methodology is well known, much is unknown or unspecified statistically. A conservative but reasonable approach to model assumptions and radiological exposure scenarios was adopted to avoid underestimating risks to biota. Specific uncertainties identified in the radionuclide models are listed below.

- It is assumed that uptake of radionuclides from soil, food, and water are similar. Radionuclides bound to soil may be less available than those in tissue or water. Many radionuclides are poorly absorbed from soils (e.g., ^{137}Cs bound to clay minerals). Therefore, assuming uptake from soil equal to uptake from food may result in a conservative estimate of actual uptake.

- The dose coefficients obtained from Eckerman and Ryman (1993) used to estimate dose rates from external exposures are developed for application in determining dose rates to humans. These dose coefficients were applied directly for wildlife receptors or adjusted based on the effective height of the receptors, but the actual dose coefficients for wildlife, given differences in size, behavior, and general morphology, may be greater or less than those developed for humans.

- The air mass loading factor of 0.1 g/m^3 used in estimating exposures from inhalation of radionuclide-contaminated dust was selected as a conservative value. Healy (1980) suggested that 0.0001 g/m^3 would be a conservative value for estimating human exposures from inhalation of dust.

- The conversion factor used in the model for below-ground exposures was derived for small volumes of tissue (e.g., a mouse or shrew) immersed in soil assumed to be contaminated to an infinite depth. The actual dose for large animals or in cases where only the first few centimeters of soil are contaminated may be higher or lower. The simplifying assumptions used in the models presented here are generally applicable, but a health physicist could be consulted to develop specific dosimetry models where a more detailed evaluation is desired.

- Absorption factors are not available for many terrestrial organisms. The approach used here was to apply values developed for similar-sized aquatic organisms (Blaylock et al. 1993) or humans (Cristy and Eckerman 1987) to wildlife species. Because size and geometry of wildlife species do not exactly match those of aquatic organisms or humans, actual absorption fractions for wildlife species may be higher or lower than those suggested here.

2.3 PROBABILISTIC EXPOSURE ESTIMATION

Contaminant exposure estimates for wildlife are frequently generated using single, conservative values (e.g., upper 95% confidence limits on the mean, maximum observed value) to represent parameters (e.g., contaminant concentration in soil, food, water, or air; ingestion rates; or diet composition) in the exposure model. These single parameter values, known as point estimates, are selected because they are believed to be protective of most individuals and their use simplifies the calculation of an exposure estimate. While the use of conservative assumptions is suitable in a screening-level assessment, the use of point estimates is not recommended in a baseline or definitive assessment. Employing point estimates for the input parameters in the exposure model does not take into account the variation and uncertainty associated with the

parameters. Contaminant exposure that endpoints may receive in any given area may therefore be either over or underestimated. Consequently, remediation may be recommended for areas where it is unnecessary, or significant risks may be overlooked. Calculation of the exposure model using point estimates also produces only a point estimate of exposure. This exposure estimate provides no information concerning the distribution of exposures or the likelihood that individuals within an area will actually experience potentially hazardous exposures. To incorporate the variation in exposure parameters and to provide a better estimate of the potential exposure experienced by wildlife, it is highly recommended that exposure modeling be performed using probabilistic methods such as Monte Carlo simulation.

A detailed discussion of Monte Carlo simulation is beyond the scope of this report. General discussion of Monte Carlo techniques are provided by Rubenstein (1981) and Law and Kelton (1982). Briefly, Monte Carlo simulation is a resampling technique frequently used in uncertainty analysis in risk assessment (Hammonds et al. 1994). In practice, distributions are assigned to input parameters in a model, and the model output is recalculated many times to produce a distribution of output parameters (e.g., estimates of contaminant exposure). Each time the model is recalculated, a value is selected from within the distribution assigned for each input parameter. As a result, a distribution of exposure estimates is produced that reflects the variability of the input parameters. To determine which input parameters most strongly influence the final exposure estimate, a sensitivity analysis may be performed (Hammonds et al. 1994). Detailed discussions of sensitivity and uncertainty analysis, and the use of Monte Carlo simulations in risk assessment, are provided by Hammonds et al. (1994) and EPA (1996). Burmaster and Anderson (1994) outline 14 principles of good practice for the use of Monte Carlo techniques in risk assessment. Initial guidance for the use and interpretation of Monte Carlo analysis in risk assessment have been developed by the EPA Risk Assessment Forum (EPA 1997) and EPA Region 8 (EPA Region 8 1995). Examples of the application of Monte Carlo techniques in wildlife exposure and risk assessment are presented in MacIntosh et al. (1994), Sample et al. (1996b), and Moore et al. (In Press). Finally, a special issue of the journal *Human and Ecological Risk Assessment* (Vol. 2, No. 4, 1996) has recently been published to commemorate the 50th anniversary of the development of Monte Carlo methods. This issue will contain multiple papers on the application and interpretation of Monte Carlo methods. Software for conducting Monte Carlo simulations include @Risk (Palisade Corporation, Newfield, New York) and Crystal Ball (Decisioneering, Inc., Denver, Colorado).

2.4 EXTRAPOLATION FROM INDIVIDUALS TO POPULATIONS

Exposure models used in a risk assessment must be appropriate for the assessment endpoints considered. The models presented in previous sections are for estimation of exposure of individual organisms, but except for threatened and endangered species, wildlife endpoints are generally considered at the population level (Suter et al. 1995). Because exposure estimates must be integrated with exposure-response information, which is expressed as organism-level responses, the use of these organism-level exposure models is appropriate.

The conversion of individual-level exposure estimates to population-level effects occurs in the risk characterization and can be made in several ways. First, it may be assumed that there is a distinct population on the site so that the exposure of the population is represented by the exposure of all of the individuals. All individuals at the site are assumed to experience equivalent exposure. This assumption is appropriate for small organisms, with limited home ranges, on large sites, particularly if the site constitutes a distinct habitat that is surrounded by inappropriate habitat. For example, a grassy site surrounded by forest or industrial development might support a distinct population of voles. The risks to that population can be estimated directly from the exposures of the individual organisms.

Another approach is to assume that a certain number of individuals are exposed to contaminants out of a larger population. The proportion of the local population exposed at levels that exceed toxic thresholds represents the proportion of the population potentially at risk. This was the logic underlying the preliminary assessment for wide-ranging wildlife on the Oak Ridge Reservation (ORR; Sample et al. 1996b). On the ORR, while most habitat for wide-ranging wildlife species exists outside of source operable units (OUs; contaminated areas), some suitable habitat is present within source OUs. The proportion of the ORR-wide population potentially at risk is represented by the number of individuals that may use habitat on source OUs. The degree to which a source OU is used (and therefore the risk that it may present) is dependent upon the availability of suitable habitat on the OU. An estimate of risks to reservation-wide populations was estimated as follows.

1. Individual-based contaminant exposure estimates are generated for each source OU using the generalized exposure model (Eq. 5). Contaminant data, averaged over the entire OU, were used in the exposure estimate.

2. Contaminant exposure estimates for each OU were compared to Lowest Observed Adverse Effects Levels (LOAELs) from Sample et al. (1996a) to determine the magnitude and nature of effects that may result from exposure at the OU. If the exposure estimate >LOAEL, then individuals at the OU may experience adverse effects.

3. Availability and distribution of habitat on the ORR and within each OU, suitable for each species considered, was determined using a satellite-generated landcover map for the ORR (Washington-Allen et al. 1995).

4. Habitat requirements for the endpoint species of interest are compared to the ORR habitat map to determine the area of suitable habitat on the ORR and within OUs.

5. The area of suitable habitat on the ORR and within OUs was multiplied by species-specific population density values (ORR-specific or obtained from the literature) to generate estimates of the ORR-wide population and the numbers of individuals expected to reside within each OU.

6. The number of individuals for a given endpoint species expected to be receiving exposures >LOAELs for each measured contaminant was totaled. This is performed using the OU-specific population estimate from step 5 and the results from step 2. This number is then compared with the ORR-wide population to determine the proportion of the ORR-wide population that is receiving hazardous exposures.

This approach provides a very simple estimate of population-level effects. It is biased because it does not take wildlife movement into account. Wide-ranging species may travel among and use multiple OUs, therefore receiving exposures greater than that estimated for a single OU. In addition, the proportion of reservation-wide population potentially at risk is limited by the proportion of suitable habitat present in source OUs. For example, if 5% of the suitable habitat for a given species is located within OUs, the proportion of the population potentially at risk cannot exceed 5%.

A third approach is to combine the results of Monte Carlo simulation of exposure with literature-derived population density data to evaluate the likelihood and magnitude of population-level effects

on wildlife. The number of individuals within a given area likely to experience exposures >LOAELs can be estimated using cumulative binomial probability functions (Dowdy and Wearden 1983). Binomial probability functions are estimated using the following equation

$$b(y;n;p) = \binom{n}{y} p^{y} (1-p)^{n-y} , \tag{13}$$

where

y	=	the number of individuals experiencing exposures >LOAEL,
n	=	total number of individuals within the watershed,
p	=	probability of experiencing an exposure in excess of the LOAEL,
b (y; n; p)	=	probability of y individuals out of a total of n, experiencing an exposure >LOAEL, given the probability that exceeding the LOAEL = p.

By solving Eq. 13 for y = 0 to y = n, a cumulative binomial probability distribution may be generated that can be used to estimate the number of individuals within an area that are likely to experience adverse effects. This approach was used to estimate the risks that PCBs and mercury in fish presented to the population of piscivores in watersheds on the ORR (Sample et al. 1996b). Monte Carlo simulations were performed to estimate watershed-wide exposures. It was assumed that wildlife were more likely to forage in areas where food is most abundant. Density or biomass of fish at or near locations where fish bioaccumulation data were collected were assumed to represent measures of food abundance. (Biomass data were preferred but were unavailable for all watersheds. Where unavailable, density data were used.) The relative proportion that each location contributed to overall watershed density or biomass data was used to weight the contribution to the watershed-level exposure. The watershed-level exposure was estimated to be the weighted average of the exposure at each location sampled within the watershed. In this way, locations with high fish densities or greater fish biomass contribute more to exposure than do locations with lower density or biomass. Because the watersheds were large enough to support multiple individuals, the weighted average exposure estimate was assumed to represent the exposure of all individuals in each watershed. While simplistic, this approach is believed to provide a better estimate of population-level effects than the previously described method. Use of this method, however, requires exposure data from multiple, spatially disjunct areas and data suitable to weight the potential exposure at each area.

Freshman and Menzie (1996) present an additional approach for extrapolating to population-level effects. Their Population Effects Foraging (PEF) model estimates the number of individuals within a local population that may be adversely affected. The PEF model is an individual-based model that allows animals to move randomly over a contaminated site. Movements are limited by species-specific foraging areas and habitat requirements. The model estimates exposures for a series of individuals, and then sums the number of individuals that receive exposures in excess of toxic thresholds (Freshman and Menzie 1996).

3. PARAMETERS FOR ESTIMATION OF EXPOSURE

Species-specific and contaminant-specific parameter values are required for implementation of any of the models outlined previously. This section summarizes methods for estimation of exposure parameters (e.g., inhalation rates and food, water, and soil ingestion rates) and contaminant uptake into selected wildlife food types. In addition, life history summaries for selected species of interest at DOE sites are presented.

3.1 ESTIMATING EXPOSURE PARAMETERS

Implementation of the exposure model presented in Eq. 4 requires the specification of certain parameters. Although some parameters such as body weight must be obtained from the literature for each endpoint species and others such as soil, water, or air contaminant concentrations and area contaminated are site-specific and must be measured, general methods are available for estimating food and water consumption rates, inhalation rates, and home range/territory size.

3.1.1 Body Weight

Body weight is an extremely important parameter in the estimation of exposure. Not only is it a factor in determining the exposure rate, but because metabolism and body weight are related, body weights may be used to predict food and water consumption rates. On a per individual basis, larger animals consume more food or water than do smaller animals. However, because larger animals have lower metabolic rates than smaller ones, smaller animals have higher food and water consumption rates per unit body weight. This means that smaller animals will experience greater oral exposure per unit body weight than will larger animals.

Body weights for selected terrestrial wildlife are reported in EPA (1993). Additional sources include: Dunning (1984, 1993), Burt and Grossenheider (1976), Silva and Downing (1995), the Mammalian Species series, published by the American Society of Mammalogists, and the Birds of North America series, published by the American Ornithologists Union and the Philadelphia Academy of Natural Sciences.

3.1.2 Estimation of Food and Water Consumption Rates

Field observations of food, water, or soil consumption rates are the best data to use to estimate exposure. With very few exceptions, these data are unavailable for most wildlife species. The second best data to use to estimate exposure are media consumption rates for wildlife species derived from laboratory studies. These data are limited because the influence of ambient conditions, such as activity regimes or environmental variables (temperature, humidity, etc.), on metabolism (and therefore consumption rates) are difficult to approximate in a laboratory setting.

In the absence of experimental data, food consumption values can be estimated from allometric regression models based on metabolic rate. Nagy (1987) derived equations to estimate food consumption (in kg dry weight) for various groups of birds and mammals

$$I_{fd} = (0.0687(BW)^{0.822})/BW \quad \text{Placental Mammals,} \tag{14}$$

$$I_{fd} = (0.0306(BW)^{0.564})/BW \quad \text{Rodents,} \tag{15}$$

$$I_{fd} = (0.0875(BW)^{0.727})/BW \quad \text{Herbivores,} \tag{16}$$

$$I_{fd} = (0.0514(BW)^{0.673})/BW \quad \text{Marsupials,} \tag{17}$$

$$I_{fd} = (0.0582(BW)^{0.651})/BW \quad \text{All Birds,} \tag{18}$$

and

$$I_{fd} = (0.0141(BW)^{0.850})/BW \quad \text{Passerine Birds,} \tag{19}$$

where

I_{fd} = food ingestion rate (kg food [dry weight]/ kg body weight/d),
BW = body weight (kg live weight).

Food ingestion rates estimated using these allometric equations are expressed as kilograms of dry weight. Because wildlife do not generally consume dry food (unless being maintained in the laboratory), food consumption must be converted to kilograms of fresh weight by adding the water content of the food. Percent water content of wildlife foods are listed in Table 4. Additional data may be obtained from the literature (e.g., Bell 1990, Redford and Dorea 1984, Odum 1993, and Holmes 1976). Calculation of food consumption in kilograms of fresh weight is performed as follows.

$$I_{ff} = \sum_{i=1}^{m} (P_i \; x \; \frac{I_{fd}}{1 - WC_i}) \; , \tag{20}$$

where

I_{ff} = total food ingestion rate (kg food [fresh weight]/kg body weight/d),
m = total number of food types in the diet,
P_i = proportion of the ith food type in the diet,
WC_i = percent water content (by weight) of the ith food type.

Water consumption rates can be estimated for mammals and birds from allometric regression models based on body weight (Calder and Braun 1983)

$$I_w = (0.099(BW)^{0.90})/BW \quad \text{Mammals,} \tag{21}$$

and

$$I_w = (0.059(BW)^{0.67})/BW \quad \text{Birds,} \tag{22}$$

where

I_w = water ingestion rate (L water/kg body weight /d),
BW = body weight (kg live weight).

Table 4. Percent water content of wildlife foods[a]

	Food type	Percent water content		
		Mean	STD	Range[b]
Aquatic invertebrates	Bivalves (w/o shell)	82	4.5	
	Crabs (w/shell)	74	6.1	
	Shrimp	78	3.3	
	Isopods, amphipods			71-80
	Cladocerans			79-87

Table 4. (continued)

	Food type	Percent water content		
		Mean	STD	Range[b]
Aquatic Vertebrates	Bony fishes	75	5.1	
	Pacific herring	68	3.9	
Aquatic plants	Algae	84	4.7	
	Aquatic macrophytes	87	3.1	
	Emergent vegetation			45-80
Terrestrial invertebrates	Earthworms (depurated)	84	1.7	
	Grasshoppers,crickets	69	5.6	
	Beetles (adult)	61	9.8	
Mammals	Mice, voles, rabbits	68	1.6	
Birds	Passerines (w/typical fat reserves)			68
	Mallard duck (flesh only)			67
Reptiles and amphibians	Snakes, lizards			66
	frogs, toads	85	4.7	
Terrestrial plants	Monocots: young grass			70-88
	Monocots: mature dry grass			7-10
	Dicots: leaves	85	3.5	
	Dicots: seeds	9.3	3.1	
	Fruit: pulp, skin	77	3.6	

[a] From EPA (1993).
[b] Single values indicate only one value available.

3.1.3 Estimation of Inhalation Rates

Similar to food and water ingestion, allometric equations, based on body mass, have also been developed to estimate inhalation rates of resting mammals (Stahl 1967) and nonpasserine birds (Lasiewski and Calder 1971)

$$I_a = (0.54576(BW)^{0.8})/BW \quad \text{Mammals,} \tag{23}$$

and

$$I_a = (0.40896(BW)^{0.77})/BW \quad \text{Non-passerine Birds.} \tag{24}$$

where

I_a = inhalation rate (m^3 air/kg body weight /d),
BW = body weight (kg live weight).

The applicability of Eq. 24 for estimating inhalation rates of passerines is not known. However, the similarity between the models for mammals and birds suggests that Eq. 24 is likely to be suitable for passerines.

3.1.4 Soil Consumption

In addition to consuming food and water, many wildlife consume soil. Soil consumption may occur inadvertently while foraging (i.e., predators of soil invertebrates ingesting soil adhering to worms, grazing herbivores consuming soil deposited on foliage or adhering to roots) or grooming, or purposefully to meet nutrient requirements. Diets of many herbivores are deficient in sodium and other trace nutrients (Robbins 1993). Ungulates, such as white-tailed deer (*Odocoileus virginianus*) have been observed to consume soils with elevated sodium levels, presumably to meet sodium needs (Weeks 1978). Because soils at waste sites may contain very high contaminant concentrations, direct ingestion of soil is potentially a very significant exposure pathway. In contrast to food and water consumption, generalized models do not exist with which to estimate soil ingestion by wildlife. Beyer et al. (1994) report soil consumption estimates for 28 wildlife species. Additional data concerning soil consumption are reported in Arthur and Alldredge (1979), Garten (1980c), Thornton and Abrahams (1983), Arthur and Gates (1988), and Calabrese and Stanek (1995).

3.1.5 Estimation of Home Range and Territory Size

Home ranges and territories represent the spatial areas occupied by wildlife. These areas provide each species with food, water, and shelter and may or may not be defended. Home range or territory size is a critical component in estimating exposure. Species with limited spatial requirements (e.g., small home ranges or territories) may live exclusively within the bounds of a contaminated site and therefore may experience high exposure. Conversely, species with large home ranges may travel among and receive exposure from multiple contaminated sites.

Multiple factors may influence home range or territory size. These factors include habitat quality, prey abundance, and population density. Methods have been developed to estimate home range size. McNab (1963) observed that home range size in mammals was a function of body weight

$$HR = 6.76\,(BW)^{0.63}, \tag{25}$$

where

HR = home range (acres),
BW = body weight (kg live weight).

Differences in home range requirements were observed between "hunters" (includes species that rely on widely distributed foods, e.g., granivores, frugivores, insectivores, and carnivores) and "croppers" (species that rely on foods that are spatially more concentrated, e.g., grazing and browsing herbivores; McNab 1963). Home ranges of "hunters" may be as much as 4 times greater than that of "croppers" of the same body mass. Home ranges for each group may be estimated using the following models

$$HR_h = 12.6\,(BW)^{0.71}, \tag{26}$$

and

$$HR_c = 3.02(BW)^{0.69}, \tag{27}$$

where

HR_h = home range for hunters (acres),
HR_c = home range for croppers (acres).

Note: 1 acre = 0.4047 ha = 4,047 m^2.

More recent research by Harestad and Bunnell (1979) produced the following relationships between body mass and home range in mammals:

$$HR_{herb} = 0.002\ (bw)^{1.02}, \tag{28}$$

$$HR_{omn} = 0.59(bw)^{0.92}, \tag{29}$$

and

$$HR_{carn} = 0.11(bw)^{1.36}, \tag{30}$$

where

HR_{herb} = home range for herbivores (ha),
HR_{omn} = home range for omnivores (ha),
HR_{carn} = home range for carnivores (ha),
bw = body weight (g).

A strong positive relationship also exists between body mass and territory or home range size among birds (Schoener 1968). Predators tend to have larger territories than omnivores or herbivores of the same weight. Territory size also increases more rapidly with body weight among predators than among omnivores or herbivores. Schoener (1968) believes these relationships reflect the higher density of available food for omnivores and herbivores. While Schoener (1968) developed regression models describing the relationship between body size, home range size, and foraging habits, all parameters needed to implement the models are not presented. A summary of home range or territory sizes for 77 species of land birds (and source references) are listed however.

3.2 ESTIMATION OF CONTAMINANT CONCENTRATIONS IN WILDLIFE FOODS

To estimate the magnitude of contaminant exposure that wildlife may experience, contaminant concentrations in food items preferred by endpoint species are needed. These data may be acquired either by direct measurement or estimation.

Direct measurement consists of the collection and analysis of contaminant concentrations in food items. Because direct measurement provides information on the actual contaminant loading in on-site biota, this approach contributes the least uncertainty to exposure estimates and is therefore the preferred approach. For various reasons however (biota phenology incompatible with sampling schedule; insufficient time, personnel, or finances to support field sampling, etc.), direct measurement of contaminant concentrations in biota may not be feasible. When direct measurement of contaminants in biota are not possible, estimation is the only alternative.

Contaminant loads in biota may be estimated using a variety of methods, ranging from mechanistic process models to simple, empirical uptake factors. While mechanistic models for estimation of contaminant concentrations in biota may give more accurate estimates than uptake factors, they generally require considerable information, much of which may not be available in a risk assessment context. Examples of complex contaminant uptake models for plants and fish are presented in Lindstrom et al. (1991) and Thomann and Connolly (1984), respectively. Because of their data requirements, complex models are generally taxa- and location-specific and may not be widely applicable.

The simplest model for estimation of contaminant loads in biota is uptake factors. Uptake factors consist of ratios of the concentration of a given contaminant in biota to that in soil. (The model assumes that exposure to the food item is primarily from contaminants in soil.) In practice, if the contaminant concentration in soil is known (which is likely in almost all ecological risk assessments), the concentration in biota may be estimated by multiplying the soil concentration by the uptake factor. Because contaminant uptake is influenced by characteristics of the organism and by the properties of the contaminant, separate uptake factors are recommended for each contaminant and taxonomic group being considered. Bioavailability of contaminants for uptake can also be influenced by soil conditions. For example, Corp and Morgan (1991) observed that while high amounts of soil organic matter reduced the bioavailability of lead to earthworms, low soil pH increased bioavailability.

The use of uptake factors depends on the assumption that the concentration of chemicals in organisms is a linear, no-threshold function of concentrations in soil. It will not be the case if the chemical in question is well regulated by the organism, either because it is an essential nutrient or because it is a toxicant with effective inducible mechanisms for metabolism or excretion. Such well-regulated chemicals will (at least within the effective concentration range for the mechanism) have nearly constant concentrations, regardless of soil concentrations. Various complex patterns are also possible because of lack of induction at low concentrations, saturation kinetics at high concentrations, toxicity at high concentrations, or other processes. Despite chemical behavior that suggests that alternative models would be more appropriate, uptake factors are commonly used in risk assessments.

In this section, we briefly review methods and models for estimating contaminant concentrations in earthworms and plants. In addition, uptake factors and regression models based on literature-derived data are presented for selected analytes in earthworms and plants. Additional uptake factors and regression models based on literature-derived data for small mammals and sediment-associated invertebrates are presented in Sample et al. (1997a) and Jones et al. (1997), respectively.

3.2.1 Earthworms

Earthworms are considered to be representative of soil invertebrates or terrestrial detritivores in many ecological risk assessments. This is in part because of their importance. Earthworms can constitute a large fraction of the biomass of soil invertebrates, they are important in the formation of soils in temperate environments, and they are a significant fraction of the diet of some vertebrates. In addition, earthworms appear to be more highly exposed to soil contaminants than other soil and litter invertebrates (Davis and French 1969; Ma 1994). Finally, uptake of chemicals from soil by earthworms has been much better studied than uptake by other soil invertebrates but is still much less studied than accumulation by aquatic invertebrates or vertebrates. Although there is some information available on the kinetics of earthworm uptake (Belfroid et al. 1994b), all available operational models are based on equilibrium partitioning with soil or soil pore water. Given the slow kinetics of soil transformation and transport processes relative to air and water, equilibrium is a reasonable assumption.

Soil/Worm Model

If paired soil and earthworm concentrations are available from the site of concern or the literature, a basic soil/worm equilibrium partitioning model may be used.

$$C_v = K_{sv} C_s, \tag{31}$$

where

C_v = concentration in worms (vermes)(mg/kg),
K_{sv} = worm/soil partitioning coefficient (kg soil/kg worm),
C_s = concentration in surface soil (mg/kg).

Values of K_{sv} (equivalent to the uptake factors described previously) are available in the literature for some chemicals (Table 5) but may be highly variable because of soil properties and the form of the contaminant. Steady state may be assumed for field studies but should be demonstrated for laboratory studies. Steady state was reached for a variety of organochlorine chemicals in 10 days (Belfroid et al. 1995). When site-specific values or literature values from a similar soil and contaminant form are available, this is the preferred model.

When site-specific data are collected to derive K_{sv} values, it is important to ensure the quality and relevance of the data. In particular, it is important that the soil and worms be collected from the same location and that the soil be from the surface layer (the A horizon, tilled layer, or equivalent) where the worms would have been exposed. Also, it is important to depurate (i.e, void their gut contents) the worms for three reasons. First, soil ingestion by vermivorous wildlife is accounted for separately in the exposure model, so use of undepurated worms would reduce the accuracy of the model. Second, the mass of ingested material is variable, so it introduces extraneous variance in the K_{sv} estimate. Finally, the bias introduced by the gut contents is not consistent. If the chemical is bioaccumulated by worms to concentrations greater than in soil (i.e., $K_{sv} > 1$), the C_v is underestimated, but if concentrations are greater in soil than worms, C_v is overestimated.

Table 5. Summary of sources of soil-earthworm uptake factors (K_{sv}) and uptake models

Study Location	Analytes with K_{sv} values	Analytes with Models	Reference
Pennsylvania, USA	Cd, Cu, Pb, Ni, and Zn	Cd	Beyer et al. 1982
Maryland, USA		Pb, Cu, Cd, and Se	Beyer et al. 1987
Finland	Al, Cd, Cu, Fe, Hg, Mn, V, and Zn		Braunschweiler 1995
Wales, Great Britain	Pb	Ca, Cd, Cu, Pb, and Zn	Corp and Morgan 1991
Warsaw, Poland	Cd, Cu, Pb, Zn		Czarnowska and Jopkiewicz 1978
Germany	Cd, Pb, and Zn		Emmerling et al. 1997
Denmark		Se	Nielsen and Gissel-Nielsen 1975
Netherlands	Cd, Cu, Mn, Ni, Pb, and Zn		Hendriks et al. 1995
Netherlands	Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn	Pb and Zn	Ma 1982
Netherlands		Cd, Cu, Pb, and Zn	Ma et al. 1983
Seveso, Italy	TCDD		Martinucci et al. 1983

Table 5. (continued)

Study Location	Analytes with K_{sv} values	Analytes with Models	Reference
Models fit to data from multiple locations.		Cd, Cu, Ni, Pb, and Zn	Neuhauser et al. 1995
Montana, USA	As, Cd, Cu, and Zn		Pascoe et al. 1996
Illinois, USA	Cd, Cr, Cu, Ni, and Pb		Pietz et al. 1984
Reading, Great Britain	Cd, Cu, Pb, and Zn	Cd, Cu, Pb, and Zn	Spurgeon and Hopkin 1996
Tennessee, USA	Cd, Pb, Zn		Van Hook 1974

[a] TCDD = 2,3,7,8 Tetrachloro Dibenzo-p-dioxin

Soil/Water/Worm Model

It has been proposed that invertebrates are in equilibrium with the aqueous phase of soil. This is consistent with the soil/worm model if the solid, aqueous, and biotic phases of the soil are all in equilibrium. This approach has been used for sediments by the EPA and others (DiToro et al. 1991). It is well supported for sediment invertebrates and is supported by some evidence for earthworms and possibly other soil invertebrates (van Gestel and Ma 1988; Connell 1990; Lokke 1994). However, it has been suggested that the model may underestimate accumulation of a few chemicals for which the dietary route is dominant (Belfroid et al. 1994a). In addition to potentially making extrapolations between soils more accurate than soil/worm partitioning, it has the advantages of making available for use the large literature on water/biota partitioning factors (bioconcentration factors) and the numerous QSARs for water/biota partitioning. However, it adds the burden of estimating soil pore water concentrations. The conventional formula is

$$C_w = C_s / K_d \,, \tag{32}$$

where

K_d = the soil (or sediment)/water partitioning coefficient (L/kg sediment),
C_w = water concentration (mg/L).

Values of K_d are available from the literature for many metals and some organics but are highly variable (Baes et al. 1984). If literature K_d values are used, this model is not expected to be more accurate than Eq. 31, but K_d values are available for some chemicals for which K_{sv} is not.

For nonionic organic compounds

$$K_d = f_{oc} K_{oc} \,, \tag{33}$$

or

$$K_d = f_{om} K_{om} \,, \tag{34}$$

where

f_{oc} = fraction organic carbon in the soil (unitless),
K_{oc} = water/soil organic carbon partitioning coefficient (kg/kg or L/kg),
f_{om} = fraction organic matter in the soil (unitless),
K_{om} = water/soil organic matter partitioning coefficient (kg/kg or L/kg),

This formula adjusts for the organic content (expressed as either organic matter or organic carbon content), which is the major source of variance among soils in the uptake of neutral organic chemicals. This normalization makes this model more accurate than Eq. 31 for neutral organic chemicals. For ionic organic chemicals, Van Gestel et al. (1991) recommend correcting the coefficient (K_{oc} or K_{om}) by dividing by the fraction nondissociated (f_{nd}), which is estimated from

$$f_{nd} = 1/(1+ 10^{pH-pKa}), \tag{35}$$

where

pK_a = the negative log of the dissociation constant.

When K_{oc} and K_{om} are both unavailable, they may be estimated from QSARs. The model used by the EPA was developed from sediments (DiToro et al. 1991)

$$\log_{10}(K_{oc}) = 0.983 \log_{10}(K_{ow}) + 0.00028, \tag{36}$$

where

K_{ow} = octanol/water partitioning coefficient (unitless).

Van Gestel et al. (1991) provide a formula for K_{om} that is based on soils rather than sediments:

$$\log_{10}(K_{om}) = 0.89 \log_{10}(K_{ow}) - 0.32 . \tag{37}$$

Values for K_{ow} are available in the literature for most organic chemicals, or they can be calculated from QSARs. K_{ow}s for selected chemicals are presented in Table 6 .

From these formulas, C_v can be calculated as

$$C_v = K_{bw} C_w , \tag{38}$$

where

K_{bw} = biota/water partitioning coefficient (L/kg organism).

K_{bw} values for chemicals in earthworms may be assumed equivalent to bioconcentration factors for aquatic invertebrates from the literature. Alternatively, QSARs can be used to estimate this factor. The model developed by Connell and Markwell (1990) for uptake by earthworms of 32 "lipophilic" organic chemicals ($\log K_{ow}$ 1.0-6.5) is

$$\log K_{bw} = \log K_{ow} - 0.6 \ (n = 60, r = 0.91). \tag{39}$$

It has been suggested that for lipophilic compounds, earthworm accumulation should also be a function of lipid content of the worms (Connell and Markwell 1990). This is not a component of the standard sediment model and makes no contribution to predictive accuracy in practice because the site-specific lipid content of worms is unknown in nearly all cases and would vary in an unquantified manner seasonally and among species. However, based on a study of marine sediment oligochaetes (Markwell et al. 1989), Menzie et al. (1992) recommend a model for earthworms in soil that contains soil organic content and worm lipid content but not K_{ow} or any other property of the chemical

$$K_{sv} = L\,(0.66 f_{oc})^{-1}, \tag{40}$$

where

L = proportion lipid in worms (unitless).

L was estimated by Menzie et al. (1992) to be 0.02, but Connell and Markwell (1990) used 0.0084 for theoretical calculations.

This model predicts that all chemicals have equal concentrations in earthworms at a site, which was not far from true for the contaminants of concern at the site where it was applied. There, the mean bioconcentration factors for four DDT residues and total chlordane ranged from 0.10 to 0.35, and the estimated mean bioconcentration factor for all chemicals was 0.25. This model is not recommended because the addition of L adds nothing without information that is seldom available (lipid content of test organisms) and because the deletion of K_{ow} is not well justified. It is discussed here because it has been widely adopted in the United States for estimating earthworm concentrations.

Finally, Connell (1990) proposed an extremely reduced formula

$$K_{sv} = 0.44(K_{ow})^{0.05}. \tag{41}$$

This model shows worm concentrations to be a weak function of K_{ow} but not of any soil or worm property. It would be appropriate only if the site soils were similar to the test soils used in the study from which this formula was derived (Lord et al. 1980).

Table 6. Octanol-water partition coefficients for selected chemicals

Chemical and form	Log K_{ow}	Source
Acetone	-0.24	EPA 1995
Aldrin	6.5	EPA 1995
Aroclor 1016	5.6	ATSDR 1989
Aroclor 1242	5.6	ATSDR 1989
Aroclor 1248	6.2	ATSDR 1989
Aroclor 1254	6.5	ATSDR 1989
Benzene	2.13	EPA 1995
beta-BHC	3.81	EPA 1995
BHC-mixed isomers	5.89	EPA 1995
Benzo(a)pyrene	6.11	EPA 1995
Bis(2-ethylhexyl)phthalate	7.3	EPA 1995
Carbon tetrachloride	2.73	EPA 1995
Chlordane	6.32	EPA 1995
Chlordecone (kepone)	5.3	EPA 1995
Chloroform	1.92	EPA 1995
o-Cresol	1.99	EPA 1995
DDT and metabolites	6.53	EPA 1995
1,2-Dichloroethane	1.47	EPA 1995

Table 6. (continued)

Chemical and form	Log K_{ow}	Source
1,1-Dichloroethylene	2.13	EPA 1995
1,2-Dichloroethylene	1.86	EPA 1995
Dieldrin	5.37	EPA 1995
Diethylphthalate	2.5	EPA 1995
Di-n-butyl phthalate	4.61	EPA 1995
1,4-Dioxane	-0.39	EPA 1995
Endosulfan	4.1	EPA 1995
Endrin	5.06	EPA 1995
Ethanol	-0.31	EPA 1992
Ethyl acetate	0.69	EPA 1995
Formaldehyde	-0.05	EPA 1995
Heptachlor	6.26	EPA 1995
Lindane (gamma-BHC)	3.73	EPA 1995
Methanol	-0.71	EPA 1995
Methoxychlor	5.08	EPA 1995
Methylene chloride	1.25	EPA 1995
Methyl ethyl ketone	0.28	EPA 1995
4-Methyl 2-pentanone	1.19	EPA 1992
Pentachloro-nitrobenzene	4.64	EPA 1995
Pentachlorophenol	5.09	EPA 1995
2,3,7,8-Tetrachloro-dibenzodioxin	6.53	EPA 1995
1,1,2,2-Tetrachloro-ethylene	2.67	EPA 1995
Toluene	2.75	EPA 1995
Toxaphene	5.5	EPA 1995
1,1,1-Trichloroethane	2.48	EPA 1995
Trichloroethylene	2.71	EPA 1995
Vinyl chloride	1.5	EPA 1995
Xylene (mixed isomers)	3.2	EPA 1995

All earthworm concentration values in these models are on a fresh weight basis for depurated worms. However, earthworm concentrations in the literature may be reported as fresh or dry weights. Water content of earthworms are reported to range from 82 to 84% (EPA 1993). Concentrations may also be reported for undepurated worms, but there is no basis for correcting those values because of the variability in mass of ingested material.

Soil-Earthworm Uptake Factors

Empirical soil-earthworm uptake factors (K_{sv}) and uptake models have been developed from field data for selected chemicals, primarily metals (Table 5). Most of these studies report uptake from a limited number of locations or represent only a small range of soil concentrations. To best evaluate the relationship between concentrations of contaminants in soil and those in earthworms, a broad range of soil concentrations is needed.

To determine how contaminant uptake varied across locations, contaminant levels, and soil conditions, a literature search was performed for studies that reported chemical concentrations in co-located earthworm and soil samples. Data were obtained for eleven chemicals: arsenic, cadmium, chromium, copper, mercury, lead, manganese, nickel, zinc, PCBs, and TCDD. To ensure relevancy of the data to field situations, only field studies in which resident earthworms were collected were considered. All earthworm tissue burdens were therefore

assumed to be at equilibrium with soil concentrations. Because soil residues in the earthworm gastrointestinal (GI) tract may be highly variable and therefore may significantly bias body burden measurements, only depurated earthworms were included. Samples in which the GI tract had been dissected or manually flushed were also considered suitable. To ensure comparability of data, only 'total' chemical analyses of both soil and earthworms (e.g., resulting from extractions using concentrated acids) were included. Data resulting from DTPA, acetic acid, and other mild extraction methods were excluded. The mean (or composite) soil and earthworm value reported for each sampling location evaluated in each study was considered an observation. If data for multiple earthworm species were reported at a site, each was considered a separate observation. Soil and earthworm data in the database were reported as mg/kg dry weight. If studies reported earthworms in terms of wet weight concentrations, dry weight concentrations were estimated assuming a 84% water content (EPA 1993). Summaries of the analytical methods and data presented for each study included in the database are presented in Appendix A. Summary statistics were calculated for K_{sv} for each chemical (Table 7). To facilitate the use of the UFs in probabilistic risk evaluations, the distribution of the UFs for each analyte was evaluated using a distribution-fitting program (BestFit; Palisade Corp. 1994a). The data were fit to normal and lognormal distributions. Goodness of fit was determined using Kolmogorov-Smirnov tests.

To evaluate if there was a linear relationship between the contaminant concentration in soil and that in earthworms, simple regressions were performed using SAS PROC REG (SAS Inst. Inc. 1988). Contaminant concentrations in both soil and earthworms were natural-log transformed prior to regression analyses. Because data concerning the number of individuals included in composites or means were not available for all observations, no weighting of observations was applied. Simple linear regression models of ln-earthworm concentration on ln-soil concentration were developed for each analyte (Table 8). Plots of the cumulative frequency distributions of the K_{sv} values and scatterplots of soil concentration versus earthworm concentration are presented for each chemical in Figs. 1-11.

With the exception of As and Ni, the distribution of all UFs was best described by the lognormal distribution (Table 7); As and Ni were best fit by a normal distribution. Median UFs for 6 chemicals (As, Cr, Cu, Mn, Ni, and Pb) were <1, indicating no biomagnification (Table 7). Median UFs>1 were observed for the remaining 5 chemicals (Cd, Hg, Zn, PCB, and TCDD; Table 7). [Note: the mean and standard deviation of the natural-log-transformed UFs are presented as parameters for describing the UF distributions for those analytes best fit by a lognormal distribution. While the untransformed UFs are best fit by a lognormal distribution, the natural-log-transformed UFs are normally distributed. These parameters may be used in two ways. They may be applied to normal distribution functions in Monte Carlo simulation software; however the output from the sampling from this distribution must be back-transformed (e.g., e^y, where y=sampling result). Alternatively, they may be incorporated into the LOGNORM2 function in the @RISK Monte Carlo simulation software (Palisade Corp. 1994b). Use of the LOGNORM2 function requires no back-transformation. Comparable results are obtained using either approach]

Regression of ln earthworm on ln soil produced significant model fits for all chemicals except Cr (Table 8). With the exception of Ni, slopes of all regression models were positive (Table 6; Figs. 1a through 11a). Intercepts differed significantly from 0 for all chemicals except Hg, Mn, and Pb (Table 8). r^2 values for the significant models ranged from 0.22 (Cu) to 0.94 (TCDD; Table 8).

Except for chromium, either K_{sv} or regression models could be used to estimate chemical concentrations in earthworm tissues. In the case of chromium, because the regression was not significant, the model should not be used; K_{sv} should be used instead. Because uptake tends to decrease at higher soil concentrations (Fisher and Koszorus 1992), regression models may give more accurate results than K_{sv} values. Comparison of the accuracy and precision of the K_{sv} values and regression models, using independent data, is presented in Sample et al. (1997).

It should be noted that K_{sv} and regression models estimate the tissue concentration in earthworms in mg/kg of dry weight. These values must be converted to mg/kg of wet weight before they are employed in exposure estimation

$$C_{wet} = C_{dry} * P_{dry}, \tag{42}$$

where

C_{wet} = wet weight concentration,
C_{dry} = dry weight concentration,
P_{wet} = proportion dry matter content of worm or other tissue.

3.2.2 Plants

Uptake of contaminants by plants is often dependent on the concentration in soil. In general, uptake increases with soil concentration until the contaminant becomes toxic to the plant (McBride 1995). Instances of apparent saturation have been observed however. For example, the cadmium content in foliage of American sycamore increases with soil concentration until it reaches 50 mg/kg (Carlson and Bazzaz 1977). Contaminants that are also nutrients may be regulated by plants such that uptake varies little relative to soil concentration. Nutrients and chemicals that mimic them are often taken up by active processes, rather than in transpiration water. The various forms of particular metals (e.g., chromium and mercury) complicates the estimation of uptake. Some investigators have observed that the uptake of monovalent cations follows Michaelis-Menten kinetics (Baker 1983), but general or specific models for the uptake of metals by plants are not well developed. Estimation of uptake of metals and other inorganics from soil by plants is generally performed using uptake factors (K_{sp} = plant/soil partitioning coefficient; kg soil/kg plant).

Models for the uptake of organic chemicals by plants are more common, probably because plant physiology plays a greater role in determining uptake of inorganic contaminants. Also, interest in herbicides and in predicting the uptake of pesticides has contributed to research on organic chemical uptake. These models range from the simple ranking of potential for uptake, based on the octanol-water partition coefficient (Scheunert et al. 1994) to the transport of water through xylem and phloem of a single or three-leafed plant, as determined by compartment volumes, cell wall thicknesses, diffusion, and partition coefficients of cell membranes (Boersma et al. 1988, 1991). Fugacity-dependent models include those of Trapp et al. (1990) and Paterson et al. (1994).

Several simple models have been developed to estimate concentrations of organic contaminants in plant tissues. Briggs et al. (1983) studied the uptake of contaminants by plants roots. They observed the following relationship

$$C_r = BCF_r * C_{sw}, \tag{43}$$

and

$$\log BCF_r = 0.77(\log K_{ow}) - 1.52, \tag{44}$$

where

C_r = concentration of chemical in roots (mg/kg fresh wt.),
BCF_r = bioconcentration factor for roots(unitless),
C_{sw} = concentration of chemical in soil water (mg/L).

In similar work with barley, Topp et al. (1986) developed the following model

$$\log BCF_r = 0.63(\log K_{ow}) - 0.959. \tag{45}$$

Table 7. Summary statistics for literature-derived soil-to-biota uptake factors (K_{sv} and K_{sp})

Taxa	Analyte	N	Mean	Standard Deviation	Minimum	Median	90th Percentile	Maximum	Mean of Natural Log-transformed values	Standard Deviation of Natural Log-transformed values	Distribution
Earthworms	As	36	0.2656	0.2116	0.0164	0.2361	0.5214	0.9250			normal
	Cd	114	27.1682	37.5895	0.4286	14.2603	66.0377	190.0000	2.58768	1.28036	lognormal
	Cr	48	0.7080	1.1496	0.0212	0.1607	2.7000	5.3680	-1.48636	1.5555	lognormal
	Cu	103	0.9283	0.9135	0.0130	0.6364	2.2807	4.8890	-0.57464	1.14691	lognormal
	Hg	15	8.5537	11.0986	0.0488	3.9334	30.0000	33.0000	1.16596	1.77202	lognormal
	Mn	16	0.0742	0.0551	0.0249	0.0605	0.1646	0.2280	-2.80288	0.62809	lognormal
	Ni	17	0.9200	0.7418	0.0333	0.7778	1.8881	2.8330			normal
	Pb	119	6.3297	26.7336	0.0007	0.2250	4.3243	228.2610	-1.10093	2.05196	lognormal
	Zn	123	8.2364	11.0731	0.0247	3.7816	25.0000	49.5100	1.03218	1.83458	lognormal
	PCB	16	14.1790	14.4186	4.3333	10.6667	23.4945	65.2270	2.40307	0.64066	lognormal
	TCDD	19	11.7409	9.8083	1.1905	11.0108	22.2290	42.0678	2.1132	0.8918	lognormal
Plants	As	110	0.5529	1.4515	0.000056	0.09791	1.2176	9.074	-2.80737	2.60632	lognormal[a]
	Cd	289	2.0147	3.6572	0.015928	0.9	4.6	35.944	-0.09243	1.29423	lognormal[a]
	Pb	204	0.3413	0.9959	0.000113	0.10235	0.615	10.601	-2.27508	1.5376	lognormal
	Ni	163	0.7235	2.4507	0.000632	0.03827	1.6667	22.214	-2.8878	2.1832	lognormal[a]
	Se	237	20.5818	75.8523	0.033376	1.83973	26.3	627	0.72426	1.91585	lognormal[a]

[a] Data not fit well by either normal or lognormal distributions, however, closest fit provided by lognormal.

Table 8. Results of regression analyses on literature-derived soil-biota uptake data

Taxa	Analyte	N	B0±SE	B1±SE	r^2	P model fit
Earthworms	As	36	-1.747±0.3542***	0.9884±0.1804***	0.47	0.0001
	Cd	114	2.8216±0.0766***	0.5512±0.03343***	0.71	0.0001
	Cr	48	2.3957±0.653***	-0.146±0.1863^{NS}	0.01	0.44
	Cu	103	1.8059±0.1528***	0.2414±0.04503***	0.22	0.0001
	Hg	15	0.0781±0.2594^{NS}	0.3369±0.0915**	0.51	0.0028
	Mn	16	-0.043±1.3719^{NS}	0.5759±0.2096*	0.35	0.016
	Ni	17	7.033±0.9409***	-1.548±0.3097***	0.62	0.0002
	Pb	119	0.0752±0.4153^{NS}	0.7612±0.07586***	0.46	0.0001
	Zn	123	5.0981±0.1384***	0.2373±0.0239***	0.45	0.0001
	PCB	16	1.7903±0.2358***	1.2909±0.09404***	0.93	0.0001
	TCDD	19	3.533±0.810***	1.182±0.074***	0.94	0.0001
Plants	As	110	-1.915±0.556***	0.673±0.183***	0.11	0.0004
	Cd	289	0.040±0.078^{NS}	0.849±0.030***	0.74	0.0001
	Pb	204	-1.625±0.364***	0.864±0.073***	0.41	0.0001
	Ni	163	-1.663±0.463***	0.754±0.087***	0.32	0.0001
	Se	237	0.518±0.163**	1.136±0.070***	0.53	0.0001

model: ln(y)=B0+B1(ln[x]), where y = concentration in biota (mg/kg dry wt.), x = concentration in soil (mg/kg dry wt.).
^{NS} Not Significant: p>0.05.
* p<0.05.
** p<0.01.
*** p<0.001.

Travis and Arms (1988) observed that the bioconcentration factor for aboveground foliage for nonpolar organic contaminants was inversely proportional to the log K_{ow}

$$\log BCF_f = 1.588 - 0.578(\log K_{ow}), \tag{46}$$

where

BCF_f = bioconcentration factor for aboveground vegetation (unitless).

Topp et al. (1986) found that bioconcentration factors for organic contaminants in total plants (roots plus foliage) were best described by the molecular weight of the chemical

$$\log BCF_t = 5.943 - 2.385(\log MW), \tag{47}$$

where

BCF_t = bioconcentration factor for total plant (root plus aboveground vegetation; unitless).
MW = molecular weight of chemical (g/mol).

Additional models for the estimation of contaminant uptake by plants by other pathways (e.g., rainsplash, [illegible] uptake of vapor-phase or particle-bound contaminants) are summarized in Paterson et al. (1990), McK[illegible] (1993), and Hope (1995).

With the exception of Eq. 46, all plant BCF models presented here estimate chemical concentrat[illegible] terms of wet weight. If Eq. 46 is used, dry weight concentrations may be converted to wet weight using [illegible] and water content data obtained from Table 4 or the literature.

In contrast to earthworms, while there have been numerous field and laboratory studies of the uptake of contaminants by plants, few empirical models or K_{sp} values for plants are reported in the literature. A report by Baes et al. (1984) provides point estimates of K_{sp} for all inorganic elements.

To determine how contaminant uptake by plants varied with contaminant levels, an analysis similar to that performed for earthworms and summarized above was performed for plants (Efroymson et al. 1997). Literature was reviewed for five chemicals: arsenic , cadmium, lead, nickel, and and selenium (summary of each paper reviewed is presented in Appendix B). Soil and plant contaminant concentration data were extracted from each paper. Data points represented different locations and plant species. Within studies, replicates were averaged. Experimental treatments in which secondary soil contaminants, aerial contaminants, or other additions were made were not included in the determination of K_{sp}. Studies in which concentrations of contaminants in soil were determined by a partial extraction with diethylene triamine pentaacetic acid (DTPA) or very weak acids or water were excluded from analysis. Although concentrations of DTPA-extracted contaminants from soils sometimes correlate with those taken up by plants (Sadiq 1985), this estimate of bioavailability has been observed not to work for some metals (Sadiq 1985, 1986) or for soils of varying pH (Miles and Parker 1979). Also, studies in which concentrations of analytes in soil or plants were estimated visually from a figure were used only if estimates could be made within about 10%. Studies were included in the analysis even if no correlation between concentrations of contaminants in soils and plants was observed in the study.

K_{sp} values were calculated for each paired soil-plant observation. Summary statistics were calculated for the K_{sp} for each chemical (Table 7). Results of the regression analyses are presented in Table 8. Plots of the cumulative frequency distributions of K_{sp} and scatterplots of soil concentration vs plant concentration are presented for each chemical in Figs. 12 - 16. The distribution of K_{sp} for lead was best described by the lognormal distribution (Table 7). The distributions for the other four analytes, while differing significantly from both the normal and lognormal distribution, was best fit by the lognormal distribution. With the exception of selenium, median K_{sp} for all chemicals was <1 (Table 7). Significant regressions with increasing trends were found for all analytes (Table 8). Additional regression models that incorporate soil pH are presented in Efroymson et al. (1997).

3.3 LIFE HISTORY PARAMETERS FOR SELECTED SPECIES

To estimate contaminant exposure by terrestrial wildlife using the models described above, species-specific values for the parameters are needed. Because of large within-species variation in values for life-history parameters, data specific to the site in question provides the most accurate exposure estimates and should be used whenever available. Because availability of site-specific life history data is extremely limited, published values from other areas within an endpoint species range must generally be used to estimate exposure.

Life history parameters that determine contaminant exposure have been outlined for eight mammals and five birds. These species were selected because they are likely to occur at DOE facilities (species occurrence will vary according to location of site however) and are considered to be potential endpoints at selected DOE facilities. To avoid repetition, it was decided to focus on species other than those reported in the "Wildlife Exposure Factors Handbook" (EPA 1993), which presents life history data for 15 birds, 11 mammals, and 8 reptiles or amphibians (Table 9). Summaries of life history parameters for selected wildlife species on the ORR are presented in Sample and Suter (1994). Other sources of life history summaries include the Mammalian Species series (published by the American Society of Mammologists) and the Birds of North America series (published by the American Ornithologists Union and the Philadelphia Academy of Natural Sciences). The Mammalian Species series currently addresses over 300 mammal species, while Birds of North America series addresses 240. Additional information on the Birds of North American may be obtained from the Internet: http://www.acnatsci.org bna

Table 9. Summary of species presented in the "Wildlife Exposure Factors Handbook" (EPA 1993)

Birds	Mammals	Reptiles or amphibians
Great Blue Heron	Short-tailed Shrew	Snapping Turtle
Canada Goose	Red Fox	Painted Turtle
Mallard Duck	Raccoon	Eastern Box Turtle
Lesser Scaup	Mink	Racer
Osprey	River Otter	Northern Water Snake
Red-Tailed Hawk	Harbor Seal	Eastern Newt
Bald Eagle	Deer Mouse	Green Frog
American Kestrel	Prairie Vole	Bullfrog
Northern Bobwhite Quail	Meadow Vole	
American Woodcock	Muskrat	
Spotted Sandpiper	Eastern Cottontail Rabbit	
Herring Gull		
Belted Kingfisher		
Marsh Wren		
American Robin		

3.3.1 Little Brown Bat (*Myotis lucifugus*)

Little brown bats are in the order Chiroptera, family Vespertilionidae. The genus *Myotis* includes approximately 80 species; *M. lucifugus* includes six subspecies (Fenton and Barclay 1980). As with most vespertilionids, the little brown bat is strictly insectivorous (Vaughan 1978).

Distribution

The little brown bat is one of the most abundant bats throughout the northern United States and Canada (Harvey 1992). It is widely distributed throughout North America. Its range extends from east to west coasts and from the mountains of northern Mexico to Alaska (Burt and Grossenheider 1976; Fenton and Barclay 1980).

Body Size and Weight

Female little brown bats are somewhat larger than males (Fenton and Barclay 1980). Reported body mass may range from 3.1 to 12 g (Silva and Downing 1995) but averages 7 to 9 g (Burt and Grossenheider 1976). Body weight varies throughout the year, remaining relatively constant from March through August then increasing dramatically in September through October, prior to hibernation (LaVal et al. 1980). Body weights

for little brown bats from several locations are presented in Table 10. Additional data on body weights are reported in Silva and Downing (1995).

Table 10. Body weights (g) for the little brown bat, *Myotis lucifugus*

Location	Sex	N	Mean	Range	Comments	Reference
Massachusetts	not stated	4	7.5±1.1[a]			Gould 1955
New Mexico	Female (ad)[b]	5	8.47±0.81	7.25-9.43	Collected 19	Ewing et al.
	Male (ad)	3	6.96±0.27	6.57-7.20	Aug.; data also	1970
	Female (yy)[c]	4	6.78±0.21	6.61-7.14	presented for 1	
	Male (yy)	2	5.74±0.06	5.69-5.80	and 15 Sept.	
Alberta, Canada	not stated		10.3	7.4-11.6		Silva and Downing 1995
Indiana	Male		6.15	3.1-10		Silva and Downing 1995
	Female		6.15	3.2-14.4		
Indiana	Male	6	6.03			Stones and Wieber 1965
	Female: nonpregnant, nonlactating	40	6.99			
	Female: pregnant	6	10.27			
	Female: lactating	13	7.77			

[a] mean±standard deviation.
[b] adult.
[c] young of year.

Food Habits and Diet Composition

Little brown bats are strict insectivores, detecting insects using ultrasonic calls (Fenton and Barclay 1980). Although insects are generally captured in flight, some may be taken from the surface of water or vegetation (Fenton and Bell 1979). Foraging is opportunistic; little brown bats have been observed to exploit insect swarms attracted to artificial lights (Fenton and Morris 1976) or large insect hatches (Vaughan 1980). While the diet composition may be highly variable, aquatic insects (e.g., Chironomidae and Trichoptera) are the primary food in most areas studied (Table 11; Fenton and Barclay 1980; Anthony and Kunz 1977; LaVal et al. 1980). However, in Alaska the diet consisted primarily (71.1% by volume) of small moths (Whitaker and Lawhead 1992). Insects consumed generally range from 3 to 10 mm in size (Anthony and Kunz 1977). Additional data concerning diet preferences of little brown bats may be found in Barclay (1991), Belwood and Fenton (1976), Kunz and Whitaker (1983), and Whitaker et al. (1981).

Table 11. Diet composition of little brown bats

Location	Prey Taxon	Percent volume	Percent frequency	Reference
Western Oregon (n=67)	Chironomidae	38.4	62.7	Whitaker et al. 1977
	Unidentified Diptera	10.4	28.4	
	Tipulidae	2.4	7.5	
	Culicidae	0.4	1.5	
	Dipterous larvae	0.1	1.5	
	Insect internal organs	10.6	11.9	
	Isoptera	8.9	13.4	
	Trichoptera	8.4	10.4	
	Unidentified insects	6.3	26.9	
	Unidentified Lepidoptera	3.7	10.4	
	Lepidopterous larvae	1.4	1.5	
	Formicidae	2.3	6.0	
	Unidentified Hymenoptera	0.4	1.5	
	Scarabidae	1.5	1.5	
	Unidentified Coleoptera	0.4	3.0	
	Unidentified Hemiptera	1.5	3.0	
	Cercopidae	1.0	1.5	
	Cicadellidae	0.4	3.0	
	Unidentified Homoptera	0.4	1.5	
	Tettigonidae	0.5	1.5	
	Gryllidae	0.1	1.5	
	Hemerobiidae	0.4	1.5	
Nova Scotia adults (n=28)	Coleoptera	7.7		Belwood and Fenton 1976
	Trichoptera	34.6		
	Chironomidae	58.8		
	Other insects	3.8		
Nova Scotia subadults (n=27)	Coleoptera	9.4		Belwood and Fenton 1976
	Trichoptera	26.6		
	Lepidoptera	15.9		
	Neuroptera	11.6		
	Chironomidae	19.5		
	Other Diptera	7.7		
	Other insects	9.2		
Watertown, New York; adults (n=12)	Coleoptera	1.2		Belwood and Fenton 1976
	Trichoptera	18.2		
	Lepidoptera	4.2		
	Chironomidae	76.4		
Watertown, New York; subadults (n=12)	Coleoptera	6.6		Belwood and Fenton 1976
	Trichoptera	29.6		
	Lepidoptera	19.9		
	Neuroptera	3.5		
	Chironomidae	35.5		
	Other insects	4.9		
Western Maryland (n=33)	Coleoptera		63.6	Griffith and Gates 1985
	Diptera		54.5	
	Hemiptera		3.0	
	Homoptera		36.4	
	Hymenoptera		39.4	
	Lepidoptera		60.6	
	Neuroptera		24.2	
	Psocoptera		15.2	
	Trichoptera		15.2	

Table 11. (continued)

Location	Prey Taxon	Percent volume	Percent frequency	Reference
New Hampshire (n=62)	Chironomidae		85.5	Anthony and Kunz 1977
	Lepidoptera		85.5	
	Culicidae		77.4	
	Tipulidae		67.7	
(Paper provides additional breakdown by sex, date, and age)	Coleoptera		59.7	
	Mycetophilidae		54.8	
	Ephemeroptera		51.6	
	Hymenoptera		33.9	
	Trichoptera		32.3	
	Neuroptera		19.4	
Indiana (n=16)	Unidentified Lepidoptera	21.6	31.3	Whitaker 1972
	Unidentified Trichoptera	13.1	25.0	
	Unidentified Diptera	11.9	31.3	
	Cicadellidae	11.6	43.8	
	Delphacidae	8.8	25.0	
	Coleopterous larvae	6.3	6.3	
	Ichneumonidae	3.8	12.5	
	Carabidae	3.4	18.8	
	Reduviidae	2.8	12.5	
	Scarabidae	2.5	6.3	
	Unidentified Coleoptera	2.2	18.8	
	Tipulidae	1.9	12.5	
	Hemerobiidae	1.9	6.3	
	Chironomidae	1.6	12.5	
	Cerambycidae	1.6	6.3	
	Formicidae	1.3	12.5	
	Chrysomelidae	0.9	6.3	
	Chrysomelidae, *Diabrotica sp.*	0.9	6.3	
	Nitidulidae	0.9	6.3	
	Miridae	0.6	6.3	
	Gryllidae	0.3	6.3	
	Unidentified insects	0.3	6.3	

Food Consumption Rate

Little brown bats maintained in captivity (at 92°F) and fed mealworms consumed 1 to 4 g food/d, with the greatest consumption observed for pregnant and lactating females (Stones and Wiebers 1965). Food consumption was also greater in summer as opposed to winter. Coutts et al. (1973) observed an average food consumption rate of 0.15 g/g/d for three males and six postlactating females. Feeding rates for bats in the field are likely to be higher. For example, Gould (1955) reports food consumption rates for four "more successful" bats to be 7.7±2.6 g/g/h (mean±STD). If 3.5 h/d are spent foraging (Anthony and Kunz 1977), this would translate to a daily consumption rate of 1.12±0.37 g/g/d. This is consistent with Barclay et al. (1991) who suggest that bats may consume their body weight in food per night to meet metabolic needs. Anthony and Kunz (1977) reported daily food consumption rates in New Hampshire to be 2.4±1.1 g/d (mean±STD), 3.7±0.5 g/d, and 1.8±0.5 g/d, for pregnant, lactating, and juvenile little brown bats, respectively. Assuming body weights reported in Table 10, these observations translate to 0.23±0.11g/g/d, 0.48±0.06 g/g/d, and 0.29±0.07 g/g/d

Water Consumption Rate

A single little brown bat maintained in the laboratory was observed to consume 0.86 mL of water per day (O'Farrell et al. 1971). The average weight of this individual over the course of the study was [illegible] Therefore the daily water consumption was 0.11 L/kg/d. In another laboratory study, average water consumption

of male and female little brown bats maintained in the laboratory was observed to be 0.18 L/kg/d (Coutts et al. 1973). Kurta et al. (1989) estimated the drinking water consumption rate of free-ranging pregnant and lactating bats to be 0.177 L/kg/d and 0.205 L/kg/d, respectively. These observations are comparable to water ingestion estimated using Eq. 21. Assuming a body weight of 7.5 g, water ingestion by little brown bats is estimated to average 0.16 L/kg BW/d. (Note: If other body weight values are used, the water ingestion rate should be recalculated.)

Soil Ingestion Rate

No published data were found concerning soil ingestion by little brown bats. As an aerial insectivore, however, soil ingestion is assumed to be negligible.

Respiration Rate

No literature data were found describing inhalation by little brown bats. Using Eq. 23 and assuming a body weight of 7.5 g, the average inhalation rate of little brown bats is estimated to be 1.45 m^3/kg BW/d. If other body weight values are used, the inhalation rate should be recalculated.

Metabolism

Energy utilization by little brown bats is highly efficient. Of 4.15±0.67 kcal/d ingested, only 0.37±0.1 kcal/d was excreted, representing an energy utilization of 91.2±1.5% (O'Farrell et al. 1971). Metabolic rates for little brown bats have been reported to range from 1.47 mL O_2/g BW/h (O'Farrell and Studier 1970) to 2.89±0.89 mL O_2/g BW/h (Altman and Dittmer 1974). Little brown bats enter hibernation September-May in northern portions of their range and November-March in southern areas (Fenton and Barclay 1980).

Habitat Requirements

Little brown bats use three distinct types of roosts: day, night, and hibernation. Day and night roosts are used by active bats in spring, summer, and fall, while hibernation roosts (hibernacula) are used during winter (Fenton and Barclay 1980). Day roosts generally consist of dark or dimly lit locations (buildings, hollow trees, under bark, occasionally in caves) with the appropriate humidity and temperature to mitigate daytime water loss (Fenton and Barclay 1980). Night roosts are occupied after the initial feeding bout of the evening. They may be located in the same building as day roosts but in different locations. Night roosts are generally confined spaces into which the bats pack themselves, possibly for improved thermoregulation (Fenton and Barclay 1980). Hibernacula generally consist of caves or abandoned mines and are used throughout the bat's range. (Harvey et al. 1991) High humidity (>90%) and temperatures above freezing characterize most hibernacula (Fenton and Barclay 1980).

Little brown bats forage primarily in open habitat, frequently over bodies of water (Fenton and Bell 1979; Barclay 1991; Saunders and Barclay 1992). Areas with dense vegetation or other obstructions to flight are avoided (Barclay 1991; Saunders and Barclay 1992). In Missouri, foraging along forest edges has been observed (LaVal et al. 1977).

Home Range

Although no information was found in the literature concerning the home range of little brown bats, the gray bat, a congeneric species, may travel as far as 12 km from roost caves to foraging sites (LaVal et al. 1977).

Population Density

No data were found documenting population density values. Populations may be limited by the availability of roost sites but not by food (Fenton and Barclay 1980). In summer, females form maternity colonies

of hundreds to thousands of individuals (Harvey 1992). Location of males in summer is not well known; it is suspected that they are solitary and scattered in a variety of roost types (Harvey 1992).

Population Dynamics/Survival

Population age structures and survival rates for little brown bats are poorly defined (Fenton and Barclay 1980). While individuals up to 30 years old have been reported (Keen and Hitchcock 1980) and 10-year-old bats are not uncommon, longevity is generally 1.5 years for males and 1.17 to 2.15 years for females (Fenton and Barclay 1980). Annual survival rates in Ontario were estimated to be 0.816 and 0.708 for males and females, respectively (Keen and Hitchcock 1980). Cockrum (1956) presents additional data on longevity.

Reproduction and Breeding

Fertilization occurs in spring, after females leave hibernation. The gestation period is 50-60 days. Only one young is produced per year (Fenton and Barclay 1980). Growth is rapid; young bats can thermoregulate by day 9.5 and are flying in three weeks. Buchler (1980) observed first flights of juveniles at 19-20 days of age. A detailed study of reproduction, growth, and development was performed by O'Farrell and Studier (1973).

Behavior

In New Hampshire, Anthony and Kunz (1977) observed bimodal foraging activity; the first feeding period was before midnight (2200-2400 h) while the second was before dawn (0330-0500 h). In contrast, Saunders and Barclay (1992) found activity was greatest within one hour of sunset.

3.3.2 Great Basin Pocket Mouse (*Perognathus parvus*)

Pocket mice are in the order Rodentia, family Heteromyidae. Pocket mice are the smallest members of this family that includes kangaroo mice and kangaroo rats. A key characteristic of the family is fur-lined cheek pouches (Burt and Grossenheider 1976). Members of this family are all adapted to arid conditions, many, including pocket mice, do not require drinking water (Vaughan 1978; Burt and Grossenheider 1976). *P. parvus* is a semifossorial granivorous species of arid or semiarid habitats (Verts and Kirkland 1988).

Distribution

Pocket mice (*Perognathus spp.*) are found only in western North America, west of the Mississippi river. *P. parvus* occurs throughout the Great Basin region, from southern British Columbia to northern Arizona (Burt and Grossenheider 1976; Verts and Kirkland 1988).

Body Size and Weight

Pocket mice are approximately the size of the house mouse (*Mus musculus*) with longer tails and smaller ears (Scheffer 1938). Males are slightly larger than females; total lengths of males and females from Utah were 174 and 172 mm, respectively (Verts and Kirkland 1988). Tail length is 110 to 120% of body length. Body weights for male and female pocket mice from several locations are presented in Table 12. O'Farrell (1975a) observed that body weights of males increase with increasing elevation.

Table 12. Body weights (g) for the Great Basin pocket mouse, *Perognathus parvus*

Location	Sex	N	Mean	Minimum	Maximum	Reference
Washington	Male	10	17.25			Scheffer 1938
	Female	10	14.3			

Table 12. (continued)

Location	Sex	N	Mean	Minimum	Maximum	Reference
Nevada	Male	10	25.4	21.5	31.0	Verts and Kirkland 1988
	Female	10	20.5	16.5	28.5	
Washington	Male: 500 ft	18	17.4±0.3[a]			O'Farrell et al. 1975
	Male: 1500 ft	12	18.3±0.3[a]			
	Male: 2500 ft	11	17.6±0.4[a]			
	Male: 3500 ft	12	19.1±0.5[a]			
Washington	Male	12	17.66±1.32[b]	15.52	19.62	Schreiber 1978
	Female	12	15.82±1.34[b]	13.16	17.47	

[a] mean±standard error.
[b] mean±standard deviation.

Food Habits and Diet Composition

Although the diet of *Perognathus parvus* consists primarily of seeds (Scheffer 1938; Martin et al. 1951; Kritzman 1974), insects may be consumed in spring, before seeds become available (Kritzman 1974; O'Farrell et al. 1975). When grass seeds were ripe, they represented 88% of the seeds in cheek pouches of mice in eastern Washington (Kritzman 1974). Food preferences of pocket mice from several locations are listed in Table 13.

Table 13. Diet composition of pocket mice

Location	Foods consumed (%)	Comments	Reference
California	Poison ivy (10-25) Filaree (10-25) Deervetch (10-25) Ryegrass (2-5) Oats (2-5) Nightshade (2-5) Bitterbrush (2-5) Saltbrush, knotweed (1/2-2)	Data are for pocket mice in general. Scientific names not reported. Values in parentheses refer to percentage use as reported by the authors. Data from spring, fall, and winter only.	Martin et al. 1951
Western Prairies and Mt-Deserts	Mesquite (10-25) Locoweed (5-10) Creosote (5-10) Beeplant (5-10) Pigweed (5-10) Cedar (5-10) Fescuegrass (2-5) Saltbush (2-5) Pricklypear (2-5) Bromegrass (2-5) Morning-glory (2-5) Bristlegrass (2-5) Sunflower (2-5) Plantain (2-5) Deervetch (2-5) Barley (2-5) Russianthistle (2-5) Nightshade, knotweed, sagebrush (1/2-2)	Data are for pocket mice in general. Values in parentheses refer to percentage use as reported by the authors. Data from throughout year.	Martin et al. 1951

Table 13. (continued)

Location	Foods consumed (%)	Comments	Reference
Eastern Washington	*Amsinckia* seeds (2.5) *Cryptantha* seeds (0.5) *Salsola* seeds (6.3) *Aster* seeds (0.4) *Franseria* seeds (0.1) *Descurania* pods (3.3) *Agropyron* seeds (5.5) *Bromus* seeds (45.6) *Festuca* seeds (20.0) *Gilia* seeds (0.8) *Microsteris* (11.5) Root nodules (0.3) Stem and leaf pieces (2.9) Insect larvae (0.2)	Contents of cheek pouches from 52 *P. parvus* collected May-October 1969. Data presented as frequency of occurrence over all samples.	Kritzman 1974

Food Consumption Rate

Schreiber (1978) estimates the daily energy requirements for male and female *P. parvus* in Washington in winter to be 2.36 and 2.63 kcal, respectively. In contrast, energy requirements in spring are 6.96 and 6.55 kcal for adult males and females, respectively. Based on estimated daily maintenance energy requirements and caloric content of cheatgrass seeds, Schreiber (1978) estimated the daily food consumption rate. Mean (±STD) ingestion for 8 individuals (4 male, 4 female) was 0.076±0.023 g/g/d. Females consumed somewhat more food/g than males (females: 0.079±0.026 g/g/d; males: 0.073±0.020 g/g/d).

Water Consumption Rate

Pocket mice generally do not require water other than that contained in their food (Scheffer 1938, Kritzman 1974, Vert and Kirkland 1988). Schmidt-Nielson et al. (1948) studied water conservation in desert rodents, including *Perognathus baileyi*. Mice survived well and gained weight when maintained for up to six weeks on a dry diet with no drinking water. In contrast, white rats and woodrats (*Neotoma*) maintained under similar conditions lost weight and had all died by 21 and 9 days, respectively (Schmidt-Nielson et al. 1948). Water balance is maintained by excreting concentrated urine, obtaining water from food and water generated through metabolism (Vert and Kirkland 1988); consequently drinking water is not required.

Soil Ingestion Rate

Data concerning soil ingestion by *P. parvus* was not located in the literature. Beyer et al. (1994) report soil ingestion by burrowing rodents (woodchucks and prairie dogs) to range from <2 to 7.7% of their diet. As a burrowing rodent, soil ingestion by *P. parvus* is likely to be comparable to these values.

Respiration Rate

No literature data were found describing inhalation by *P. parvus*. Using Eq. 23 and assuming a body weight of 18 g for males and 16 g for females (Table 12), the average inhalation rate is estimated to be 1.22 m^3/kg BW/d for males and 1.25 m^3/kg BW/d for females. If other body weight values are used, the inhalation rate should be recalculated.

Metabolism

The bioenergetics of *P. parvus* was studied by Schreiber (1978). Annual energy intakes for males and females was estimated to be 2550 kcal/y and 2462 kcal/y, respectively. Summer torpor reduces energy

demand by 3%. In winter, the reduction was 40-43% lower than summer, because of more extensive torpor. Metabolic rates for active, resting, nesting, and torpid *P. parvus* are related to ambient temperature and may be estimated as follows:

$$M_{active} = 11.5-0.24T_a, \quad (48)$$
$$M_{resting} = 8.6-0.24T_a, \quad (49)$$
$$M_{nest} = 7.0-0.165T_a, \quad (50)$$

and

$$M_{torpor} = 0.38+0.014T_a, \quad (51)$$

where

M_{active} = metabolic rate for active individuals (mL O_2/g/h),
M_{resing} = metabolic rate for resting individuals (mL O_2/g/h),
M_{nest} = metabolic rate for individuals in nests (mL O_2/g/h),
M_{torpor} = metabolic rate for torpid individuals (mL O_2/g/h),
T_a = ambient temperature (°C).

Schreiber (1978) also presents models for estimating annual energy expenditure.

Habitat Requirements

P. parvus prefers arid to semiarid environments that are predominantly sandy and dominated by sagebrush (Verts and Kirkland 1988). O'Farrell (1975b) describes the habitat requirements in Washington to be shrub-steppe with light-textured soils. Abundance of *P. parvus* is greater at sites with abundant seed-producing annuals and lower in perennial grasslands or locations where springtime soil temperatures <40°F are extensive (O'Farrell 1975a). While *P. parvus* were captured at all elevations on the Hanford Reservation from 500-3500 ft., 37% of all individuals were collected at lower elevations (e.g., 500 ft.; O'Farrell 1975a).

Home Range

The home range of male *P. parvus* in Washington ranged from 0.156 to 0.4 ha, while those for females ranged from 0.05 to 0.23 ha (O'Farrell et al. 1975). Home range size is inversely related to population density. In southern British Columbia, home ranges range from 0.066 to 0.09 ha (Schreiber 1978). In related species, Blair (1953) reports home ranges of male and female *P. merriami* to be 1.88 and 5.87 acres (0.76 and 2.4 ha), respectively. Average home ranges of male *P. penicillatus* in New Mexico were 2.72±0.48 acres (1.1±0.2 ha), with a maximum of 5.54 acres (2.24 ha). In contrast, average home range of females was 1.09±0.14 acres (0.44±0.06 ha), with a maximum of 1.43 acres (0.58 ha; Blair 1953).

Population Density

Average peak autumn population density in Washington was 118.5 individuals/ha, but ranged from a high of 162 to a low of 76.3 (O'Farrell et al. 1975). Annual average population densities of 28.5/ha (peak of 42/ha) and 82.3/ha have been reported for southeast Washington and the Yakima Valley, respectively (Verts and Kirkland 1988). Schreiber (1978) suggests that at high densities, *P. parvus* may become food stressed. He estimates the maximum sustainable density to be 39-83 individuals/ha.

Population Dynamics/Survival

One, two, and three-year survival rates of *P. parvus* in Washington are reported to be 56-80%, [illegible] 19%, and 2-3%, respectively (O'Farrell et al. 1975). The highest winter survival was observed among juveniles born when precipitation, food supply, and reproduction was lowest. Summer population size [illegible]

highly correlated to October-April precipitation (O'Farrell et al. 1975). This rainfall stimulates growth and reproduction in vegetation and consequently affects small mammal numbers.

Reproduction and Breeding

Under favorable conditions, *P. parvus* generally have two litters per female per year; only one during poor years (Kritzman 1974). Duration of the breeding season varies from four months (April-July) to six months (March-August depending on elevation (i.e., shorter at higher elevations; O'Farrell 1975). Scheffer (1938) suggests that the gestation period is 21 to 28 days. Litter sizes average approximately five (Scheffer 1938; Duke 1957) and may range from two to eight (Scheffer 1938, Speth et al. 1968). Males become sexually active in spring (before May) and remain active through August (Speth et al. 1968). O'Farrell et al. (1975) observed the first signs of estrus in females in April, first pregnancies in May, and last pregnancies in August.

Behavior

P. parvus is semifossorial, spending a considerable amount of time underground. Burrows, approximately 25 mm in diameter, ending in a ball-shaped chamber, are constructed 13-30 cm below the soil surface (Scheffer 1938). Burrows may extend as deep as 1 m (Verts and Kirkland 1988). While *P. parvus* is generally nocturnal or crepuscular, individuals may be active during the day (Scheffer 1938). Activity is suppressed by inclement weather.

Social Organization

P. parvus is not considered social, individuals occupy separate nests in the wild (Scheffer 1938, Verts and Kirkland 1988). Conspecifics housed together will fight initially but later tolerate each other (Scheffer 1938). In contrast, *P. parvus* attacks other rodent species it may be housed with (Verts and Kirkland 1988).

3.3.3 Pine Vole (*Microtus pinetorum*)

Pine voles are in the order Rodentia, family Cricetidae. Related species include the meadow vole (*M. pennsylvanicus*) and prairie vole (*M. ochrogaster*). The pine vole is a semifossorial herbivore of wooded habitats (Burt and Grossenheider 1976).

Distribution

The pine vole occurs throughout much of the eastern United States. Its range extends from the Atlantic coast to eastern Texas, north to Wisconsin, southern Ontario, and southern New England (Burt and Grossenheider 1976; Smolen 1981; Johnson and Johnson 1982).

Body Size and Weight

The body form of the meadow vole is cylindrical and slender with reduced eyes, ears, and tail, consistent with a semifossorial lifestyle (Smolen 1981). The body length of adults averages approximately 120 mm (Smolen 1981). Female pine voles are generally slightly larger than males (Table 14; Smolen 1981). Body weights of pine voles from several locations are listed in Table 14.

Table 14. Body weights (g) for the pine vole, *Microtus pinetorum*

Location	Sex	N	Mean	Range	Reference
Virginia	Male	11	25.4±1.5	23.4-28.2	Cengel et al. 1978[a]
	Female: nonpregnant	11	24.8±1.8	21.6-27.9	
New York and New Jersey	Adults: sex not differentiated	25	25.6	22-37	Benton 1955
Vermont	Adults: sex not differentiated	4	26.1	20.6-30.3	Miller 1964
Connecticut	Adults	18	23.9	20.5-29.0	Miller and Getz 1969
	Sub-adults	10	19.0	16.0-21.0	
	Juveniles	4	13.5	12.0-14.5	
Louisiana	Adults	2	25.6	25.2-26.0	Lowery 1974
Indiana	Female		27.2	22.7-33.8	Silva and Downing 1995
	Male		25.5	23.3-29.5	
Georgia	Male	17	24.2	14.5-28.6	Smolen 1981
	Female	6	27.4	23.1-30.8	

[a]Values represent mean and range of means from 11 separate observations

Food Habits and Diet Composition

Pine voles are primarily herbivores; however, snails (Martin et al. 1951) or beetles (Benton 1955) may be consumed. Hamilton (1938) reports that pine voles feed largely on succulent roots and tubers. In New York and New Jersey, the diet of pine voles consists of bulbs, tubers, roots, seeds, fruit, bark, and leaves (Benton 1955). Diet varies by season: grass roots and stems are eaten in summer, fruit and seeds in fall, and bark, roots, and stored foods in winter (Benton 1955). Pine voles may be a serious pest in orchards, eating the bark and roots of fruit trees (Johnson and Johnson 1982, Swihart 1990). Lists of species of plants consumed are presented in Smolen (1981) and Martin et al. (1951). A summary of food habitats of pine voles in North Carolina and Virginia is presented in Table 15.

Table 15. Diet composition of pine voles

Location	Date	Food type	Percent volume	Percent frequency	Comments	Reference
North Carolina (n=11)		*Endogone* (fungus)	0.4	54.5		Linzey and Linzey 1973
		Unidentified vegetation	78.5	100		
		Fruit	0.2	9.1		
		Unidentified seeds	20.6	36.4		
		Hair	T	36.4		
		Pebbles	0.3	36.4		

Table 15. (continued)

Location	Date	Food type	Percent volume	Percent frequency	Comments	Reference
Virginia (n=5/date and location)	July -M	Grass	20		Values extrapolated from histogram	Cengel et al. 1978
		Forb	78			
		Bulb	2			
	September-M	Grass	60			
		Forb	36			
		Root	2			
		Apple fruit	2			
	September-A	Grass	15			
		Forb	81			
		Root	2			
		Bulb	2			
	November-M	Grass	80			
		Forb	16			
		Root	2			
		Bulb	2			
	November-A	Grass	30			
		Forb	63			
		Root	2			
		Bulb	5			
	January-M	Grass	82			
		Forb	2			
		Root	9			
		Apple fruit	7			
	January-A	Grass	4			
		Forb	88			
		Root	8			
	March-M	Grass	85			
		Root	13			
		Bulb	2			
	March-A	Grass	20			
		Forb	65			
		Root	15			
	May-M	Grass	12			
		Forb	88			
	May-A	Grass	4			
		Forb	96			

[a]M=maintained orchard.
[b]A=abandoned orchard.

Food Consumption Rate

In a study of the efficacy of feeding repellants on consumption of apple twigs by pine voles, mean consumption (in the absence of alternate foods) was 0.051 g/g/d (Swihart 1990). While no other data concerning feeding rates in pine voles were found, data are available for related species. Among meadow voles, food intake when exposed to 14-h days was 0.095±0.002 (mean±SE) g/g/d; intake by individuals exposed to 10-h days was 0.085±0.005 g/g/d (Dark et al. 1983). Mean food consumption by prairie voles

(assumed to weigh 35 g; Burt and Grossenheider 1976) was 0.088 g/g/d and 0.12 g/g/d when ambient temperatures were 21° and 28°C, respectively (Dice 1922).

Water Consumption Rate

Odum (1944) reports the daily water consumption for a single male pine vole to be 0.3 L/kg/d. In prairie voles (*M. ochrogaster*), water consumption was 0.37 and 0.43 L/kg/d for two individuals (Chew 1951). In contrast, Dice (1922) reports mean water consumption for this same species to be 6.2±3.1 mL/individual/d. Assuming a body weight of 35 g (Burt and Grossenheider 1976), mean water consumption was 0.18±0.08 L/kg/d. Using Eq. 21 and assuming a body weight for pine voles of 25 g, water ingestion is estimated to average 0.14 L/kg BW/d. (Note: If other body weight values are used, the water ingestion rate should be recalculated.) Benton (1955) suggests that because of the high water content of their diet, pine voles may not require drinking water.

Soil Ingestion

Data concerning soil ingestion by pine voles was not located in the literature. Beyer et al. (1994), however, reports soil ingestion by meadow voles to be 2.4% of diet. Soil ingestion by pine voles is likely to be comparable or higher because of the greater fossorial nature of pine voles relative to meadow voles.

Respiration Rate

No literature data were found describing inhalation by pine voles. Using Eq. 23 and assuming a body weight of 25 g (Table 14), the average inhalation rate is estimated to be 1.14 m^3/kg BW/d If other body weight values are used, the inhalation rate should be recalculated.

Metabolism

No literature data were found concerning metabolism in the pine vole. In a related species, the montane vole (*M. montanus*), resting metabolism declined from 3.46±0.15 mL O_2/g/h at 20°C to 2.05±0.07 mL O_2/g/h at 34°C and then increased to 2.71±0.09 mL O_2/g/h at 38°C (Tomasi 1985). In meadow voles, resting metabolism was 2.7 mL O_2/g/h (Altman and Dittmer 1974).

Habitat Requirements

Throughout their range, pine voles occur in a wide variety of habitats, ranging from closed-canopy beech-maple forests with extensive litter (Miller 1964) to grassy fields with brush (Smolen 1981). Pine voles are not restricted to pine forests, as suggested by their common name; in Louisiana, they are more frequently found in hardwood stands (Lowery 1974). Key habitat requirements consist of well-drained soil with thick ground cover of litter or vegetation (Smolen 1981).

Home Range

Pine voles are very sedentary, moving only short distances (Lowery 1974). Home ranges are generally defined by the extent of their burrow system (Smolen 1981). The home range of 17 individuals in an oak-hickory woodland averaged 34.7 m in diameter (range: 13.7-85 m; Benton 1955). In New York, the average home range of 13 individuals was 19.2 m in diameter (Benton 1955). In dry upland hardwood forest, average home ranges were 33.7 m (range: 10-148) and 32.7 m (10-73 m) for females and males, respectively (Miller and Getz 1969).

Population Density

Population density in a 3-ha, dry upland site ranged from 0 to 14.6 voles/ha (Miller and Getz 1969); density in an adjacent mixed conifer-hardwood swamp was <2 voles/ha. Densities are generally greater in

orchards than in natural forests. Density estimates for an orchard in New York ranged from 80 to 120 voles/ha (Hamilton 1938).

Population Dynamics/Survival

Pine vole populations are very local and highly variable (Benton 1955). Miller and Getz (1969) observed mean survival in a high-density upland population to be 2.6 months; maximum observed survival was 12 and 10 months for 2 males and 2 females, respectively. Average survival from one year to the next is reported to be 58% for adults and 57% for juveniles (Smolen 1981).

Reproduction/Breeding

Breeding occurs from January to October in the north portion of the range (Benton 1955) but may be year-round in the south (Lowery 1974). Miller and Getz (1969) estimate the breeding season in Connecticut to extend from mid-February through mid-November. Peak breeding occurs in March and April (Benton 1955). Females are aggressors during mating, which is brief, lasting only a few seconds (Benton 1955). Gestation is estimated to be 20 to 24 days. Hamilton (1938) provides a detailed description of the development of juvenile pine voles. Litter size generally ranges from two to four (Hamilton 1938, Benton 1955). Because female pine voles have only four mammae, large litters are unsuccessful (Smolen 1981). Although litter size is unaffected by day length, juvenile growth is greater under a short photoperiod (8L:16D; Derting and Cranford 1989). Female pine voles are mature in 10 to 12 weeks and are generally breeding by 15 weeks (Smolen 1981).

Behavior

Pine voles are semifossorial, spending considerable time in subsurface burrows and surface runways (Smolen 1981). Borrows are generally 3.8-5 cm in diam. beneath leaves and litter and are rarely 30 cm deep, generally 7.6 to 10 cm at most (Hamilton 1938). In areas with thick litter, surface runways may be constructed (Smolen 1981). Surface activity is not correlated with temperature or humidity (Miller and Getz 1969). Although mostly nocturnal or crepuscular, pine voles may occasionally be active during the day (Lowery 1974). Miller and Getz (1969) report that nocturnal and crepuscular activity was only slightly greater than daytime activity.

Social Organization

Captures of multiple individuals in the same trap suggest a degree of sociability in this species (Miller and Getz 1969). Pine voles are not territorial; multiple individuals may share the same burrow system (Smolen 1981).

3.3.4 Black-Tailed Jackrabbit (*Lepus californicus*)

Black-tailed jackrabbits (also known as California jackrabbits) are in the order Lagomorpha, family Leporidae. Jackrabbits are technically hares, with their young born fully haired, unlike rabbits (Dunn et al. 1982). Three other species of jackrabbit occur in North America: the white-tailed jackrabbit (*L. townsendii*), the antelope jackrabbit (*L. alleni*), and the white-sided jackrabbit (*L. callotis*).

Distribution

The black-tailed jackrabbit is found in the western United States. It ranges from Missouri in the east to the Pacific coast, from the prairies of South Dakota to Texas, and from Washington and Idaho to Mexico in the south (Dunn et al. 1982). It has also been successfully introduced into several eastern states and may be displacing its eastern cousin, the white-tailed jackrabbit (Dunn et al. 1982).

Body Size and Weight

On average, *L. californicus* is smaller than *L. townsendii*. Total body lengths range from 465-630 mm, the tail is 50-112 mm, and the hind foot is from 112-145 mm (Dunn et al. 1982). Representative body weights for black-tailed jackrabbits appear in Table 16. Newborn black-tailed jackrabbits have a total length of 168 mm, and weigh approximately 110 g (Dunn et al. 1982).

Table 16. Body weights (kg) for the black-tailed jackrabbit, *Lepus californicus*

Location	Sex	Mean	Range	Reference
Arkansas	Both	2.3	1.8-3.6	Silva and Downing 1995
Colorado	Both	2.54		Dunn et al. 1982
California	Male	2.47	2.11-2.8	Lechleitner 1959
	Female	2.78	2.3-3.3	
Utah	Male	2.03		Goodwin and Currie 1965
	Female	2.17		

Food Habits and Diet Composition

Jackrabbits are strict herbivores, eating a variety of plants depending on availability and geographic location (Dunn et al. 1982). Black-tailed jackrabbits prefer succulent vegetation when available, with grasses and forbs being important in the summer and shrubs becoming more important in the winter (Dunn et al. 1982). Grasses and sedges may also be important food items. Additional information on foraging habits of black-tailed jackrabbits in different locations are presented in Westoby (1980), Currie and Goodwin (1966), Clark and Innis (1982), Gross et al. (1974), and Dunn et al. (1982).

Food Consumption Rate

Arthur and Gates (1988) estimated a forage intake rate of 145 g (dry weight)/d for black-tailed jackrabbits in Idaho. In Utah, Currie and Goodwin (1966) observed fall, winter, and spring food ingestion rates of 97.3 g (dry weight)/d, 111.4 g/d, and 61.3, g/d, respectively. Assuming a body weight of 2.1 kg (Goodwin and Currie 1965) and a water content for dry grass of 10% (Table 4), daily food ingestion rates are equivalent to 0.076 g/g/d (Idaho), 0.051 g/g/d (fall, Utah), 0.059 g/g/d (winter, Utah), and 0.032 g/g/d (spring Utah).

Water Consumption Rate

Black-tailed jackrabbits are well-adapted to arid environments and are able to regulate water quite efficiently. They have the ability to elevate their body temperature during the day to avoid having to dissipate the heat and hence lose water (Hinds 1977). Black-tailed jackrabbits also can concentrate urine to reduce water loss (Dunn et al. 1982). These factors suggest that black-tailed jackrabbits consume very little water and get most of their moisture from food.

Soil Ingestion

Arthur and Gates (1988) measured a mean (range) ingestion rate of soil for black-tailed jackrabbits in Idaho to be 9.7 (9.0-10.6) g /individual/d, with seasonal peaks occurring in spring and fall. This amount was equivalent to 6.3% of the total dry matter intake for black-tailed jackrabbits. Assuming a body weight of 2.54 kg (Dunn et al. 1982), soil ingestion is estimated to be 0.0038 g/g/d.

Respiration Rate

No literature data were found describing inhalation by black-tailed jackrabbits. Using Eq. 23 and assuming a body weight of 2.54 g, the average inhalation rate is estimated to be 0.45 m^3/kg BW/d. If other body weight values are used, the inhalation rate should be recalculated.

Metabolism

At rest, the body temperature of adult jackrabbits is approximately 37° to 38°C (Dunn et al. 1982). Hinds (1977) discovered that body temperatures in laboratory jackrabbits do not differ significantly with season or on a diurnal basis. Hinds (1977) observed that the summer thermoneutral zone for black-tailed jackrabbits was 26° to 34°C, with an average basal metabolism of 0.562±0.15 mL O_2 /g/h. The winter thermoneutral zone was lower (21° to 28°C), and the average basal metabolism was 0.579±0.004 mL O_2/g/h. Oxygen consumption at ambient temperatures both above and below thermoneutrality increased but at a quicker rate at lower temperatures.

In the summer, evaporative water loss averaged 0.135±0.009% body mass/h, up to ambient temperatures of 26°C. At this temperature evaporative cooling commences, and water loss increases exponentially. In the winter, the entire range of physiological responses appears to be shifted to lower temperatures, so that water loss is higher in winter. Dry heat transfer and thermal conductance were also estimated by Hinds (1977). Both *L. californicus* and *L. alleni* survive in the desert by exploiting opportunities to minimize the heat load and water expenditure, but *L. alleni* seems to be better adapted to arid conditions. Strategies used by black-tailed jackrabbits to survive in the desert include increasing their body temperature during the day to store heat, concentrating their urine, excreting dry feces, and increasing blood flow to the ears to increase convective and radiative heat loss (Dunn et al. 1982).

Habitat Requirements

Although the black-tailed jackrabbit occupies many diverse habitats, it is primarily found in association with short grass areas in the arid regions of the western United States (Dunn et al. 1982). They inhabit desert shrub areas throughout their range but have also become well adapted to many agricultural situations in western states (Dunn et al. 1982).

Home Range

The home range size of the black-tailed jackrabbit is determined by the pattern of food, cover, and water in the surrounding area (Dunn et al. 1982). In California, Lechleitner (1958) reports that home ranges are usually less than 20.2 ha, with females having larger home ranges than males. In Idaho, home range sizes of less than 16.2 ha are reported (French et al. 1965).

Population Density

Population densities vary greatly by location. Density estimates for areas of the arid southwest range from 0.2/ha in Nevada (Hayden 1966), to 0.9/ha in Utah, and to 1.2/ha in Arizona (Dunn et al. 1982). In more temperate regions, densities ranged from 3.0/ha in California (Leichleitner 1958) to as high as 34.6/ha in agricultural areas in Kansas (Dunn et al. 1982). There also appear to be cycles in population densities, with peak densities occurring every 5 to 10 years, possibly because of density-dependent factors (French et al. 1965, Dunn et al. 1982).

Population Dynamics/Survival

Several extensive studies have been performed on the demographics of black-tailed jackrabbits (Lechleitner 1959; Gross et al. 1974). There is evidence that populations are density dependent (French et al.

1965). Other researchers have also noted the tendency for population levels to cycle. In California, Lechleitner (1959) reported a preimplantation mortality of 6.7% and postimplantation mortality of 6.2%. In Utah, Gross et al. (1974) estimated preimplantation and postimplantation mortality rates of 8.0 and 3.0%, respectively. Juvenile mortality rates in Utah ranged from 24 to 71% (mean=59%; Gross et al. 1974), similar to juvenile mortality rates estimated for other locations (Dunn et al. 1982). Adult mortality rates were measured in Utah over an 8-year period, yielding mean yearly mortality rates of 56-57% with a range from 9 to 87% (Gross et al. 1974).

Reproduction/Breeding

Anatomically, male and female black-tailed jackrabbits are similar to domestic rabbits (Dunn et al. 1982). The length of their breeding season is highly variable, depending on latitude and various environmental factors. Generally, the breeding season is shorter for areas located at higher latitudes with more severe winters (French et al. 1965). This can be as short as 128 days in northern Idaho (French et al. 1965) to over 240 days in California with breeding possible all year round (Lechleitner 1959). Gross et al. (1974) report the mean gestation period to be 40 days, ranging up to 47 days depending on the geographic location and the individual. The number of litters per year can also vary from two in colder climates to as many as seven in warmer climates, with the average annual production throughout the range being about 14 young per female (Dunn et al. 1982). The black-tailed jackrabbit is like other lagomorphs in that it is an induced ovulator with a relatively well-synchronized breeding season (Lechleitner 1959; Gross et al. 1974). The litter size varies from about five in its northern range to two in its southern range (Dunn et al. 1982). Males will reach breeding age in seven to eight months, but females generally will not breed until their second year (Lechleitner 1959; Bronson and Tiemeier 1958).

Behavior

Black-tailed jackrabbits are crepuscular, generally feeding in the early morning and evening hours and overnight (Dunn et al. 1982). They prefer to eat in areas that are inconspicuous but that allow them to detect danger from a moderate distance. They often feed in the open, using hollows or open depressions (Dunn et al. 1982). Coprophagy, which is common in many lagomorphs, has also been observed in the black-tailed jackrabbit (Leichleitner 1957).

3.3.5 Mule Deer (*Odocoileus hemionus*)

Mule deer are in the order Artiodactyta, family Cervidae. Mule deer are also referred to as black-tailed deer, but this designation usually applies to the Pacific Coast subspecies. There are about seven generally recognized subspecies (Mackie et al. 1982). Mule deer are medium-sized cervids and are strictly herbivorous.

Distribution

Mule deer/black-tailed deer are found over most of North America from the 100th meridian to the Pacific coast and from southern Alaska to central Mexico (Mackie et al. 1982; Anderson and Wallmo 1984).

Body Size and Weight

Mule deer are medium-sized members of the cervid family but may vary in both size and weight depending on the geographic location of a particular population. Generally, adult males weigh between 70-150 kg (Anderson and Wallmo 1984). The largest individuals occur in the Rocky Mountains, with males averaging 152.3 cm in length and females 142.4 cm. The average weight of males and females are 74.04 kg

to 58.99 kg, respectively (Mackie et al. 1982). West-coast black-tailed deer are smaller, with adult weights for males and females as low as 50 and 32 kg, respectively (Mackie et al. 1982).

Food Habits and Diet Composition

It is difficult to generalize the typical forage of mule deer; foods eaten vary dramatically in kind, quantity, and nutritional quality as well as in digestibility from one season to another, from one year to the next, and from place to place (Mackie et al. 1982). Mule deer may use many different plants at different times, some may be eaten only in certain seasons, and some parts of plants may be selected over others. In general, diets of mule deer consist mostly of browse, whereas the diets of elk, cattle, and wild horses consist mainly of sedges and grasses (Hansen and Clark 1977). Both rumen and fecal analysis have been used to describe deer diets, and both methods give similar results (Anthony and Smith 1974). Examples of food preferences of mule deer are presented in Table 17.

Food Consumption Rate

Alldredge et al. (1974) determined food intake by mule deer in Colorado. Concentrations of ^{137}Cs in deer tissue and diets were used to develop an intake and a retention function. Average intake rates varied by season, age class, and sex (Table 18); mean intake rate was 21.9 g of air-dried forage/kg body weight/d. More specific information on mule deer forage intake rates can be found in Collins and Urness (1983) and Wickstrom et al. (1984).

Wallmo et al. (1977) used several factors including body weight, metabolic weight, activity metabolic rate, forage intake, gross energy, and dry matter digestibility to develop a model to evaluate the ability of ingested forage to supply the energy needs of mule deer. This model can be used to estimate the carrying capacity of seasonal ranges for mule deer populations (Wallmo et al. 1977).

Water Consumption Rate

Mule deer obtain much of their water through succulent forage or as dew on forage plants. This is sufficient to meet their metabolic needs during the spring, summer, and fall; in the winter snow is ingested (Mackie et al. 1982). Observations of mean water intake by penned mule deer range from 24-35 mL/kg/d in winter and 47-70 mL/kg/d in the summer (Anderson and Wallmo 1984). Water consumption by black-tailed deer ranges from 53 mL/kg/d in winter to 104 mL/kg/d in summer (Anderson and Wallmo 1984).

Soil Ingestion

Soil ingestion rates were calculated for mule deer in north central Colorado feeding in a grassland-shrub community (Arthur and Alldredge 1979). The intake varied by season, with a year-round average of 16.1 g/individual/d (Table 19). The soil ingested ranged from 0.6 to 2.1% of the deers' diets (dry matter intake). Beyer et al. (1994) report soil ingestion by mule deer to be <2% of their diet.

Respiration Rate

No literature data were found describing inhalation by mule deer. Using Eq. 23 and assuming a body weight of 57.1 kg, the average inhalation rate is estimated to be 0.24 m^3/kg BW/d. If other body weight values are used, the inhalation rate should be recalculated.

Table 17. Diet composition of mule deer

			Percentage of diet							
Location	Habitat	Season	Trees	Shrubs	Forbs	Grasses	Cactus	Fern	Other	Reference
New Mexico	SW pinyon-juniper		75		16	2.2			6.8	Boeker et al. 1972
Arizona	Sonoran desert	Spring	4.7	37.6	22.8	2.6	29.6		2.7	Short 1977
Arizona	Sonoran desert	Summer	24.1	38	22.4	0.4	14.1		1	Short 1977
Arizona	Sonoran desert	Fall	3.4	48	2.5	Tr.	44.5		1.6	Short 1977
Arizona	Sonoran desert	Winter	4.9	31.7	4.3	1	55.9		2.2	Short 1977
Colorado	Pinyon-juniper range	Winter	81.4		10.7	7.9				Bartmann et al. 1982
Colorado	Pinyon-juniper range	Winter	90.3		8.6	1.1				Bartmann et al. 1982
Colorado	Pinyon-juniper range	Winter		93.8	5.6				1.6	Bartmann et al. 1982
Colorado	Pinyon-juniper range	Winter		89.9	6.2				3.9	Bartmann et al. 1982
Colorado	Sagebrush-steppe range	Winter		62.9	31.2	5.7			0.2	Bartmann et al. 1982
Colorado	Sagebrush-steppe range	Winter		80.7	7.2	12.1				Bartmann et al. 1982
Colorado	Old-growth forest	Fall	52	3	39	3		1	2	Leslie et al. 1984
Washington	Old-growth forest	Winter	49	4	41	2		1	3	Leslie et al. 1984
Washington	Old-growth forest	Spring	61	5	8	4		19	3	Leslie et al. 1984
Washington	Old-growth forest	Summer	60	8	8	4		13	7	Leslie et al. 1984
Washington	Old-growth forest	Fall	3	26	29	7		30	5	Leslie et al. 1984
Washington	Old-growth forest	Winter	2	43	21	6		23	5	Leslie et al. 1984
Washington	Old-growth forest	Spring	25	8	50	6		3	8	Leslie et al. 1984
Utah	Clear-cut forest	Summer	5		92				3	Deschamp et al. 1979
Utah	Dry meadow	Summer	6		83	2			9	Deschamp et al. 1979
Utah	Wet meadow	Summer	4		93				3	Deschamp et al. 1979
Utah	Mature forest	Summer	20		62		18			Deschamp et al. 1979
Utah	Stagnated forest	Summer	20		65		15			Deschamp et al. 1979

Table 18. Forage intake rates (g dry forage/kg/d) for mule deer (Alldredge et al. 1974)

	Mean (±SE)
Summer	25.7 ±2.4
Winter	20.1 ±1.2
Male	22.4 ±1.8
Female	21.5 ±1.4
Subadults	31.8 ±2.3
Adults	18.2 ±0.9
Mean for all groups	21.9 ±1.1

Table 19. Soil ingestion rates (g/d) by mule deer (Arthur and Alldredge 1979)

	Mean (±SE)
Spring	29.6±20.1
Summer	7.7±10.2
Fall	8.8±6.5
Winter	18.3±10.8

Metabolism

The mean core body temperatures of captive mule deer and black-tailed deer have been calculated. The mean (range) for a yearling male *O.h. hemionus* is 37.1°C (36.3 to 42.1; Thorne 1975). For two male black-tail fawns the temperature was 38.9°C (38.4 to 39.8), and for two adult females the mean temperature was 38.3°C (37.8 to 39.3) (Cowan and Wood 1955b). Mule deer have a preferred ambient temperature range from about -9° to 7°C, but they can tolerate climates with average temperatures between -15° and 30°C, with extremes from -60° to 50°C (Mackie et al. 1982).

Mule deer are homiothermal and lack sweat glands. Thermoregulation from evaporation is difficult; therefore, alternative strategies are used to regulate body temperature (Mackie et al. 1982). Heat production, thermoregulation, and environmental stressors in mule deer are discussed by Nordan et al. (1970), Parker and Robbins (1984), and Parker (1988). Mautz and Fair (1980) observed a linear relationship between heart rate and energy expenditure

$$\text{kcal/kg}^{0.75}\text{/min} = 0.00143(\text{heart rate}) - 0.0186. \qquad (52)$$

Although using heart rates as a predictor of energy expenditure for mule deer of similar sizes seems feasible, fluctuations by time of day and ambient temperature may limit the precision of these estimates (Freddy 1984). The average maintenance energy requirement of fawns in winter was 158 kcal ME/kg$^{0.75}$/d, where

ME = metabolizable energy (Baker et al. 1979). This is the caloric intake needed to maintain body weight equilibrium and includes the unquantified inherent cost of activity and thermoregulation (Baker et al. 1979). Kautz et al. (1982) estimated this value to be between 134 and 204 kcal/kg$^{0.75}$/d for mule deer fawns. Several studies have been done on the energy costs for different mule deer activities (Kautz et al. 1982; Parker et al. 1984). The costs of bedding, standing, walking, and trotting in kcal/kg$^{0.75}$/d are 112, 164, 326, and 1,293, respectively (Kautz et al. 1982).

Habitat Requirements

Mule deer are found in all major climatic and vegetational zones of western North America. Generally, mule deer frequent semiarid, open forest, brush, and shrub lands associated with steep, broken, or otherwise rough terrain (Mackie et al. 1982). They are the most populous in mountain foothill habitats but can be found in prairie and semiarid desert habitats as well.

Home Range

Mule deer usually confine themselves to small individual home ranges, with extreme movements occurring only during migration (Mackie et al. 1982). More extreme movements may also occur as a result of severe environmental conditions. The mean annual home range size is 58.8 ha for black-tailed deer and 285.3 ha for mule deer (Anderson and Wallmo 1984). Dasmann and Taber (1956) determined the average home range to be between 640 and 1280 m in diameter for adult does and between 822 and 1280 m for adult bucks. Robinette (1966) observed similar home range sizes in Utah.

Population Density

Population densities vary by habitat type from 0.005 to 0.02 individuals/ha in open prairies and plains, to 0.015-0.045 individuals/ha in broken prairies, and to 0.04-0.07 individuals/ha in mountain regions (Mackie et al. 1982). Winter densities of deer can get much higher with values from 0.3 to 0.5 individuals/ha (Mackie et al. 1982; Anderson and Wallmo 1984; Dasmann and Taber 1956). Populations may also fluctuate from year to year, increasing or decreasing the overall densities.

Population Dynamics/Survival

The abundance of mule deer is determined both by the number of deer that can be supported by a unit of area and the amount of habitat available (Mackie et al. 1982). Local populations may be influenced by many different extrinsic factors, the most important of which are habitat and nutritional limitations. Other limiting factors include weather, diseases, parasites, predation, competition, other wild and domestic ungulates, and hunting (Mackie et al. 1982). Some papers on specific mortality rates of mule deer in Colorado, Utah and Washington are White and Bartmann (1983), Robinette et al. (1957), and Taber and Dasmann (1954).

Mortality of fetuses in mule deer has been estimated at between 3.5 and 10.5%, with postnatal mortality of 22-53% for males and 17-25% for females (Anderson and Wallmo 1984). Average longevity has not been determined, but some wild deer have been observed living to age 20 (Robinette et al. 1957).

Reproduction/Breeding

Mule deer are polygamous, with males wandering and seeking does in estrus. Males are highly aggressive during rut and are antagonistic toward others (Mackie et al. 1982). Females generally do not breed until their second year, with peak breeding occurring between November and December. Gestation usually lasts from 200 to 208 days with the peak births occurring in late June (Anderson and Wallmo 1984). Does usually have one or two fetuses with triplets occurring only about 1.4% of the time. Weaning generally occurs from about week 5 to week 16. The length of the estrous cycle in mule deer was calculated to be between 2[illegible]

and 29 days (Anderson and Wallmo 1984). Additional information on the fertility of mule deer can be found in Robinette et al. (1955).

Behavior and Social Organization

The degree of sociability in mule deer varies according to season, sex, population, and subspecies, with most being neither highly gregarious, nor strictly solitary (Mackie et al. 1982). Mule deer are most dispersed during the summer and most congregated during the winter, as suitable habitat decreases. There have been scattered reports of group territoriality (Mackie et al. 1982). Additional information on mule deer behavior can be found in Mackie et al. (1982), Kucera (1978), and Dasmann and Taber (1956).

3.3.6 Coyote (*Canis latrans*)

Coyotes are in the order Carnivora, family Canidae. Coyotes are closely related to jackals, having 19 recognized subspecies (Bekoff 1982). Coyotes tend to hunt prey alone or in pairs and are primarily carnivorous. They eat mostly small mammals but also birds, reptiles, insects, fruits, seeds, berries, and nuts (Bekoff 1982).

Distribution

Coyotes are nearctic canids, occupying many diverse habitats, including grasslands, deserts and mountains, between about 10° north latitude and 70° north latitude (Bekoff 1982). They are found throughout the continental United States and much of Canada; some use urban habitats. Coyotes have been extending their range in the past 40 years, possibly because of the extermination of the gray wolf and the destruction of wolf habitat (Thurber and Peterson 1991).

Body Size and Weight

Coyotes range in length from about 1 to 1.5 m, with a tail about 400 mm long (Bekoff 1982). Size and weight vary across different geographic locations and with different subspecies, although adult males tend to be slightly heavier and larger than adult females. The variation in body weights of male and female coyotes from different locations across North America are shown in Table 20. The birth weight of coyotes is about 240-275 g, with the body from head to tail measuring 160 mm (Bekoff 1982).

Table 20. Body weights (kg) for the coyote, *Canis latrans*

Location	Sex	N	Mean±SE	Range	Reference
Iowa	Male		13.4		Bekoff 1982
	Female		11.4		
Minnesota	Male			12-13	Bekoff 1982
	Female			11-12	
	Juvenile male			10-11	
	Juvenile female		10		
California	Male	28	11.2	8.2-12.5	Hawthorne 1971
	Female	26	9.8	7.7-12.0	
Maine	Male	28	15.8±1.24		Richens and Hugie 1974
	Female	20	13.7±1.24		
Kansas	Male		13.1		Bekoff 1982
	Female		11.0		

Table 20. (continued)

Location	Sex	N	Mean±SE	Range	Reference
Ontario	Both: 1959-1960 Both: 1983-1984	124 44	14.6±0.17 15.5±0.37		Schmitz and Lavigne 1987
Alaska	Male Female	26 28	12.9±0.2 11.1±0.2		Thurber and Peterson 1991
Arizona	Both	18	10±0.04		Golightly and Ohmart 1983
Oklahoma	Male	7	13.9	12-15.3	Halloran and Glass 1959
Connecticut and Massachusetts	Male Female			11.7-15.9 11.2-12.3	Pringle 1960

Food Habits and Diet Composition

Coyotes are opportunistic foragers (Toweill and Anthony 1988; Todd et al. 1981), consuming a wide variety of foods (Bowen 1981). Coyotes have also been shown to follow a strategy of optimal foraging (MacCracken and Hansen 1987). Coyotes are primarily carnivorous, feeding principally on birds and mammals, but also relying on insects and fruits (Fitcher et al. 1955). Selected information on diet preferences of coyotes is presented in Table 21. The evidence from the studies on stomach and scat contents of coyotes indicates that there is a seasonal shift in food habits (Korschgen 1957; Hawthorne 1972; Bowen 1981; MacCracken and Uresk 1984; Smith 1990). Only a small percentage of a coyote's diet is livestock; actual predation on livestock is rare (Bekoff 1982; Wells and Bekoff 1982).

Table 21. Diet composition of coyotes as determined by stomach content analysis

	Percentage volume						
Location	Mammals	Birds	Insects	Plants	Carrion	Misc.	Reference
12 Western states	64 (29% lagomorphs, 17% rodents, 14% livestock, 2% deer, 2% skunk and badger)	3	1	3	29		Sperry 1933
10 Western states	60 (34% lagomorphs, 15% rodents, 8% livestock, 3% deer)	3		1	36		Sperry 1934
Nebraska	78 (54% lagomorph, 12.5% livestock, 6.9% mice, 4.6% other)	17.7	0.9	1.6		1.8	Fichter et al. 1955

Table 21. (continued)

Location	Percentage volume						Reference
	Mammals	Birds	Insects	Plants	Carrion	Misc.	
Missouri (spring)	77.1 (48.6% lagomorphs, 16.5% livestock, 5.4% mice and rats, 6.6% other)	17.7 (17% poultry)	tr.	0.2	5.0		Korschgen 1957
Missouri (summer)	65 (35.2% lagomorphs, 17.5% livestock, 5.6% mice and rats, 6.7% other)	28 (27.4% poultry)	1.9	0.8	4.3		Korschgen 1957
Missouri (fall)	72.2 (47.7% lagomorphs, 7.2% livestock, 9% mice and rats, 8.3% other)	13.2 (12.8% poultry)	3.5	6.5	4.3	0.3	Korschgen 1957
Missouri (winter)	82.7 (58.1% lagomorphs, 7.6% livestock, 9.5% mice and rats, 7.5% other)	9 (8.5% poultry)	tr.	0.9	6.6	0.8	Korschgen 1957

Food Consumption Rate

Fitch (1948) conducted a captive feeding study with one adult female coyote captured in the San Joaquin Experimental Range, California. Over a one-month period, the coyote consumed a daily average of 0.54 kg of food (body weight not reported). The author observed that the coyote would have eaten even more if given the opportunity and estimated the average food consumption under natural conditions to be about 1.5 lb/d (0.68 kg/d). Huegel and Rongstad (1985) observed food consumption rates of 10-12% of body mass/day among radio-tagged coyotes in northern Wisconsin in winter. Litvaitis and Mautz (1980) estimate the annual ingestion rates of deer, hares, and mice by a 12.9 kg eastern coyote to be 167 kg, 166 kg, and 134 kg, respectively. These values are equivalent to a daily consumption rate of 0.028 to 0.035 g/g/d. Golightly and Ohmart (1983) estimated the minimum energy requirements for desert coyote to be 260 J/g/d. Assuming a diet consisting of small mammals with a caloric density of 21.6 kJ/g (Golley 1961) and a water content of 68% (Table 4), this is equivalent to daily consumption rate of 0.018 g/g/d.

Water Consumption Rate

No literature data were found describing water ingestion by coyotes. Using Eq. 21 and assuming a body weight of 16.3 kg, water ingestion is estimated to average 0.075 L/kg BW/d. (Note: If other body weight values are used, the water ingestion rate should be recalculated.)

Soil Ingestion

No literature data were found concerning soil ingestion by coyotes. Beyer et al. (1994) report soil consumption by red fox to be 2.8% of daily food consumption. Values for coyote may be comparable.

Respiration Rate

No literature data were found describing inhalation by coyote. Using Eq. 23 and assuming a body weight of 16.3 kg , the average inhalation rate is estimated to be 0.31 m^3/kg BW/d. If other body weight values are used, the inhalation rate should be recalculated.

Metabolism

Shield (1972) determined the O_2 consumption rates of several cold-acclimated Alaskan coyotes at a series of ambient temperatures; rates ranged from 7.1 mL O_2/kg/min at 20°C to 20.3 mL O_2/kg/min at -70°C. Golightly and Ohmart (1983) evaluated metabolism and body temperature of coyotes from a desert habitat in Arizona. They observed that minimum O_2 consumption occurred between 22° and 26°C and that the basal metabolic rate (BMR) within this zone was 0.0015 W/g (Golightly and Ohmart 1983). Unlike the kit fox and other desert canids, the coyote did not exhibit any distinct daily rhythms of oxygen consumption. This may be a reflection of the coyote's irregular activity patterns (Golightly and Ohmart 1983). Using BMR values to obtain the minimum energy intake requirements for coyotes, 129.6 J/g/d or 1296 kJ/d are required for a 10 kg coyote in thermal neutrality (Golightly and Ohmart 1983). The minimum energy requirements for a desert coyote were calculated to be 260 J/g/d (Golightly and Ohmart 1983).

Habitat Requirements

Coyotes are very adaptable, occupying diverse habitats ranging from forest to range to desert. Coyotes generally live in dens, built in brush-covered slopes, steep banks, rock ledges, thickets, and hollow logs. Dens of other animals, like badgers, are often used (Bekoff 1982). Coyotes need enough food for a habitat to be suitable, but because they are opportunistic feeders they have adapted well to many diverse habitats. Coyotes in Maine prefer open habitats like bogs and frozen lakes and softwood-dominated mixed habitats to hardwood and hardwood-dominated mixed habitats (Major and Sherburne 1987). In Michigan, coyotes prefer the mixed aspen-conifer and swamp conifer sites, as well as lowland brush habitat (Ozoga and Harger 1966).

Home Range

The home range size of coyotes is highly variable, depending on geography and season (Bekoff 1982). Coyotes in packs that defend ungulate carrion in the winter have compressed home ranges (1430 ha), whereas coyotes living alone or in pairs may have a home range of 3010 ha (Bekoff 1982; Bekoff and Wells 1980). Home range sizes have been reported as high as 6800 ha for male coyotes and as high as 3600 ha for females (Bekoff 1982). Coyotes do not seem to exhibit territoriality unless they are in a pack (Bekoff and Wells 1980).

Population Density

The density of coyote populations varies from year to year and by region. Fichter et al. (1955) report densities of 0.0015 individuals/ha (Fichter et al. 1955). Coyote densities in Alberta during the 1960s and 1970s varied from a low of 0.0014/ha to 0.0044/ha, depending on the abundance of their major food source, hares (Todd et al. 1981). In Michigan, densities of 0.0019/ha to 0.001/ha, have been reported (Ozoga and Harger 1966). Other studies have found population densities of 0.001/ha to 0.023/ha (Bekoff 1982).

Population Dynamics/Survival

The mortality rate of coyotes depends on their age and the level of control to which they are exposed. Pups and individuals less than one year of age have the highest mortality rate (67-68%; Bekoff 1982). Adult mortality varies from 36-45%, with about 3/4 of a coyote population being between 1and 4 years of age (Bekoff 1982). In order to maintain population stability, net survival of 33-38% is necessary (Knowlton 1972; Nellis and Keith 1976). Maximum ages of wild individuals were recorded at 13.5 years (Nellis and Keith 1976) and 14.5 years (Knowlton 1972).

Reproduction/Breeding

Anatomically and physiologically, coyotes are very similar to domestic dogs and can produce fertile hybrids with them, as well as with red and grey wolves and golden jackals (Bekoff 1982). The number of females that breed in a year is dependent on food availability. Generally, 60-90% of adult females produce litters, along with some female yearlings (Bekoff 1982). Knowlton (1972) estimated that approximately 87% of ovulated implants were represented by viable ova, with a high percentage of these developing into viable young. Gestation lasts about 63 days with an average litter size of 6 (Bekoff 1982). Litter size can vary depending on food availability. The sex ratio in the population is about 1:1 (Bekoff 1982). Young begin to eat solid foods at about 3 weeks of age and are usually weaned by around six weeks of age (Bekoff 1982). During the first eight weeks of life, pup weight increases by about 0.31 kg per week, with the pups reaching adult weight at about 9 months of age (Bekoff 1982). Emergence from the den usually coincides with pups beginning to eat solid foods.

Behavior and Social Organization

Coyotes communicate with a series of postures, gestures, tail movements, facial expressions, and vocalizations. Generally, coyotes are less social than wolves, but they will sometimes form packs. Pack formation occurs when there are large prey items to be eaten or for cooperative group defense purposes (Bekoff 1982). Coyotes may be active at various times during the day but tend to be most active around sunrise and sunset. They also exhibit seasonal differences in activity with more time spent resting during the winter to conserve energy (Bekoff 1982).

3.3.7 Kit Fox (*Vulpes macrotis*)

Kit foxes are in the order Carnivora, family Canidae. They are closely related to the swift fox (*Vulpes velox*), with their common names having been used interchangeably in the past (Samuel and Nelson 1982). They are carnivorous animals, and opportunistic feeders, but seem to rely mostly on rodents and lagomorphs in their diets (McGrew 1979). While they have been exterminated from much of their historical range, populations are returning in some areas (Samuel and Nelson 1982).

Distribution

Kit foxes are distributed throughout the desert and semiarid regions of western North America. They are historically found throughout the Sonoran, Chihuahuan, Mohave, and Painted deserts and much of the Great Basin Desert (McGrew 1979). The similar swift fox (*Vulpes velox*) is found from New Mexico to the Dakotas (Samuel and Nelson 1982).

Body Size and Weight

Kit foxes have a typical fox appearance, with a slim body, large ears relative to their body, and a long bushy tail (McGrew 1979). The kit fox has a body length of about 40 cm, with the tail being 25 to 30 cm (over 40% of the total body length) (McGrew 1979; Samuel and Nelson 1982). Their average adult weight

ranges from 1.5 to 3 kg (McGrew 1979). Weights of kit foxes from several specific locations are listed in Table 22.

Table 22. Body weights (kg) for the kit fox. *Vulpes macrotis*

Location	Sex	N	Mean ± SE	Range	Reference
Utah	Male	10	2.06	1.7-2.5	Egoscue 1962
	Female	6	1.91	1.6-2.1	
Arizona	Both: summer	11	1.77±0.06		Golightly and Ohmart 1983
	Both: winter	9	1.87±0.06		
California	Male	21	2.4±0.01		White and Ralls 1993
	Female	17	2.1±0.01		
Arizona	Male	4	1.82±0.06		Zoellick and Smith 1992
	Female	3	1.67±0.04		
	Both	7	1.76±0.05		

Food Habits and Diet Composition

Kit foxes are almost exclusively carnivorous, with primary prey being small mammals and rabbits (McGrew 1979). The endangered San Joaquin kit fox feeds almost exclusively on kangaroo rats, which are also a major food source for other subspecies of kit fox (Morrell 1972). Egoscue (1962) found that black-tailed jackrabbits (*Lepus californicus*) made up over 94% of the kit foxes' diet in Utah. These differences in diet reflect the fact that kit foxes are opportunistic feeders, although not to the extent that coyotes are (McGrew 1979). Kit foxes will supplement their diets with ground-nesting birds, reptiles, and insects but do not appear to switch to diurnal prey or move to areas of greater prey abundance when there is a decline in their primary prey species (Egoscue 1962; Morrell 1972; McGrew 1979).

Food Consumption Rate

. Adult kit foxes kept in captivity ate an average of 175 g fresh meat/d (Egoscue 1962), with males consuming 108-348 g/d and females consuming 56-292 g/d. Assuming a mean body weight of 2 kg, mean food consumption equals 0.0875 g/g/d (range = 0.028-0.174 g/g/d). The total family food requirement for the first 64 days following the birth of a litter was estimated to be 44,605 g (Egoscue 1962).

Water Consumption Rate

Kit foxes appear to obtain adequate moisture from their prey species, as they are often many kilometers away from any water source (Egoscue 1962; Morrell 1972). The fact that kit foxes do not utilize evaporative cooling methods for dissipating metabolic heat would support the idea that they are adapted to a low moisture, arid environment (Golightly and Ohmart 1983).

Soil Ingestion

No literature data were found concerning soil ingestion by kit foxes. Beyer et al. (1994) report soil consumption by red foxes to be 2.8% of daily food consumption. Values for kit foxes may be comparable.

Respiration Rate

No literature data were found describing inhalation by kit foxes. Using Eq. 23 and assuming a body weight of 1.5 to 3 kg, the average inhalation rate is estimated to range from 0.44 m^3/kg BW/d to 0.5 m^3/kg BW/d. If other body weight values are used, the inhalation rate should be recalculated.

Metabolism

Golightly and Ohmart (1983) studied metabolism and body temperatures of kit foxes and other desert canids from Arizona. The minimum summer oxygen consumption rate was observed between 19° and 31°C; minimum O_2 consumption in winter occurred between 23° and 33°C (Golightly and Ohmart 1983). BMR was 0.0034 W/g in summer and 0.0028 W/g in winter. Kit fox metabolic rates are not consistent with those of other desert-adapted species. Instead, kit foxes exhibit high thermal conductance, which may be an adaptation for dissipating heat loads by nonevaporative means. Foxes may use dens during the day and limit their activities to the night to avoid excessive heat and water loss (Golightly and Ohmart 1983). The kit fox cannot tolerate high ambient temperatures, and the den provides safety and a predictable shelter with a moderated microclimate. Kit foxes also exhibited distinct circadian rhythms in oxygen consumption and body temperature, with peak levels corresponding to early evening and early morning activity periods (Golightly and Ohmart 1983). This is unlike the coyote and allows metabolic rate and water loss to be minimized in the kit fox.

Habitat Requirements

Kit foxes prefer semiarid habitats with less than 20% ground cover, light colored loamy desert soil, and elevations lower than 1675 ft (McGrew 1979). The vegetation of these areas is a shrubby or shrub-grass combination that varies depending on the actual location.

Home Range

Home ranges of kit foxes overlap broadly with different family hunting groups hunting in the same areas but not at the same time. This suggests that no specific hunting territory is maintained or defended (Morrell 1972). Morrell (1972) estimated the home range of kit foxes in the San Joaquin Valley of California to be 260 to 520 ha. Zoellick and Smith (1992) calculated the overall average home range size to be 1120 ± 94 ha for foxes in western Arizona. The male home range averaged 1230 ± 100 ha, and the female home range averaged 980 ± 140 ha. White and Ralls (1993) calculated the home range of kit foxes in California to average 1160 ± 90 ha. White and Ralls (1993) also calculated a mean social group home range of 1370 ± 110 ha.

Population Density

In Utah, Egoscue (1956) estimated the population density of the kit fox to be 0.001 pairs/ha, or at an optimum, 0.008 individuals/ha. Zoellick and Smith (1992) found population densities of 0.0022-0.0028 individuals/ha in western Arizona. White and Ralls (1993) estimated minimum population densities of 0.0015-0.0024 individuals/ha in California. In 1959, the population of the San Joaquin kit fox was estimated to be between 1000 and 3000 total, or about 0.004 individuals/ha (Samuel and Nelson 1982).

Population Dynamics/Survival

The mortality rates of kit foxes are unknown, but their overall abundance has declined dramatically as a result of poisoning and trapping; habitat loss has contributed to the decline (Zoellick et al. 1989). [illegible] fox mortality is the result of being hit by cars and by predation by coyotes and hawks (Egoscue 1962). [illegible] of the kit fox populations that have been studied remain at a relatively stable size, presumably at a level [illegible] can be supported by the environment. Egoscue (1956, 1962) and others have often seen a slight bias [illegible]

the number of males in the adult kit fox population. Population numbers have been observed to rise or fall depending on the population of their major food source (Egoscue 1962; Morrell 1972). During a period of low food supply, Egoscue noted that average adult age was only 1.96 years.

Reproduction/Breeding

Males will generally join females at natal dens in October or November, with breeding occurring between December and February (McGrew 1979). Initial observations suggested kit foxes to be monogamous (Egoscue 1962); however, recent research indicates multiple females sharing a den with one male (Morrell 1972). Little is known about courtship behavior, but copulation appears to be similar to other canids. The gestation period in kit foxes in unknown but is assumed to be about the same as the red fox, 49-55 days (Egoscue 1962). Litters are usually born in February or March, with a litter size of 4-5 and a nearly even sex ratio (Egoscue 1962; Morrell 1972; Samuel and Nelson 1982). The male fox stays with the family and hunts for food while the female suckles the pups and rarely leaves the den (McGrew 1979). Pups emerge from the den in about a month and reach adult weight by about five months of age. The family group will split up in October, with the pups usually dispersing beyond their parents home range (Morrell 1972).

Behavior and Social Organization

Few detailed accounts exist of kit fox behavior, although there is some information on reproduction, hunting, and denning (McGrew 1979). Foxes appear to use olfactory clues, similar to other canids, and Egoscue (1962) has described several kit fox vocalizations. Morrell (1972) also described some of these vocalizations. Some of the lack of information on behaviors is because of the nocturnal habits of the kit fox. Dens are a very important part of the kit fox's life, with most having multiple entrances, anywhere from 2 to 24 (Egoscue 1962). A suitable den is a critical habitat component for the kit fox, as dens are used throughout the year (Samuel and Nelson 1982). Family groups tend to have a whole group of dens that they use almost exclusively, but this may change from year to year (Egoscue 1956, 1962). Smaller dens are used during the breeding season and larger dens are used during the winter (Samuel and Nelson 1982). Several researchers have also recently investigated the spacing patterns of kit foxes and their nightly movements (White and Ralls 1993; Zoellick et al. 1989; Zoellick and Smith 1992).

3.3.8 Weasels (*Mustela spp.*)

Weasels are in the order Carnivora, family Mustelidae. Weasels are small to medium sized predators with a characteristic elongated body form. Three species occur in North America, the long-tailed weasel (*Mustela frenata*), the short-tailed weasel (ermine or stoat; *M. erminea*), and the least weasel (*M. nivalis*) (Svendson 1982). Additional *Mustela* species in North America include the mink (*M. vison*) and the black-footed ferret (*M. nigripes*). Because exposure parameters for mink are presented in EPA (1993) and the black-footed ferret is a critically endangered species with an extremely limited distribution, neither species is discussed here.

Distribution

Long-tailed weasels occur from southern Canada, throughout the United States (except for the desert Southwest), through Central America to northern South America (Svendson 1982). Both short-tailed and least weasels have circumpolar ranges, occurring throughout the Holarctic (King 1983, Svendson 1982). In North America, short-tailed weasels occur across the Arctic, south to northern California, Nevada, Utah and Colorado in the west and south to northern Iowa, Wisconsin, Michigan and Pennsylvania in the east (Svendson 1982, Burt and Grossenheider 1976). Least weasels occur from Alaska and the Canadian Arctic south to Nebraska, Iowa, Illinois, Indiana, Ohio, and Pennsylvania to the southern Appalachians (Svendson

1982, Burt and Grossenheider 1976). Least weasels are not known to occur in the Rocky Mountains or in northern New England. The northern distribution of long-tailed weasels in North America may be limited by snow cover which restricts foraging (Simms 1979a). Southern distribution of least and short-tailed weasels may be limited by competition and interference interactions with long-tailed weasels (Simms 1979a).

Body Size and Weight

Of the three North American weasels, the long-tailed weasel is the largest (total length: 300 - 350 mm), short-tailed weasels are intermediate in size (total length: 225 - 340 mm) , and least weasels are smallest (total length: <250 mm in males; <225 mm in females) (Svendson 1982). Tail length is 40-70% of head and body length for long-tailed weasels, 30-45% for short-tailed weasels, and 25% or less for least weasels (Svendson 1982). While both long-tailed and short-tailed weasels have black-tipped tails, the least weasel does not. Summer pelage of these three species is generally brown on top and white to yellowish on the undersides. Winter coats are generally a uniform white.

Sexual dimorphism is pronounced in weasels, with males consistently larger than females. Sexual dimorphism is attributed to the polygynous mating system of weasels; small females have an energetic advantage over large females while rearing young while large males have a competitive advantage during breeding (Erlinge 1979, Moors 1980). Body weights of weasels from several locations are summarized in Table 23. Sanderson (1949) presents data on growth of a litter of long-tailed weasels from 35 to 100 days in age. Growth curves for male and female least weasels maintained in captivity for 15 weeks are presented by Heidt et al. (1968).

Table 23. Body weights (kg) for the weasels

Species	Location	Sex	N	Mean	Range	Reference
Long-tailed weasel	Nevada	Male: adult	4	0.297±0.036[a]		Brown and Lasiewski 1972
		Female: adult	4	0.153±0.003[a]		
	Montana	Male: adult	12	0.287		Wright 1947
	Indiana	Male: adult	19	0.200±0.054[a]	0.102-0.284	Mumford and Whitaker 1982
		Female: adult	6	0.094±0.010[a]	0.083-0.109	
	North America	Male: adult			0.198-0.340	Burt and Grossenheider 1976
		Female: adult			0.085-0.198	
Short-tailed weasel	New Zealand[c]	Male: adult	11	0.308±0.016[b]		King et al. 1996
		Female: adult	8	0.209±0.013[b]		
	Europe	Male: adult			0.208-0.283	King 1983
	Great Britain	Male: adult		0.320		
	Russia	Male: adult			0.134-0.191	
	North America	Male: adult			0.056-0.206	
	Minnesota	Male: adult	12		0.090-0.170	Jones et al. 1983
		Female: adult	4		0.043-0.071	
	Colorado	Female: adult	4	0.038	0.030-0.044	

Table 23. (continued)

Species	Location	Sex	N	Mean	Range	Reference
	North America	Male: adult Female: adult			0.071-0.170 0.028-0.085	Burt and Grossenheider 1976
Least weasel	Indiana	Male: adult Female: adult	26 10	0.045±0.013[a] 0.032±0.090[a]	0.026-0.068 0.022-0.052	Mumford and Whitaker 1982
	Great Plains, North America	Male: adult Female: adult	2 5	 0.042	0.055-0.063 0.032-0.050	Jones et al. 1983
	North America	Male: adult Female: adult			0.039-0.063 0.038-0.039	Burt and Grossenheider 1976

[a] Mean ± STD
[b] Mean ± SE
[c] Individuals introduced from Great Britain.

Food Habits and Diet Composition

Weasels are specialist predators of small, warm-blooded vertebrates (King 1983). Their diet consists predominantly of small mammals (50-80% of annual consumption) with larger species consuming larger-sized prey (Table 24; Svendson 1982). Other foods may be consumed, depending on season and availability. Food preferences of weasels from several locations are listed in Table 24.

Table 24. Diet composition of weasels

Species	Location	Prey taxon	Percent	Comments	Reference
Long-tailed weasel	Michigan	Small mammals *Peromyscus* *Microtus* *Tamiasciurus* Small birds	 98.3 28.2 1.0 6.8	Data represent frequency of occurrence of prey types in 294 scats from winter.	Quick 1944
	Colorado	Small mammals *Microtus* *Peromyscus* *Eutamias* *Cynomys* *Thomomys* *Cittelus* *Ochatona* Insects *Vespula* Tettigoniidae	 52.0 19.5 18.2 2.6 3.9 2.6 1.3 6.5 2.6	Data represent frequency of occurrence of prey types in 77 scats from all seasons.	Quick 1951

Table 24. (continued)

Species	Location	Prey taxon	Percent	Comments	Reference
	Iowa	*Microtus*	42.85	Data represent percent volume of prey types in 135 scats from winter and spring.	Polderboer et al. 1941
		Reithrodontomys	21.75		
		Peromyscus	10.23		
		Sylvilagus floridanus	8.42		
		Blarina	5.42		
		Mus	1.86		
		Tree Sparrow	1.02		
		Grasshopper	0.60		
		Geomys	0.60		
		Mustela nivalis	5.40		
		Unidentified matter	1.85		
	California	*Microtus*	97.9	Data represent percent occurance of prey remains by dens in winter.	Fitzgerald 1977
		Thomomys	1.0		
		Peromyscus	0.5		
		Sorex	0.5		
Short-tailed weasels	California	*Microtus*	99.1	Data represent percent occurrence of prey remains by dens in winter.	Fitzgerald 1977
		Peromyscus	0.2		
		Sorex	0.35		
		Small birds	0.35		
	Minnesota	Mice	54.5	Data represent percent volume of prey types in 80 stomachs in winter.	Aldous and Manweiler 1942
		Shrews	21.8		
		Hare	6.1		
		Porcupine	5.0		
		Birds	2.7		
		Weasel	2.5		
		Squirrel	2.5		
		Fish	1.2		
		Unknown	3.7		
	Great Britain	Mammals			King and Moors 1979
		Mice and Voles	22.0		
		Rats and Squirrels	4.8		
		Insectivores	0.6		
		Lagomorphs	28.0		
		Birds	33.3		
		Invertebrates	4.2		
Least Weasels	Great Britain	Mammals			King and Moors 1979
		Mice and Voles	55.3		
		Rats and Squirrels	2.6		
		Insectivores	1.3		
		Lagomorphs	19.1		
		Birds	14.5		
		Invertebrates	5.3		

Table 24. (continued)

Species	Location	Prey taxon	Percent	Comments	Reference
	Great Britain	Small rodents	89	Data represent frequency of occurance of prey types in 215 scats.	King 1980
		Voles	67		
		Cleithrionomys	41		
		Microtus	19		
		Unidentified vole	7		
		Mice (*Apodemus*)	16		
		Unidentified rodents	7		
		Birds	23		
		Passerines	12		
		Non-passerines	2		
		Unidentified birds	3		
		Eggs	7		
		Lagomorph	0.5		
		Mole	0.5		
	Sweden	*Microtus*	46	Data represent frequency of occurance of prey types in 148 scats.	Erlinge 1975
		Cleithrionomys	9		
		Apodemus	10		
		Arvicola	16		
		Lagomorph	15		
		Soricidae	1		
		Birds	2		
		Reptile	1		

Food Consumption Rate

Observations of three long-tailed weasels (sex not reported) indicate that four mice/day would "sustain them in apparent health" (Quick 1951). Brown and Lasiewski (1972) report the mean (±STD) metabolism of male and female long-tailed weasels to be 1.36 ±0.2 and 0.84 ±0.12 kcal/hr, respectively. Assuming that male and female weasels weigh 0.297 kg and 0.153 kg (Brown and Lasiewski 1972), respectively, the diet consists exclusively of small mammals with an energy content of 5163 kcal/kg dry weight (Golley 1961), and the water content of small mammals is 68% (EPA 1993), male and female weasels consume 0.067 and 0.080 kg food/kg BW/d. For comparison, food ingestion by male and female long-tailed weasels, estimated using Eq. 14 is 0.266 and 0.299 kg food/kg BW/d, respectively [assuming BW from Brown and Lasiewski (1972), diet consisting only of small mammals, and water content of small mammals is 68% (EPA 1993)].

No data were found concerning food ingestion by short-tailed weasels. Using Eq. 14 and assuming body weights for males and females reported by Burt and Grossenheider (1976; Table 23), a diet consisting only of small mammals with water content 68% (EPA 1993), food ingestion rates of 0.29 to 0.34 kg food/kg BW/d are estimated for males and 0.33 to 0.41 kg food/kg BW/d for females.

Food ingestion by least weasels has received more attention than that for other weasels. Golley (1960) observed food consumption of 0.41 and 0.42 kg/kg/d for a single least weasel (assumed to weigh 0.36 kg) on a diet of *Microtus* or white mice (*Mus*), respectively. Moors (1977) observed mean (± STD) food ingestion by male and female least weasels to be 0.33 ± 0.06 and 0.36 ± 0.08 kg/kg/d, respectively. The

greatest food ingestion rates are reported by Gillingham (1984); mean (± STD) ingestion by six individuals (sex not reported) was 0.56 ± 0.03 kg/kg/d.

Water Consumption Rate

Weasels require a constant supply of drinking water, drinking small amounts frequently (Svendson 1982). Long-tailed weasels are reported to consume 25 mL water/d (Svendson 1982). No other literature data were found describing water ingestion by weasels. Using Eq. 21, water ingestion rates may range from 0.11 L/kg BW/d for long-tailed weasels weighing 0.297 kg to 0.15 L/kg BW/d for least weasels weighing 0.022 kg. If other body weight values are used, the water ingestion rate should be recalculated.

Soil Ingestion

No literature data were found describing soil ingestion by any weasel species. Beyer et al. (1994) report soil consumption by red fox to be 2.8% of daily food consumption. Values for weasels may be comparable.

Respiration Rate

No literature data were found describing inhalation by weasels. Using Eq. 23, inhalation rates may range from 0.70 m^3/kg BW/d for long-tailed weasels weighing 0.297 kg to 1.17 m^3/kg BW/d for least weasels weighing 0.022 kg. If other body weight values are used, the inhalation rate should be recalculated.

Metabolism

Brown and Lasiewski (1972) found that cold-stressed long-tailed weasels lost body heat more rapidly and had metabolic rates 50-100% greater than would be expected for a 'normal' shaped animal of similar weight. Higher metabolic rates and greater thermal conductance for weasels relative to other mammals are also reported by Casey and Casey (1979) and Chappell (1980). Similarly, Iversen (1972) observed that the basal metabolic rate of small mustelids (<1 kg BW, includes both short-tailed and least weasels) was greater than that for larger mustelids (>1 kg BW). Metabolism for small mustelids was described by the following equation:

$$M = 0.958BW^{0.55} \tag{53}$$

where

M = basal metabolic rate (kcal/d)
BW = body weight (kg)

The higher metabolic rates and thermal conductance of weasels are attributed to greater surface area, shorter fur, and the inability of weasels to attain a spherical posture that would reduce heat loss (Brown and Lasiewski 1972).

Habitat Requirements

Habitat preferences of weasels are highly variable. All species tend to be most abundant in habitats with large small mammal populations and near bodies of water. Quick (1944) observed that long-tailed weasels in Michigan spent 53% of their time in crop and fallow land, 29 % in plowed fields, and 18% in forested areas. Stubble and plowed fields appeared to be preferred hunting areas. Similar observations were made by Polderboer et al. (1941). In contrast, Gamble (1981) found that long-tailed weasels preferred late seral stage habitats where prey species diversity was greatest. In southern Ontario, long-tailed weasels used habitats ranging from grassland to forest, with no apparent preference (Simms 1979b).

Short-tailed weasels occur from agricultural lowlands, woodlands, and meadows to montane habitats 3,000 - 4,000 m in elevation; dense forests and deserts are avoided (Svendson 1982). In southern Ontario, short-tailed weasels were observed to prefer early successional habitats and avoid forests (Simms 1979b).

Habitats used by least weasels include marshes, meadows, cultivated fields, brushy areas, and open woods (Svendson 1982). In Wisconsin, high marsh habitats with the water table at or near the surface for a good part of the year are preferred (Beer 1950). Erlinge (1974) observed spruce plantations and regenerating clearings to be most preferred by least weasels.

Home Range

Home ranges of weasels vary by sex, habitat, food availability and season, with smaller species having smaller home ranges (Svendson 1982). King (1975) reports home ranges for least weasels in a deciduous forest in Great Britain to be 7-15 ha for males and 1-4 ha for females. In the Bialowieza Forest of eastern Poland, home ranges for male least weasels increased from 11-37 ha during a rodent outbreak to 117-216 ha during a rodent population crash (Jedrzejewski et al.1995). Erlinge (1977) reports home ranges for male and female short-tailed weasels in Sweden to be 2-3 ha and 8-13 ha, respectively. In contrast, home ranges for short-tailed weasels in Ontario ranged from 20-25 ha and 10-15 ha for males and females, respectively (Simms 1979b). Home ranges for long-tailed weasels have been reported to range from 5-16 ha in Iowa (Polderboer et al. 1941) to 81-121 ha in Michigan and Colorado (Quick 1944, 1951).

Population Density

Weasel population densities vary considerably by season, food availability, and species (Svendson 1982). For example, densities of least weasels in the Bialowieza Forest of eastern Poland range from 0.52 to 2.73 individuals / km^2 in winter, declining to 0 to 1.9 individuals / km^2 in early spring (Jedrzejewski et al.1995). Midsummer densities varied from 4.2 to 4.8 individuals / km^2 in years of moderate prey abundance, to 10.2 individuals / km^2 during a rodent population peak, to 1.9 individuals / km^2 during the prey population crash. In a study of a 95 ha area in southern Ontario comprised predominantly of early successional habitat, Simms (1979b) observed an overall density of short-tailed weasels of 5.97 individuals / km^2. However, if only preferred habitat types are considered, density is 10.53 individuals / km^2. Svendson (1982) reports that densities of long-tailed weasels may range from 6 to 7 individuals/ km^2, while in the Rocky Mountains of Colorado, 0.77 individuals/ km^2 are reported (Quick 1951).

Population Dynamics/Survival

Population fluctuations of weasels are associated with the abundance of prey species. Keith and Cary (1991) observed that 81% of the variation in abundance of weasels (*M. frenata* and *M. erminea*) was attributed to fluctuations in the abundance of hares, voles and mice in Alberta, Canada. In the Bialowieza Forest of eastern Poland, abundance of least weasels was observed to be positively correlated with the abundance of voles and mice (Jedrzejewski et al. 1995).

Longevity of weasels is not well documented. Mean age at death for least weasels in Great Britain was 11 months (King 1975). The lifespan for short-tailed weasels in the wild is reported to be 4 to 6 years (Svendson 1982). In a study of short-tailed weasels in New Zealand, the mean age of individuals captured was 15 months; maximum longevity was 5 years (King et al. 1996). Age-specific mortality of first year individuals was 76%. In Colorado, marked adult long-tailed weasels were observed in the same area for 3 years (Svendson 1982).

Reproduction/Breeding

Both long-tailed and short-tailed weasels display delayed implantation (Svendson 1982). Fertilized ova develop to the blastocyst stage in approximately 14 days, then remain free in the uterus for the next 9 to

10 months (King 1983). Active gestation, from implantation of the embryo to parturition, takes approximately 4 weeks (King 1983). The least weasel, in contrast, does not have delayed implantation; kits are born approximately 41 days following fertilization (Svendson 1982). Additional reproductive parameters for North American weasels are summarized in Table 25.

Table 25. Summary of reproductive characteristics for North American weasels (data from Svendson 1982)

Species	Age at sexual maturity	Breeding season	Gestation	Litter size	Number litters/year
Long-tailed weasel	♂: 1 yr ♀: 3-4 months	July-August	~ 278 days; 27 days implantation	6-9	1
Short-tailed weasel	♂: 1 yr ♀: 3-4 months	July-August	~ 270 days; 21-28 days implantation	6-9	1
Least weasel	♂: 3-4 months ♀: 3-4 months	All year	~ 41 days; no delayed implantation	3-6	2-3

Behavior

Weasels are active year-round and do not hibernate (Svendson 1982). While commonly considered to be nocturnal, weasels tend to be most active during the daytime (Svendson 1982). Erlinge (1980) observed seasonal changes in daily activity; short-tailed weasels tended to be nocturnal in winter and diurnal in summer.

3.3.9 Green Heron (*Butorides virescens*)

The green heron (also known as the green-backed heron) is in the order Ciconiformes, family Ardeidae. This small, compact wading bird is part of a world-wide complex of related species, considered by some to be a single species (Davis and Kushlan 1994). This species is notable in that it has been observed to use a variety of baits and lures to catch prey.

Distribution

In eastern North America, the green heron occurs from the Atlantic Coast to the Great Plains, from southeastern Canada to the Gulf Coast and Florida (Davis and Kushlan 1994). In the west, it is found along the Pacific Coast to Vancouver Island. Range of the green heron is limited by aridity, altitude, and high latitude (Davis and Kushlan 1994).

Body Size and Weight

The green heron is small and stocky (41-46 cm long) with neck and legs shorter than those in other herons (Davis and Kushlan 1994). Dunning (1993) reports the mean body weight of green herons from Florida to be 212±5.92 g (mean±STD; n=34; sex not stated). In Louisiana, the mean body weight of 16 adults and 14 juveniles was 241 g and 219 g, respectively (Davis and Kushlan 1994). Meyerriecks (1962) reports body weights for two males and a female to be 158 g, 191.6 g, and 181.5 g, respectively.

Food Habits and Diet Composition

The diet of green herons consists primarily of fish (40 to >90%; Table 26). Other prey items include crayfish and other crustaceans, insects, spiders, and amphibians. Fish consumed are generally small in size

In Michigan, Alexander (1977) observed the following size distribution of fish consumed: 0 to 25.4 mm, 60%; 25 to 51 mm, 37%; 51 to 76 mm, 1.1%; and 76 to 100 mm, 2.2%. Prey consumed by herons in Louisiana ranged from 10 to 100 mm (Davis and Kushlan 1994).

Table 26. Diet composition of green herons

Location	Prey taxon	Percent volume	Percent frequency	Reference
Louisiana (n=27) data from late summer	Fish	93	93	Davis and Kushlan 1994
	Mosquitofish (*Gambusia affinis*)	1	11	
	Shiners (*Notropis* spp.)	2	7	
	Sunfish (*Lepomis* spp.)	35	26	
	Pirate perch (*Aphredoderus sayanus*)	2	4	
	Threadfin shad (*Dorosoma petenense*)	53	48	
	Crustacea	1	22	
	Crayfish (Cambarinae)	1	11	
	Prawns (*Palaemonetes kadiakensis*)	<1	11	
	Insecta	6	63	
	Coleoptera	<1	4	
	Hemiptera	1	19	
	Odonata	2	48	
	Orthoptera	3	26	
	Arachnida	1	22	
	Water spiders (*Dolomedes* spp.)	1	22	
Throughout United States (N=255)	Noncommercial fishes	38.52		Meyerriecks 1962
	Food fishes	5.91		
	Undetermined fish fragments	0.96		
	Crustaceans	20.64		
	Insects	23.65		
	Spiders and other invertebrates	10.32		
Michigan (n=12)	Fish	67		Alexander 1977
	Red belly dace	7.7[a]		
	Creek chub	3.3[a]		
	Darter	3.3[a]		
	Brook stickleback	62.2[a]		
	Fathead minnow	13.3[a]		
	Mudminnow	7.7[a]		
	Largemouth bass	2.2[a]		
	Crustaceans	1		
	Insects	9		
	Amphibians	10		
	Vegetation	3		
	Unidentified	10		

[a] Values represent percent of total fish species consumed.

Food Consumption Rate

Kushlan (1978) developed a model for estimation of daily food ingestion rates by herons

$$\log I_f = 0.966 (\log BW) - 0.64 , \qquad (54)$$

where

I_f = food ingestion rate (g fresh wt. /individual/d),
BW = body weight (g).

Assuming a body weight of 212 g (Dunning 1993), green herons are estimated to consume 0.19 g/g/d. This estimate is comparable to that observed for two nestling green herons, just prior to fledging (16% of body

mass/d; Junor 1972). In contrast, Alexander (1977) estimates that green herons in Michigan consume 50% of their body mass in food per day. No data are presented to support this estimate, however.

Water Consumption Rate

No literature data were found describing water consumption by green herons. Using Eq. 22 and assuming a body weight of 212 g (Dunning 1993), the average water ingestion rate is estimated to be 0.098 L/kg BW/d If other body weight values are used, the water consumption rate should be recalculated.

Soil Ingestion

Data concerning soil ingestion by green herons were not located in the literature. As a piscivorous, nonfossorial species, soil ingestion is likely to be negligible.

Respiration Rate

No literature data were found describing inhalation by green herons. Using Eq. 24 and assuming a body weight of 212 g (Dunning 1993), the average inhalation rate is estimated to be 0.58 m^3/kg BW/d. If other body weight values are used, the inhalation rate should be recalculated.

Metabolism

No literature data on metabolism were located.

Habitat Requirements

Green herons are highly flexible, using almost any available fresh or salt water habitat within their range (Meyerriecks 1962). Their primary requirement is dense vegetation. Green herons forage in swamps, marshes, riparian zones along creeks or human-made ditches, pond or lake edges, etc. (Davis and Kushlan 1994). These herons generally avoid open flats frequented by other, longer-legged herons.

Home Range

Davis and Kushlan (1994) report that green herons defend feeding territories from conspecifics; however, specific data on home range and territory size in this species are lacking.

Population Density

Because green herons are generally solitary and widely dispersed, population density estimates are problematic (Davis and Kushlan 1994).

Population Dynamics/Survival

There are few data on survivorship or longevity in green herons. Banding records indicate longevity of at least 7 years (Davis and Kushlan 1994). Limited data on populations indicate somewhat increasing abundance in the eastern United States, with range expansions at its northern and western limits (Davis and Kushlan 1994).

Reproduction/Breeding

Data on reproduction in green herons was derived from Bent (1926), Meyerriecks (1962), DeGraaf et al. (1981), and Davis and Kushlan (1994). Green herons may nest singly or in colonies. Nests are frequently in trees or shrubs near water, typically 3 to 4.5 m in height. In New York, eggs may be present from April 29 to August 4. Clutch sizes range from three to six eggs but are typically four to five eggs. Incubation lasts 19 to 21 days. Hatching success averages 78.9%. The nestling period lasts 16 to 17 days. Green herons

produce one clutch/year in northern latitudes, two per year in the south. Green herons are sexually mature at one year of age but generally do not breed until their second year.

Behavior

Green herons use the fewest number of feeding behaviors reported for North American day herons (Davis and Kushlan 1994). Of 36 potential behavior types, green herons used only 15. Green herons are also less active than other herons. Green herons are known to bait for fish using bread crusts, feathers, insects, worms, sticks, and plastic (Davis and Kushlan 1994).

Green herons are migratory in the northern parts of their range (Meyerriecks 1962). Migration generally occurs at night, either singly or as flocks of 50 or more individuals.

Social Organization

Green herons are not social outside the breeding season (Meyerriecks 1962). They are typically solitary foragers. During the breeding season, they may nest singly or form small colonies of up to 30 pairs (DeGraaf et al 1981). Green herons may also be found as part of mixed breeding colonies with other heron species (Davis and Kushlan 1994).

3.3.10 Burrowing Owl (*Speotyto cunicularia*)

The burrowing owl is in the order Strigiformes, family Strigidae. This owl is unique among North American owls in that it is diurnal, forms loose colonies, and is very tolerant of human activity (Haug et al. 1993).

Distribution

The burrowing owl has a very broad distribution in the Americas. This species occurs in suitable habitat throughout western North America, from southern Canada to southern Mexico (Johnsgard 1988, Haug et al. 1993). Populations also occur in southern Florida, the western Caribbean islands, and in Central and South America to Tierra del Fuego.

Body Size and Weight

The burrowing owl is a small owl with total body lengths of males and females ranging from 19 to 25 cm (Haug et al. 1993). Earhart and Johnson (1970) report that, in contrast to other North American owls, male borrowing owls are longer winged and heavier than females. More recent data do not support this observation (Haug et al. 1993). Body weights of borrowing owls from several locations are presented in Table 27.

Table 27. Body weights (g) for burrowing owls, *Speotyto cunicularia*

Location	Sex	N	Mean	Reference
Colorado	Male	38	146.3 ± 1.9 [a]	Haug et al. 1993
	Female	31	156.1 ± 3.6	
Florida	Male	111	148.8 ± 1.5	
	Female	162	149.7 ± 1.7	
Throughout North America	Male	31	158.6 (120-228)[b]	Earhart and Johnson 1970
	Female	15	150.6 (129-185)	

[a] mean± SE.
[b] mean (range).

Food Habits and Diet Composition

Burrowing owls are opportunistic feeders, foraging on arthropods, small mammals, and small birds (Earhart and Johnson 1970; Johnsgard 1988; Haug et al. 1993; Table 28). Diets vary by season, according to availability of prey (Thomsen 1971; Marti 1974; Haug et al. 1993). Food habits of burrowing owls from several locations are summarized in Table 28. Size of prey taken by burrowing owls is small; mean weight is 3 g with 91.2 % being ≤ 1 g (Marti 1974). Vegetation observed in diet of owls from California is attributed to stomach contents of prey (Thomsen 1971; Table 28).

Table 28. Diet composition of burrowing owls

Location	Prey taxon	Percent	Comments	Reference
California	Meadow vole	27.63	Values represent mean total biomass observed in pellets over four seasons	Thomsen 1971
	Jackrabbit	2.28		
	Pocket gopher	1.25		
	Norway rat	0.38		
	House mouse	0.25		
	Hoary bat	0.025		
	Western meadowlark	0.075		
	Blackbird	0.1		
	Shorebirds	0.05		
	Unidentified birds	3.05		
	Toad	0.28		
	Jerusalem cricket	11.25		
	Unidentified orthoptera	0.075		
	Coleoptera	16.05		
	Isopoda	0.05		
	Sand and dirt	4.13		
	Stones	0.9		
	Vegetation	32.35		
Idaho	Mammals	68	Values represent percent biomass in pellets	Gleason and Craig 1979
	Birds	1		
	Amphibians	3		
	Arachnids	4		
	Insects	25		

Table 28. (continued)

Location	Prey taxon	Percent	Comments	Reference
Colorado	Mammals		Values represent	Marti 1974
	Sylvilagus spp.	0.23	mean percent of	
	Perognathus spp.	0.17	numbers observed	
	Reithrodontymys spp.	1.17	in pellets collected	
	Peromyscus maniculatus	5.38	over six months	
	Microtus ochrogaster	2.03		
	Other mammals	0.22		
	Birds	0.12		
	Reptiles	0.03		
	Crayfish	0.38		
	Insects			
	Gryllidae	9.78		
	Acrididae	9.37		
	Cicindelidae	0.30		
	Carabidae	50.27		
	Scarabidae	10.13		
	Silphidae	2.83		
	Tenebrionidae	3.15		
	Curculionidae	1.40		
	Other insects	2.58		
	Spiders	0.23		

[a] Mean and range of observations from three locations.

Food Consumption Rate

Coulombe (1970) reports the mean (±STD) daily energy expenditure by burrowing owls in summer (21-26°C) and winter (10°C) to be 0.18 ± 0.05 kcal/g/d and 0.14 ± 0.036 kcal/g/d, respectively. Using Eq. 20 [assuming a diet of 90.7% invertebrates and 9.3% small mammals (Table 28; Marti 1974), caloric densities and water content of invertebrates and small mammals of 5.278 kcal/g and 76.3% (Bell 1990) and 5.163 kcal/g (Golley 1961) and 68% (Table 4), respectively], mean daily food consumption by burrowing owls is estimated to be 0.046 g/g/d in summer and 0.036 g/g/d in winter. These estimates are substantially lower than that estimated using the same assumptions and Eq. 18 (summer = 0.165 g/g/d; winter = 0.153 g/g/d).

Water Consumption Rate

No literature data were located concerning water ingestion rates for burrowing owls. Using Eq. 22, owls weighing 0.15-0.16 kg are estimated to consume approximately 0.11 L/kg/d.

Soil Ingestion

Sand, dirt, and rocks accounted for 0.12 to 15% of the volume of pellets of burrowing owls from California (mean ± STD: 5.0 ± 5.9;Thomsen 1971).

Respiration Rate

Burrowing owls are adapted to high CO_2 and low O_2 concentrations they experience in burrows. While respiration rates for bobwhite increased sharply in response to decreasing O_2 concentration, that for burrowing owls remained constant (Boggs and Kilgore 1983). Average (±SE) respiration rates for resting

burrowing owls under normal conditions is 129 ± 4.5 mL/min. or 1.12 m^3/kg/d (Boggs and Kilgore 1983). This measured value is almost twice that estimated using Eq. 24: 0.6 m^3/kg/d for owls weighing 0.15-0.16 kg.

Metabolism

The metabolism and physiology of burrowing owls was extensively studied by Coulombe (1970). Oxygen consumption varied in relation to ambient temperature and was described by the following

$$VO_2 = 1.44 - 0.0324\,(T_A - 13.66) \text{ for } T_A < 25°C, \quad (55)$$

$$VO_2 = 1.05 \pm 0.56\ (\bar{x} \pm 95\%\ CI) \text{ for } T_A\ 25\text{-}37°C, \quad (56)$$

and

$$VO_2 = 1.32e^{0.0911\,(T_A - 41.27)} \text{ for } T_A > 37°C, \quad (57)$$

where

VO_2 = oxygen consumption in cm^3/g/h,
T_A = ambient temperature.

Habitat Requirements

The typical habitat of burrowing owls consists of dry, open, treeless plains, heavily grazed or low-quality grassland, or desert vegetation (Johnsgard 1988; Haug et al. 1993). Other areas include golf courses, cemeteries, road-sides, airports, vacant lots, etc. (Haug et al. 1993). Borrowing owls are frequently associated with burrowing mammals (MacCracken et al. 1985; Rich 1986; Green and Anthony 1989; Desmond and Savidge 1996). Although the presence of burrows appears to be a critical requirement for western owls, owls in Florida usually excavate their own burrows (Haug et al. 1993). In Saskatchewan, burrowing owls foraged in grass-forb areas but avoided croplands and grazed pasture (Haug and Oliphant 1990).

Home Range

Although the mean home range size of owls in Saskatchewan was 241 ha (range = 14-481 ha; Haug and Oliphant 1990), 95% of all movement occurred within 600 m of nest burrows. Territories are generally limited to the immediate area around burrows; adjacent pairs may share foraging ranges (Johnsgard 1988). In California, Thomsen (1971) observed a mean territory size of 0.8 ha (range: 0.04-1.6 ha).

Population Density

Nest density is probably influenced by the availability of nest burrows (Johnsgard 1988). In the Imperial Valley of California, mean (±STD) density was 0.035±0.018 individuals/ha (range: 0.003-0.06; Coulombe 1971). Desmond and Savidge (1996) report that burrowing owl densities varied according to the size of the prairie dog towns they were associated with; small towns (<35 ha) had 0.1-30 owls/ha while large towns (≥35 ha) had 0.03-0.4 owls/ha. Densities of owls, within owl clusters in large prairie dog towns ranged from 0.9-2.5 owls/ha. As the size of the prairie dog town increased, the abundance of owls increased, but their density decreased (Desmond and Savidge 1996).

Population Dynamics/Survival

Evidence suggests that burrowing owl populations are declining across much of their range (Haug et al. 1993). The annual survival of burrowing owls in California was 30% for juveniles and 80% for adults (Thomsen 1971). Longevity in excess of 8 years has been reported (Haug et al. 1993).

Reproduction/Breeding

Data on reproduction in burrowing owls was derived from Martin (1973), Johnsgard (1988), Green and Anthony (1989), and Haug et al. (1993). Burrowing owls nest in underground burrows that they may or may not excavate themselves. Eggs may be present from mid-March to May. Clutch sizes range from 3 to 12 eggs but are typically 6 to 8 eggs. Incubation lasts 27 to 30 days. Hatching success ranges from 55 to 90.3%. The nestling period lasts 40 to 45 days. Generally, only one clutch/year is produced. Burrowing owls are sexually mature at 1 year of age.

Behavior

Burrowing owls are migratory only in the northern part of their range; birds in Florida and southern California are sedentary (Johnsgard 1988). While burrowing owls are generally crepuscular in their foraging (Coulombe 1971), hunting has been observed during both day and night. Insects are generally hunted by day and small mammals at night (Haug et al. 1993). Thomsen (1971) observed dust bathing in this species.

Social Organization

Burrowing owls are semicolonial, forming loose colonies (Haug et al. 1993). Migrant birds, however, are solitary.

3.3.10 Cooper's Hawk (*Accipiter cooperii*)

The Cooper's hawk is in the order Falconiformes, family Acciptridae. Cooper's hawks are generally woodland species. They are intermediate in size between the other two congeneric accipiters in North America: the sharp-shinned hawk (*A. striatus*) and the northern goshawk (*A. gentilis*; Rosenfield and Bielefeldt 1993).

Distribution

The Coopers's hawk is found in forested areas throughout the conterminous United States, southern Canada, and south to central Mexico (Rosenfield and Bielefeldt 1993).

Body Size and Weight

The Cooper's hawk is medium sized (approximately that of a crow), with short, rounded wings and a long, rounded tail (Rosenfield and Bielefeldt 1993). Males are significantly smaller than females (Storer 1966). Birds in the eastern United States are larger than birds in the western United States. Body weights of Cooper's hawks are presented in Table 29.

Table 29. Body weights (g) for the Cooper's hawk, *Accipiter cooperii* [a]

Location	Status	Sex	N	Mean ± STD
Eastern United States	Migrant	Male	51	349 ± 20
		Female	57	529 ± 36
	Breeding	Male	15	338 ± 20
		Female	31	566 ± 40
	Juvenile, migrant	Male	53	335 ± 26
		Female	58	499 ± 40
Western United States	Migrant	Male	177	281 ± 19
		Female	416	439 ± 35
	Breeding	Male	48	280 ± 19
		Female	20	473 ± 41
	Juvenile, migrant	Male	183	269 ± 22
		Female	310	399 ± 36
	Juvenile, breeding [b]	Male	9	276 ± 26
		Female	5	486 ± 29

[a] All data from Rosenfield and Bielefeldt (1993).
[b] nonbreeding, summer birds.

Food Habits and Diet Composition

The diet of Cooper's hawks has been well studied. Sherrod (1978) and Rosenfield and Bielefeldt 1993) provide reviews of literature concerning diet composition. In general, Cooper's hawks are reported to forage primary on medium-sized birds (approximately 60-80%), with small mammals making up the remainder. However, Bielefeldt et al. (1992) suggest that the methods used in most dietary studies overestimate the proportion of birds in the diet and that small mammals may constitute the primary food. Species consumed include the American robin, jays, northern flicker, European starling, grouse, quail, pheasant, crows, doves, sparrows, chipmunks, hares, squirrels, deer mice, and bats. The diet composition of Cooper's hawks from several locations is presented in Table 30.

Table 30. Diet composition of Cooper's hawks

Location	Prey taxon	Percent	Comments	Reference
Northwestern Oregon	Birds ($\bar{x}_{size}$= 79.2g)	74	Diet composition determined from prey remains at nests. Species composition listed in appendix	Reynolds and Meslow 1984
	Mammals ($\bar{x}_{size}$= 296.4g)	25		
Eastern Oregon	Birds ($\bar{x}_{size}$= 123.7g)	47		
	Mammals ($\bar{x}_{size}$= 147.5g)	43		

Table 30. (continued)

Location	Prey taxon	Percent	Comments	Reference
Northwest Washington	Birds Mammals	85 15	Diet composition determined by direct observation of prey deliveries to nests. Primary prey types were American robin and California quail	Kennedy and Johnson 1986
Michigan	Birds Mammals	84.4 15.6	Diet composition determined by analysis of gullet contents of nestlings and residues in nests	Hamerstrom and Hamerstrom 1951
New York and Pennsylvania	Birds Mammals	81.8 18.2	Diet composition determined from pellets prey remains at nests. Primary prey types were starlings, flickers, eastern meadowlarks, and chipmunks.	Meng 1959
Wisconsin	Birds Mammals	52 (42-60)[a] 48 (40-58)[a]	Diet composition determined by crop content analysis	Bielefeldt et al. 1992
Michigan	Birds Mammals	29 71		

[a] Mean and range of observations from three locations.

Cooper's hawks take prey ranging in size from 37 to 85% of their body weight (Rosenfield and Bielefeldt 1993). Mean prey size taken by Cooper's hawks in eastern and western Oregon was 134.7 g and 136.3 g, respectively (Reynolds and Meslow 1984). Males generally take smaller prey than females (Rosenfield 1988). In Washington, the percentage of prey taken that was < 91 g was 81% for males and 65% for females (Kennedy and Johnson 1986).

Food Consumption Rate

Craighead and Craighead (1969) observed a food consumption of 0.197 g/g/d for a single male maintained in captivity during fall and winter. Average consumption by two females and a male, during spring and summer, was 0.165 g/g/d (range = 0.16 to 0.173; Craighead and Craighead 1969). Using Eq.s 18 and 20, food ingestion rates of Cooper's hawks are estimated to range from 0.1 g/g/d to 0.13 g/g/d [assuming body weights of 566 g and 280 g (Table 26) and water content of birds and mammals of 68% (Table 4)].

Water Consumption Rate

No literature data were located concerning water ingestion rates for Cooper's hawks. Using Eq. 22, water ingestion rates of Cooper's hawks are estimated to range from 0.07 L/kg/d to 0.09 L/kg/d [assuming body weights of 566g and 280g (Table 26)].

Soil Ingestion

No literature data were located concerning soil ingestion by Cooper's hawks. Soil ingestion is likely to be negligible and consist only of that associated with prey that are consumed.

Respiration Rate

No literature data were located concerning inhalation rates for Cooper's hawks. Using Eq. 24, inhalation rates of Cooper's hawks are estimated to range from 0.47 m^3/kg/d to 0.55 m^3/kg/d [assuming body weights of 566g and 280g (Table 26)].

Metabolism

While generally viewed as "sit and wait" predators, accipiters are more active than previously thought. Consequently, their metabolic rates are generally higher than those observed in other Falconiformes (Kennedy and Gessaman 1991). Mean metabolic heat production of male and female Cooper's hawks at rest are 2516.25 and 2655.50 mW, respectively (Kennedy and Gessaman 1991).

Habitat Requirements

Cooper's hawks are a forest species, occurring in deciduous, mixed, and evergreen forests; floodplain forests; and wooded swamps (DeGraaf et al. 1981; Rosenfield and Bielefeldt 1993). Forest edges are often used and may serve as primary hunting sites. They have also been observed to use urban habitats (Clark 1977). Nesting habitat in Oregon was intermediate in both age and density of trees, relative to those used by sharp-shinned (younger and denser) and goshawks (older and more open; Reynolds et al. 1982). In the central Appalachians, the nest habitat of Cooper's hawks was characterized as mature forest with well developed understory and herb layer (Titus and Mosher 1981).

Home Range

Cooper's hawks require considerable space. Home ranges during the breeding season may range from 400 to 1800 ha (Rosenfield and Bielefeldt 1993). The mean size of winter ranges of four Cooper's hawks in Michigan was 192 ha (range=67 to 435 ha; Craighead and Craighead 1969). Summer home range size for this population was highly variable, ranging from 18 to 531 ha; but mean size (203 ha) was comparable to that in winter.

Population Density

Density data for Cooper's hawks are based on the abundance of nests. As a consequence, the data are biased because nonbreeding individuals are not represented. Regardless, available data indicate this species to be diffuse throughout its range. Craighead and Craighead (1969) report densities of 0.017 pairs/ha in Michigan and 0.046 pairs/ha in Wyoming. In Oregon, mean density was 0.00045 pairs/ha (Reynolds and Wight 1978).

Population Dynamics/Survival

Although eastern populations declined in the mid-1900s and the species is listed as threatened or endangered in several eastern states, evidence suggests the presence of recovering breeding populations in many areas (Rosenfield and Bielefeldt 1993). Mean age at death reported from banding data was 16.3 months, with maximum longevity being 12 years. Mortality in the first year is 72 to 78%, then 34 to 37% in subsequent years (Rosenfield and Bielefeldt 1993).

Reproduction/Breeding

Data on reproduction in Cooper's hawks was derived from DeGraaf et al. (1981), Palmer (1988), and Rosenfield and Bielefeldt (1993). Cooper's hawks nest in extensive forests, woodlots of 4 to 8 ha, and occasionally in isolated trees. Nests are constructed of sticks, placed in a main crotch or on a horizontal limb against the trunk of live trees, typically 10.7 to 13.7 m in height. Eggs may be present from May to June. Clutch sizes range from three to six eggs, but are typically four to five eggs. Incubation lasts 34 to 36 days.

Hatching success ranges from 74 to 96%. The nestling period lasts 30 to 34 days for eastern birds and 27 to 30 days for western birds. Only one clutch/year is produced. Cooper's hawks generally do not breed until they are at least 2 years old.

Behavior

Cooper's hawks are diurnal, spending approximately 20% of the day hunting (Rosenfield and Bielefeldt 1993). Birds from the northern portion of their range are migratory, although some stay resident year-round, even in Canada (Palmer 1988). Southern birds may be locally migratory or more or less resident, leaving high elevations for more protected low elevations during winter.

Social Organization

Outside of the breeding season, Cooper's hawks are solitary. Small groups may form during migration, but these are incidental and are not the result of social interactions (Rosenfield and Bielefeldt 1993).

3.3.11 Western Meadowlark (*Sturnella neglecta*)

The western meadowlark is in the order Passeriformes, family Emberizidae. This bird is one of the most abundant and widely distributed birds in North America. It is similar in appearance to the eastern meadow lark (*Sturnella magna*), differing only in song (Lanyon 1994).

Distribution

Western meadowlarks range throughout western North America, west of the Mississippi River to the Pacific Coast (Lanyon 1994). They occur from the southern half of British Columbia, Alberta, Saskatchewan, and Manitoba in the north, to central Mexico in the south.

Body Size and Weight

The western meadowlark is a medium-sized terrestrial songbird, approximately 24 cm in length (National Geographic Society 1987) with a long, slender bill, short tail, and long legs (Lanyon 1994). Males meadowlarks weigh more than females. Body weights for western meadowlarks from different locations throughout their range are presented in Table 31.

Table 31. Body weights (g) for the western meadowlark, *Sturnella neglecta*

Location	Sex	N	Mean	Reference
South Dakota	Male	3[a]	111.9±2.2[b]	Wiens and Rotenberry 1980
	Female	3[a]	86.3±3.0[b]	
Texas	Male	3[a]	110.9±3.0[b]	
	Female	3[a]	90.1±1.1[b]	
Washington	Male	4[a]	113.2±1.5[b]	
	Female	4[a]	94.2±3.5[b]	
Nevada	Male	3	111.5±0.8	

Table 31. (continued)

Location	Sex	N	Mean	Reference
Saskatchewan	NS[c]	NS	103	Wiens and Innis 1974
Colorado	NS	NS	110	

[a] Number of sampling dates.
[b] mean ± standard deviation of means for n sampling dates.
[c] Not stated.

Food Habits and Diet Composition

Western meadowlarks are ground foragers that consume both plant material (primarily seeds) and invertebrates (Bent 1958; Lanyon 1994; Rotenberry 1980). Bent (1958) reports the diet to consist of approximately 30% plant and 70% insect foods. Food preferences of western meadowlarks are summarized in Table 32. The mean size of insects consumed by western meadowlarks in Washington ranges from 7.7 to 14.6 mm (Rotenberry 1980).

Table 32. Diet composition of western meadowlarks

Location	Prey taxon	Percent volume	Reference
Throughout North America (n=1920)	Plant material	36.7	Lanyon 1994
	Grain	30.8	
	Weed seeds	5.3	
	Miscellaneous	0.6	
	Arthropods	63.3	
	Coleoptera	21.3	
	Orthoptera	20.3	
	Lepidoptera	12.2	
	Hemiptera	1.7	
	Hymenoptera	5.6	
	Diptera	0.1	
	Arachnida	0.2	
	Miscellaneous insects	1.9	

Table 32. (continued)

Location	Prey taxon	Percent volume	Reference
Washington (n=23)[a]	Angiospermae		Rotenberry 1980
	Graminae	1.6	
	Miscellaneous forbs	0.3	
	Arachnida		
	Araneida	0.7	
	Solpugida	0.6	
	Insecta		
	Coleoptera		
	Curculionidae	14.8	
	Tenebrionidae	14.4	
	Scarabidae	5.2	
	Carabidae	7.6	
	Larvae	0.6	
	Miscellaneous	0.8	
	Hymenoptera		
	Formicidae	2.1	
	"Wasps"	1.5	
	Lepidoptera		
	Larvae	10.3	
	Diptera		
	Asilidae	0.4	
	Miscellaneous	0.3	
	Neuroptera	0.8	
	Hemiptera	1.1	
	Orthoptera	29.6	
	Homoptera		
	Cicadidae	7.4	
	Miscellaneous	0.3	

[a] Values represent means from 4 sampling dates.

Food Consumption Rate

Bryant (1914, cited in Lanyon 1984) estimates that daily food consumption by western meadowlarks is approximately three times its stomach capacity. Mean dry mass per stomach in Washington ranges from 0.35 to 1.3 g (mean±STD: 0.79±0.40; Rotenberry 1980). Assuming a body weight of 108.8 g and a diet consisting almost exclusively of insects (Rotenberry 1980) with a water content of 76.3% (Bell 1990), the mean daily food ingestion by western meadowlarks is estimated to be 0.028±0.014 g/g/d. This estimate is comparable to that obtained using Eqs. 19 and 20: 0.026 g/g/d (assuming body weight=108.8 g, diet=100% insects, water content= 76.3%).

Water Consumption Rate

Pierce (1974) reports *ad libitum* water consumption by western meadowlarks to be 18.6% of their body weight per day (0.186 L/kg/d). Minimum water consumption for weight maintenance was 66% of the *ad libitum* rate. This is equivalent to that estimated using Eq. 22 and assuming a body weight of 108.8 g (0.12 L/kg BW/d).

Soil Ingestion

Western meadowlarks are reported to ingest grit, probably to aid in digestion or as a source of inorganic nutrients (Gionfriddo and Best 1996). Grit was observed in 44% of the stomachs considered. The mean particle size in stomachs of adults was 1.4 mm with 2±3 particles/stomach (Gionfriddo and Best 1996). Data relating grit ingestion to food ingestion rate were not found in the literature, however. Consequently, estimation of a soil ingestion rate from these data is problematic.

Respiration Rate

No literature data were located concerning inhalation rates for western meadowlarks. Eq. 24, although developed for nonpasserine birds, may be used; however, significant uncertainty in the resulting estimate must be acknowledged.

Metabolism

Nocturnal and diurnal resting metabolic rates for western meadowlarks are 1.73 and 1.97 mL O_2/g/h, respectively (Pierce 1974). These values are low relative to other birds and represent adaptations to hot, open environments.

Habitat Requirements

Western meadowlarks are common in open habitats including native grasslands, pastures, hay and alfalfa fields, weedy borders, cropland, roadsides, orchards, and, occasionally, desert grasslands (Lanyon 1994). In areas where their ranges overlap, western meadowlarks generally prefer more arid habitats than eastern meadowlarks (Lanyon 1956; National Geographic Society 1987).

In an extensive study of habitat associations and avian communities in a shrub-steppe environment in Washington, Wiens and Rotenberry (1981) found western meadowlarks to be broadly distributed over most of the available habitat. While the density of meadowlarks did not correlate well with overall habitat variation, density was positively correlated with sagebrush, grass, and litter cover and negatively with bare ground.

Home Range

Male western meadowlarks defend multipurpose territories in which they forage, breed, and raise young (Lanyon 1994). Territories in Wisconsin varied from 1.2 to 6.1 ha but were generally 2.8 to 3.2 ha. Kendeigh (1941) reports territories to range from 4 to 13 ha in Iowa. Schaef and Picman (1988) report a mean territory size of 7 ha in Manitoba.

Population Density

Wiens and Rotenberry (1981) report densities of western meadowlarks in shrub-steppe habitat in Washington ranging from 0.02 to 0.88 individuals/ha. In an Iowa prairie, Kendeigh (1941) observed approximately 0.05 birds/ha. In a state-wide census of breeding birds in North Dakota, Stewart and Kantrud (1972) estimated the mean density of western meadowlarks to be 0.11 pairs/ha.

Population Dynamics/Survival

In good habitat, western meadowlarks can be very abundant. Stewart and Kantrud (1972) estimate western meadowlarks to be the fourth most abundant breeding bird in the North Dakota (behind horned larks, chestnut-collared longspur, and red-winged blackbirds). The state-wide population was estimated to be over 2×10^6 pairs. Although the longevity of captive birds ranges from 3 to 5 years, some individuals have lived as long as 10 years (Lanyon 1994). Survivorship in wild populations is unknown.

Reproduction/Breeding

Data on reproduction in western meadowlarks was derived from Bent (1958) and Lanyon (1994). Western meadowlarks make well-concealed nests on the ground, often in a shallow depression and frequently in thick vegetation. Eggs may be present from April to July, throughout the range. Clutch sizes range from 3 to 6 eggs but average 4.8 eggs. Incubation lasts 13 to 14 days, rarely 15 to 16 days. A hatching success of 53% has been reported in British Columbia. The nestling period lasts 10 to 12 days. Western meadowlarks may raise up to two clutches/year. Sexual maturity is reached in one year.

Behavior

Although western meadowlarks will tolerate other ground-nesting species in their territories, they aggressively defend against both conspecifics and eastern meadowlarks (in areas where both species are sympatric; Lanyon 1994).

Social Organization

During fall and winter, western meadowlarks form loose flocks of up to 200 individuals. The flocks may include eastern meadowlarks (Lanyon 1994).

3.3.12 Swallows

Swallows are in the order Passeriformes, family Hirundinidae. Eight species of swallows occur in North America: tree swallow (*Tachycineta bicolor*), violet-green swallow (*Tachycineta thalassina*), purple martin (*Progne subis*), bank swallow (*Riparia riparia*), northern rough-winged swallow (*Stelgidopteryx serripennis*), cliff swallow (*Hirundo pyrrhonota*), cave swallow (*Hirundo fulva*), and barn swallow (*Hirundo rustica*) (National Geographic Society 1987). All are aerial foraging species that forage over open fields or bodies of water (Imhof 1976).

Distribution

Swallow species are found throughout North America. Tree, bank, northern rough-winged, cliff, and barn swallows breed across the northern 3/4 of the United States into Canada and Alaska (except the rough-winged, which extends only to southern Canada; National Geographic Society 1987). Violet-green swallows occur in the west, from Alaska to Mexico. Purple martins breed east of the Rocky Mountains and along the Pacific Coast. Cave swallows occur only in Texas and southern New Mexico (West 1995).

Body Size and Weight

Swallows are small, long-winged birds. Body lengths range from approximately 13 cm for bank swallows to 20 cm for purple martins. Body masses for North American swallow species range from <15 to approximately 50 g (Table 33).

Food Habits and Diet Composition

The diet of swallows consists primarily of insects; however, some plant matter may be consumed (Beal 1918). The diet composition of swallow species in North America is summarized in Table 34. Flies (Diptera) are generally very important food items for swallows, comprising as much as 40% of the diet of some species (Quinney and Ankney 1985; Blancher and McNicol 1991; Table 34). Chironomid midges are an important food item of tree swallows, accounting for 33% of the diet of nestlings (Blancher and McNicol 1991). Because many swallows forage extensively over water (Brown and Brown 1995; DeJong 1996; Robertson et al. 1992; DeGraaf et al. 1981), aquatic prey constitute a significant portion of their diet.

Blancher and McNicol (1991) found prey of aquatic origins to account for 64.9, 71, and 54.9% of the diet of nestling, egg-laying female, and other adult tree swallows, respectively. Swallows generally consume small insects. Quinney and Ankney (1985) report that 99% of the insects consumed by tree swallows are ≤ 10 mm in length. Blancher and McNicol (1991) observed that ~90% of prey were ≤25 mm in length.

Food Consumption Rate

Brown and Brown (1995) report that cliff swallows forage at a rate of 3.4, 3.8, and 3.5 kcal/h during nest building, incubation, and nestling periods, respectively. Female tree swallows in New Brunswick, Canada, were observed to require 5.73±1.40 kJ/g/d (mean±STD; n=10; Williams 1988). Assuming that the diet consists exclusively of insects (Quinney and Ankney 1985) and that the energy and water content of insects is 22.09 kJ/g dry weight and 76.3%, respectively (Bell 1990), daily food consumption by tree swallows is 0.198±0.048 g/g/d.

Water Consumption Rate

No literature data were located concerning water ingestion rates for swallows. Estimated water ingestion rates among swallows may range from 0.24 L/kg BW/d to 0.16 L/kg BW/d (based on Eq. 22 and body weights of 15 and 50 g). In practice, water ingestion rates should be recalculated using body weights for species of interest.

Soil Ingestion

Swallows are reported to ingest grit, probably to aid in digestion or as a source of inorganic nutrients (Barrentine 1980; Mayoh and Zach 1986). Although Barrentine (1980) found grit in 80% of the stomachs of nestling barn swallows, the occurrence of grit in the stomachs of adults was only 22% (Gionfriddo and Best 1996). Among nestlings, particles ranged from 0.84 to 4 mm in diameter, with 4.8±4.5 (mean±STD) particles/stomach (Barrentine 1980). In contrast, the mean particle size in stomachs of adults was 1.2 mm, with 1±4 particles/stomach (Gionfriddo and Best 1996). Grit was found in 35 and 20% of the stomachs of nestling and adult tree swallows, respectively (Mayoh and Zach 1986). The number of particles and the mass of grit was greater in nestings than adults: the number of particles was 10.2±2.2 (mean±SE) in nestlings vs 0.8±0.8 in adults and mass (mg) was 17.2±2.6 in nestlings vs 6.1±6.1 in adults. Data relating grit ingestion to food ingestion rate was not found in the literature, however. Consequently estimation of a soil ingestion rate from these data is problematic.

Respiration Rate

No literature data were located concerning inhalation rates for swallows. Eq. 24, although developed for nonpasserine birds, may be used; however, significant uncertainty in the resulting estimate must be acknowledged.

Metabolism

Williams (1988) studied the field metabolism of tree swallows during the breeding season to evaluate whether aerial foraging species have higher energy requirements that other species. Resting and night-time basal metabolic rates were determined to be 79.3±12.6 and 59.5 mL O_2/h, respectively, for birds weighing 21.6±1.9 g. The results indicated that swallows have higher metabolic rates than birds with less energy-intensive lifestyles (e.g., ground foraging species). Additional information on the metabolism of swallows is included in a bioenergetics-based model of PCB accumulation by nestling tree swallows (Nichols et al. 1995).

Habitat Requirements

As aerial foraging species, all swallows require open areas that do not inhibit flight activities. Areas that may be used include open fields, farmland, suburban yards, marshes, bodies of water, riparian edge, broken forest, etc. (DeGraaf et al. 1981; Brown and Brown 1995; Robertson et al. 1992; Bent 1942; West 1995; DeJong 1996). Preferred habitats are generally near water. Some habitats are avoided, for example dense forest, desert, and alpine areas (Brown and Brown 1995). Prior to human development, nests were placed on cliffs or within tree cavities. Now, many human-made structures such as bridges or buildings may be used for nesting. Proximity to a mud source for nest building may also be a requirement for some species (Brown and Brown 1995). Purple martins originally nested in tree cavities but now rely extensively on human-made multiroom nest boxes (DeGraaf et al. 1981). As a cavity nester, tree swallows need dead trees (Robertson et al. 1992). Bank and northern rough-winged swallows frequently use burrows in earthen banks near water bodies (DeJong 1996; Stoner 1936; DeGraaf et al. 1981).

Home Range

Prior to incubation, tree swallows may travel up to 60 km from nest to forage. However, during incubation and nesting, males may travel 4-5 km and females 2-3 km in search of food (Robertson et al. 1992). Bank and barn swallows generally forage within 0.8 km or less from nest sites (Stoner and Stoner 1941; DeGraaf et al. 1981). Among cliff swallows, foraging is generally restricted to a 1.5-km radius around the colony; however, birds may travel up to 6 km to forage (Brown and Brown 1995).

Population Density

Because of their colonial nature and patchy distribution, densities of swallows can be highly variable, difficult to estimate, and dependant on habitat and availability of suitable nest sites. Additionally, density estimates based on breeding pairs are biased because nonbreeding floaters are not accounted for (Robertson et al. 1992). Some representative density estimates follow. Densities of foraging barn swallows of 0.64 individuals/ha have been reported in Illinois (DeGraaf et al. 1981). Breeding densities for barn swallows range from 0.077 pairs/ha in 'favorable' habitat in South Dakota to 0.27 pairs/ha in mixed agricultural/residential habitat in Maryland (DeGraaf et al. 1981). Among tree swallows, breeding densities have been reported to range from 3.5 to 500 pairs/ha, the later estimate resulting from nest boxes placed at an artificially high density (DeGraaf et al. 1981). The breeding density of northern rough-winged swallows in Michigan was approximately 0.18 pairs/ha (Lunk 1962).

Population Dynamics/Survival

First-year mortality among swallows is high: 68, 79, and 83% for cave, tree, and cliff swallows, respectively (West 1995; Robertson et al. 1992; Brown and Brown 1995). After the first year, survivorship improves, ranging from 40 to 60% (Robertson et al. 1992; Brown and Brown 1995). For rough-winged swallows, a 33% adult survival is required for population maintenance (DeJong 1996). Maximum longevity in swallows ranges from 5 years (rough-winged swallows; DeJong 1966) to 11 years (cliff and tree swallows; Robertson et al. 1992; Brown and Brown 1995).

Reproduction/Breeding

Reproductive parameters for North American swallows are summarized in Table 35. Reproductive success for rough-winged swallows in Michigan are reported to be 73, 61, and 65% for hatching, fledging, and overall nesting, respectively (Lunk 1962). Success rates for tree swallows are somewhat higher: hatching success = 88.4%, fledging success = 80.2, and overall nesting success = 78.8% (Robertson et al. 1995).

Behavior

Most North American swallows are migratory, traveling to winter ranges in the southern United States, Mexico, and South America (DeJong 1996; West 1995; Robertson et al. 1992; Brown and Brown 1995). Many swallows drink water while in flight, tipping their bills into water during low flight (DeJong 1996; Robertson et al. 1992; Brown and Brown 1995).

Social Organization

Swallows are generally considered highly social, gregarious birds. Many swallows are colonial, congregating in large breeding colonies. Bank swallow colonies may include 10 to more than 300 nests (DeGraaf et al. 1981). Cliff swallows are the most colonial; colonies of 1000 nests are common, with 3700 nests in the largest colony (Brown and Brown 1995). Rough-winged swallows are the least social (DeJong 1996), commonly forming groups of 3 to 12 individuals. These swallows nest singly or in small groups of 2 to 25 pairs, often at edges of bank swallow colonies.

Table 33. Body weights (g) for swallows

Species	Location	Sex and age	N	Mean	Range	Reference
Cave swallow	Yucatan, Mexico	Male: adult	3	19.0		West 1995
		Female: adult	3	17.7		
	Texas	Both: adult	25	20.4	18.4-22.3	Dunning 1993
Northern rough-winged swallow	Pennsylvania	Both: adult	47	15.9±0.58	10.3-18.3	Dunning 1993
	Not stated	Male: adult	9	14.59±0.54		DeJong 1996
		Female: adult	6	13.3±0.63		
Tree swallow	Southern Ontario	Male: adult > 2 years	86	20.4±1.5	17-24	Robertson et al. 1992
		Female: adult > 2 years	134	21.5±1.7	18-25.5	
	Pennsylvania	Both: adult	82	20.1±1.58	15.6-25.4	Dunning 1993
Cliff swallow	Nebraska	Male: adult during nesting	6797	23.9		Brown and Brown 1995
		Female: adult during nesting	3566	24.15		
	California	Both: adult	88	21.6±2.04	17.5-26.7	Dunning 1993
Purple martin	Maine	Both: adult	22	49.4±1.49		Dunning 1993
Violet-green swallow	California	Male: adult	16	14.4	13.0-16.3	Dunning 1993
		Female: adult	15	13.9	12.5-15.2	
Barn swallow	Morocco	Male: adult	1337	16.2	12.1-28.2	Dunning 1993
		Female: adult	994	15.8	11.0-24.8	
Bank swallow	New York	Both: adult	249	14.6	12.0-18.6	Stoner 1936

Table 34. Diet composition of swallows in North America

Species	Location	Taxa	Percent volume	Percent frequency	Comments	Reference
Purple martin	Throughout the United States and Canada (n=205)	Hymenoptera	23		Other consists of Ephemeroptera, spiders, and sowbugs	Beal 1918
		Diptera	16.09			
		Hemiptera/Homptera	14.58			
		Coleoptera	12.53			
		Lepidoptera	9.39			
		Orthoptera	1.09			
		Odonata	15.1			
		Other	8.09			
Cliff swallow	Throughout United States (N=375)	Ants	8.24		Other consists of Odonata, Ephemeroptera, spiders, and snails	Beal 1918
		Other Hymenoptera	20.51			
		Diptera	13.95			
		Hemiptera/Homptera	26.32			
		Coleoptera	26.8			
		Orthoptera	0.71			
		Other	2.97			
Barn swallow	27 states and Canada (n=467)	Ants	9.89			Beal 1918
		Other Hymenoptera	12.82			
		Diptera	39.49			
		Hemiptera/Homptera	15.1			
		Coleoptera	15.63			
		Lepidoptera	2.39			
		Orthoptera	0.51			
		Odonata	4			

Table 34. (continued)

Species	Location	Taxa	Percent volume	Percent frequency	Comments	Reference
Tree swallow	22 states and Canada (n=343)	Ants	6.37		90% of plant material consumed consisted of fruit of waxberry (*Myrica carolinensis*). Other consisted primairily of spiders	Beal 1918
		Other Hymenoptera	7.58			
		Diptera	40.58			
		Coleoptera	14.39			
		Lepidoptera	5.02			
		Orthoptera	0.37			
		Odonata	4			
		Other	4.64			
		Plant Material	16.9			
Violet-green swallow	Arizona, California, Oregon, Colorado, Wyoming, and Alaska. (N=110)	Ants	9.42		Other consisted primarily of Ephemeroptera	Beal 1918
		Other Hymenoptera	17.48			
		Diptera	19.36			
		Hemiptera/Homptera	35.96			
		Coleoptera	10.57			
		Lepidoptera	3.12			
		Other	4.09			

Table 34. (continued)

Species	Location	Taxa	Percent volume	Percent frequency	Comments	Reference
Bank swallow	21 states and Canada (n=394)	Ants	13.39		Other consists of Ephemeroptera (which accounted for 43% of diet in April), spiders, and snails	Beal 1918
		Other Hymenoptera	20			
		Diptera	26.63			
		Hemiptera/Homptera	7.96			
		Coleoptera	17.9			
		Lepidoptera	2.21			
		Odonata	2.11			
		Other	10.53			
	New York (n=64)	Coleoptera	36.13			Stoner 1936
		Diptera	31.59			
		Homoptera	17.81			
		Hemiptera	6.13			
		Hymenoptera	5.66			
		Ephemeroptera	1.66			
		Other	1.02			
Northern rough-winged swallow	15 states and Canada (n=136)	Ants	11.99		Other consists of Odonata, Ephemeroptera, spiders, and snails	Beal 1918
		Other Hymenoptera	18.91			
		Diptera	32.89			
		Hemiptera/Homptera	14.9			
		Coleoptera	14.83			
		Lepidoptera	1.11			
		Orthoptera	0.12			
		Other	5.04			
		Plant Material	0.21			

Table 35. Summary of reproductive characteristics for North American swallows

Species	Nest habitat	Egg dates	Clutch size	Number of clutches per year	Incubation period	Nestling period	Age of first breeding	References
Purple martin	Tree cavities, multiroom bird houses	May 21-July 13 (New York)	3 to 8, typically 4 to 5	1	16 to 18 days	26 to 31 days	1 year	DeGraaf et al. 1981
Cliff swallow	Mud cups on cliffs, cave entrances, buildings, bridges, culverts	May 20-5, June peak in Nebraska	1 to 6, typically 3 to 4	1	10 to 19 days, typically 13 to 15 days	20 to 26 days	1 year	Brown and Brown 1995
Barn swallow	Mud cups on human-made structures, especially buildings (barns)	May 11-August 3 (New York)	4 to 6, typically 4 to 5	1 to 2 in warmer areas	Approx. 15 days	16 to 23 days	1 years	DeGraaf et al. 1981
Tree swallow	Tree cavities or nest boxes	Laying starts in early May	2 to 8, typically 4 to 7	1, rarely 2	11 to 19 days, typically 14 to 15 days	15 to 25 days, typically 18 to 22 days	1 year, if possible	Robertson et al. 1992
Violet-green swallow	Tree cavities or nest boxes	May 1-July 1 (California)	4 to 7, typically 4 to 5	1	13 to 14 days	Approx. 23 days	No data	Bent 1942
Bank swallow	Burrows in earthen banks	May 15-July 13 (New York)	4 to 6, typically 5	Up to 2	14 to 16 days	18 to 22 days	1 year	Stoner 1936; DeGraaf et al. 1981
Northern rough-winged swallow	Burrows in earthen banks	Mid-May to mid-June	4 to 8, typically 4 to 6	1	15.5 to 16.5 days	17 to 21.5 days	1 year	DeJong 1996
Cave swallow	Mud cups on cliffs, cave entrances, buildings, bridges, culverts	April-July (New Mexico)	3 to 5, occasionally 1 to 2	2	No data	20 to 23 days	1 yr	West 1995

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FIGURES

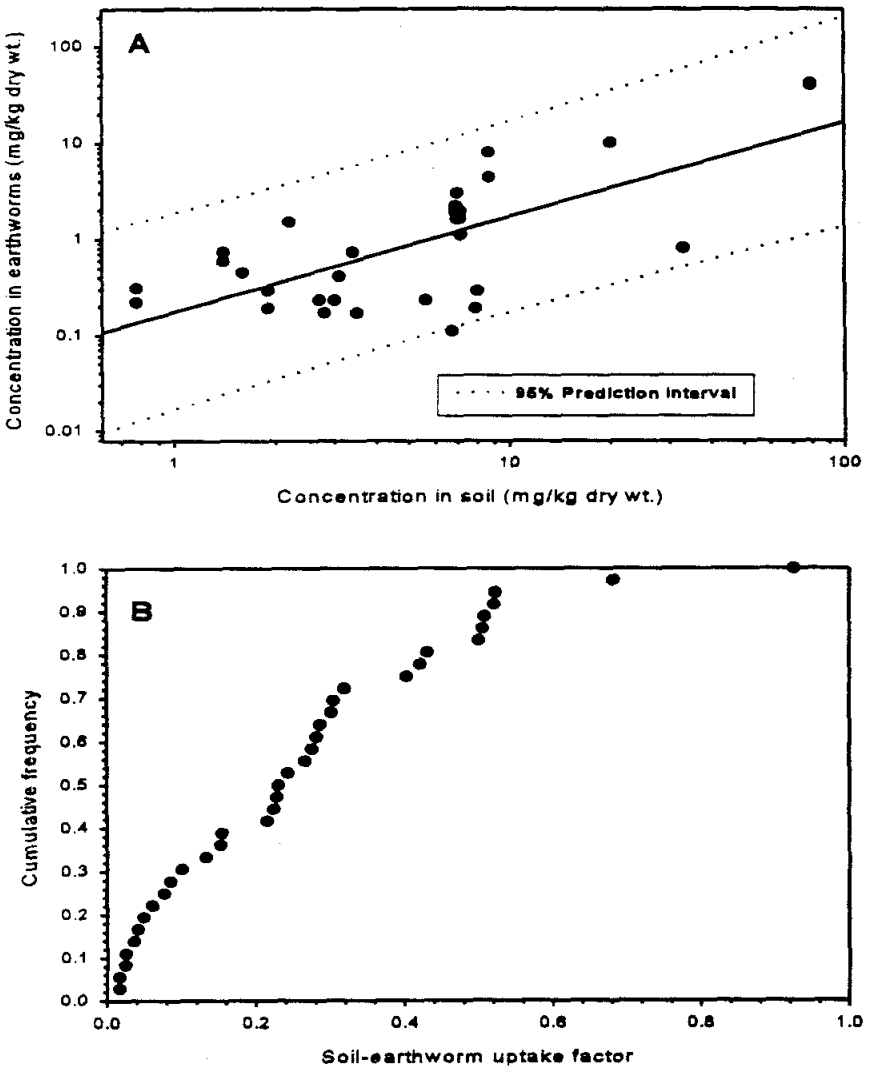

Figure 1. Literature-derived data on accumulation of As by earthworms. A) log-log scatterplot of As concentration in soil versus As concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

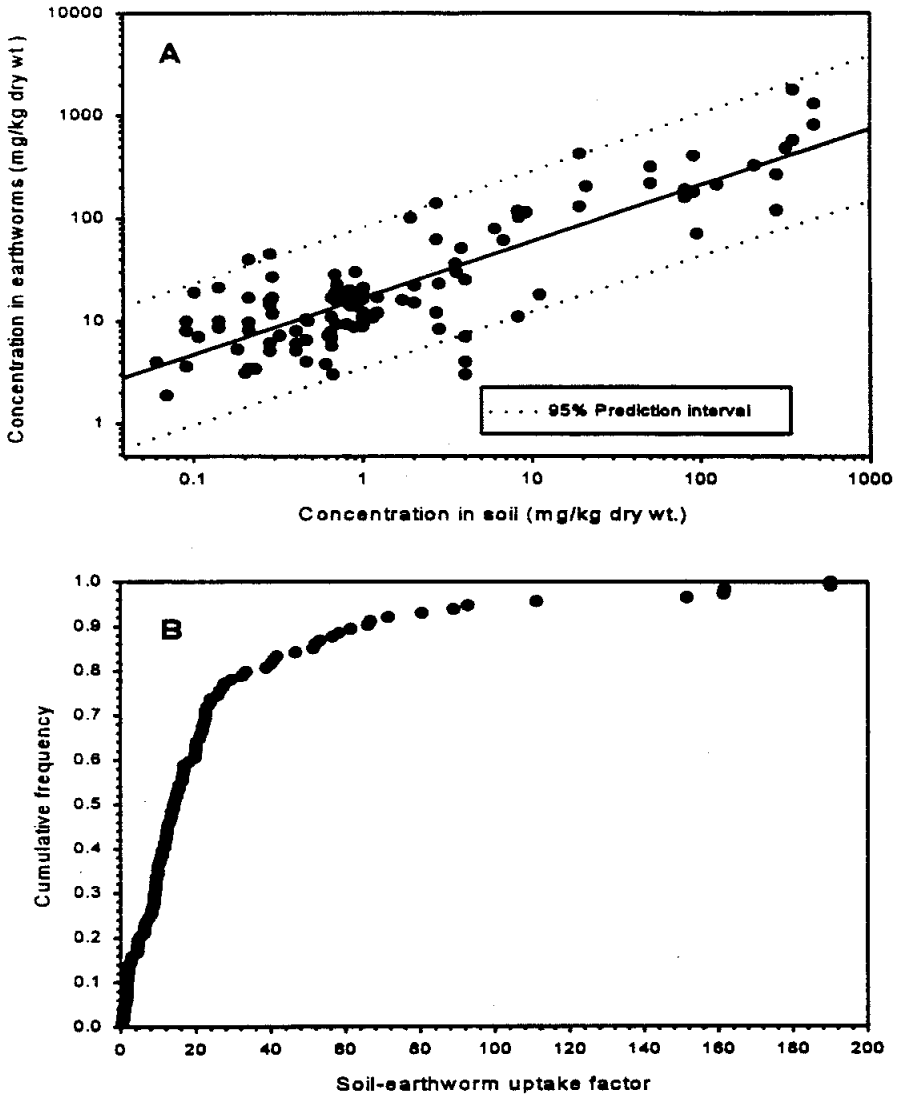

Figure 2. Literature-derived data on accumulation of Cd by earthworms. A) log-log scatterplot of Cd concentration in soil versus Cd concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7.Summary of studies considered to evaluate accumulation are presented in Appendix A.

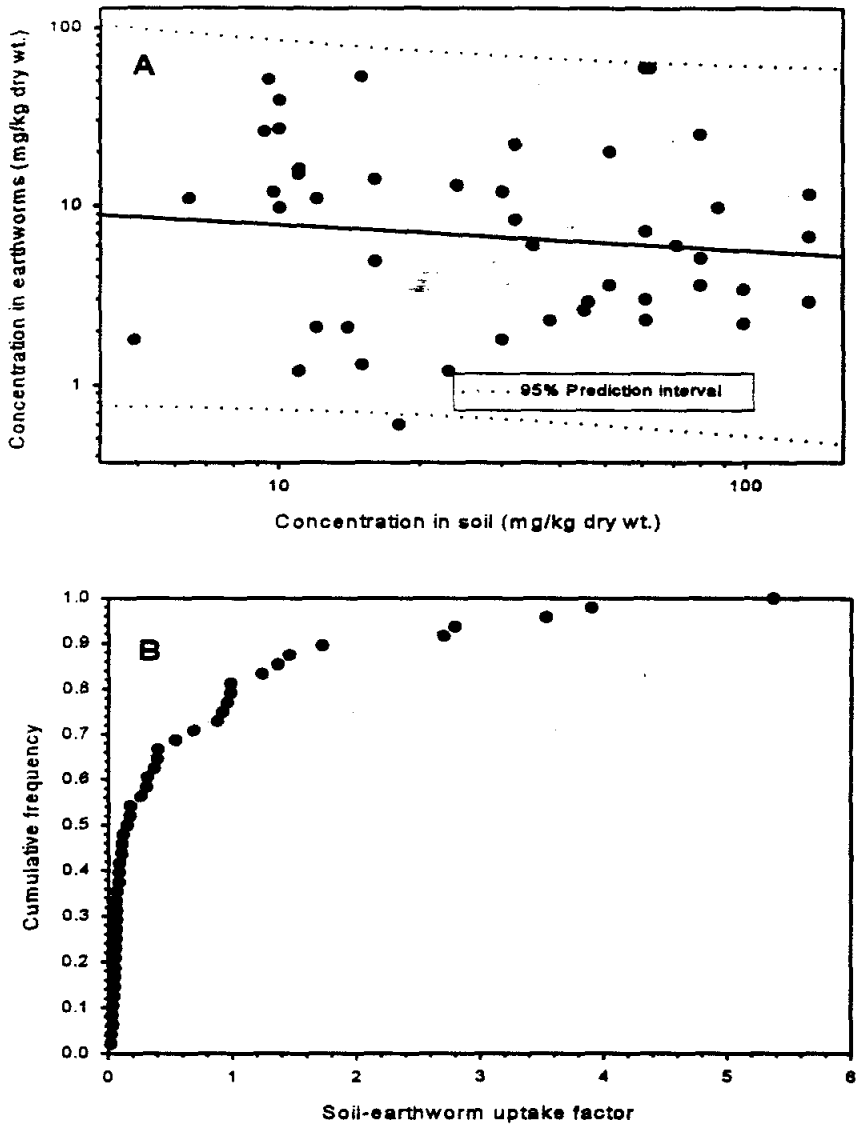

Figure 3. Literature-derived data on accumulation of Cr by earthworms. A) log-log scatterplot of Cr concentration in soil versus Cr concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

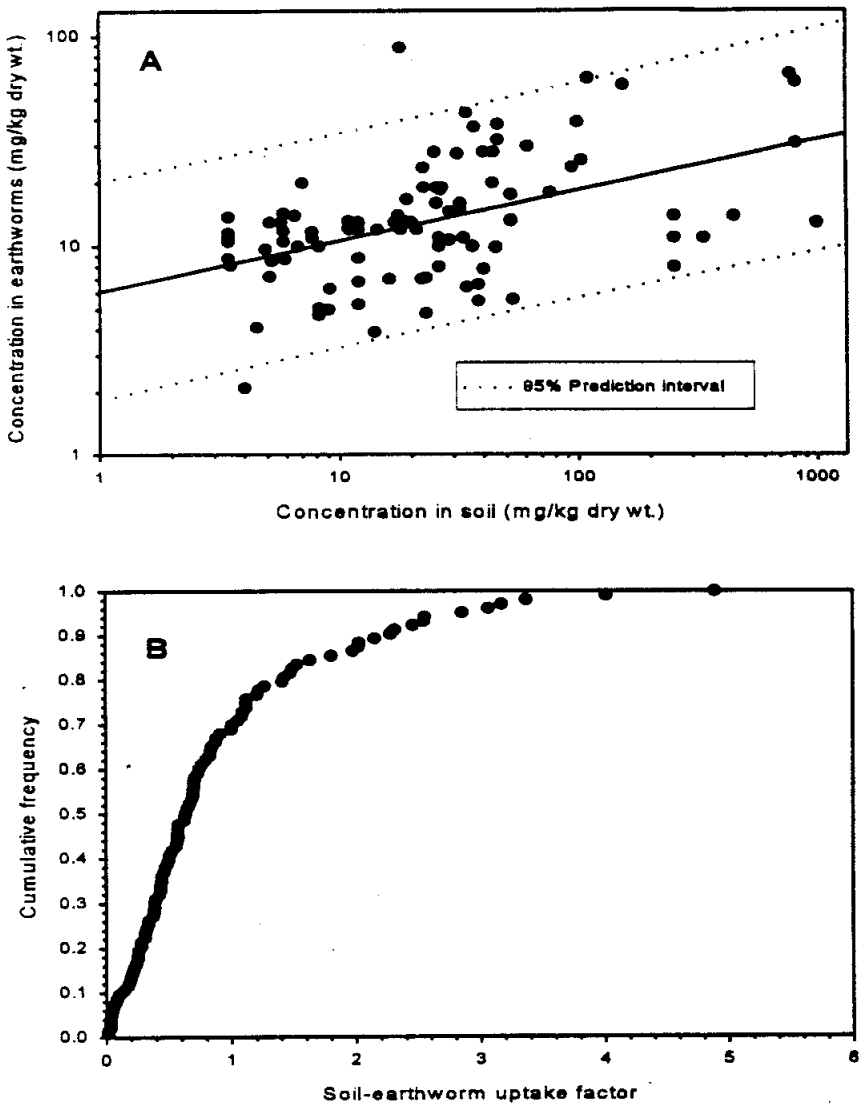

Figure 4. Literature-derived data on accumulation of Cu by earthworms. A) log-log scatterplot of Cu concentration in soil versus Cu concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

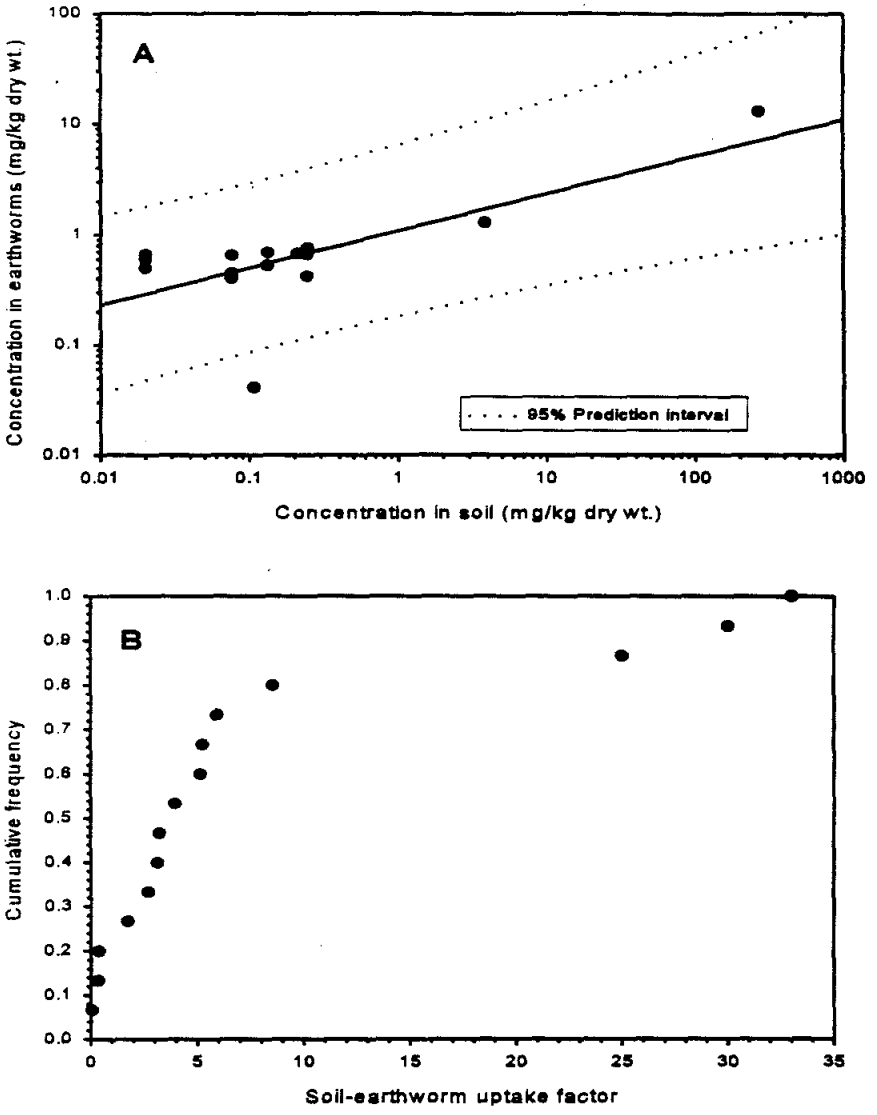

Figure 5. Literature-derived data on accumulation of Hg by earthworms. A) log-log scatterplot of Hg concentration in soil versus Hg concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

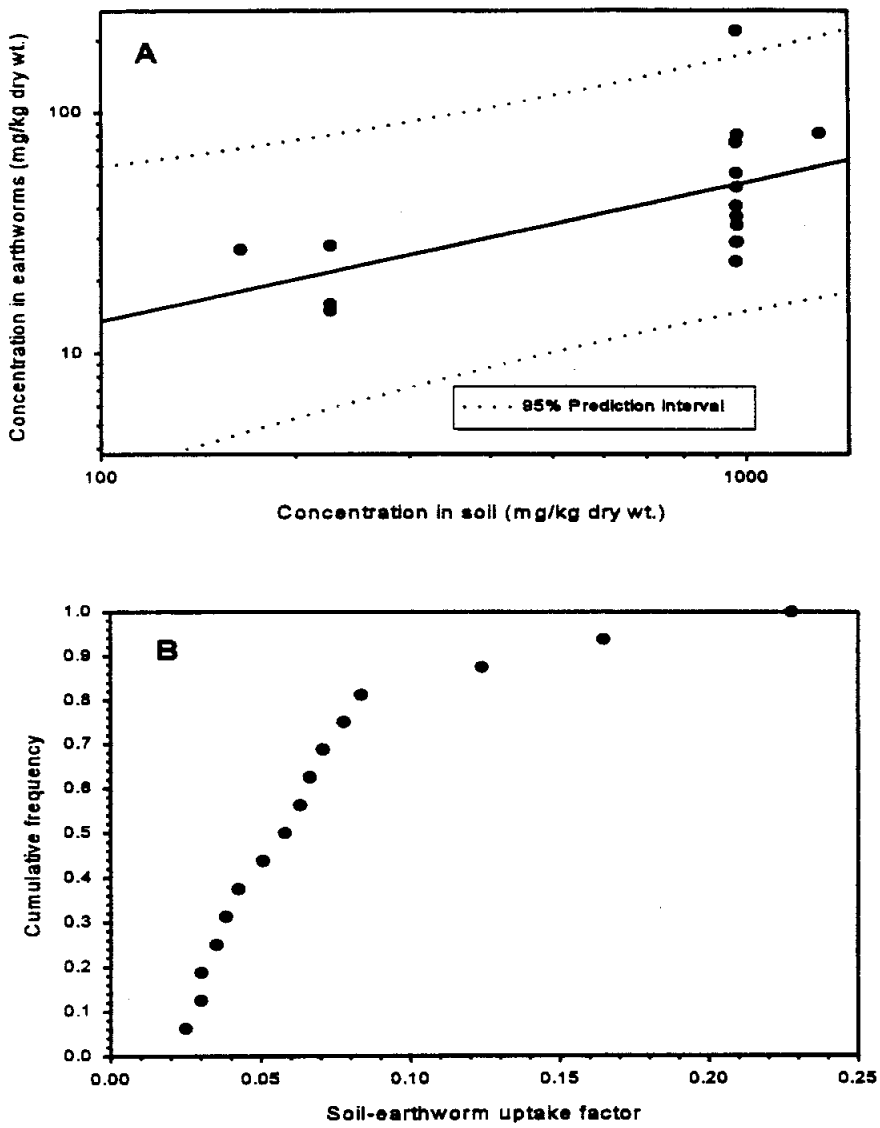

Figure 6. Literature-derived data on accumulation of Mn by earthworms. A) log-log scatterplot of Mn concentration in soil versus Mn concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

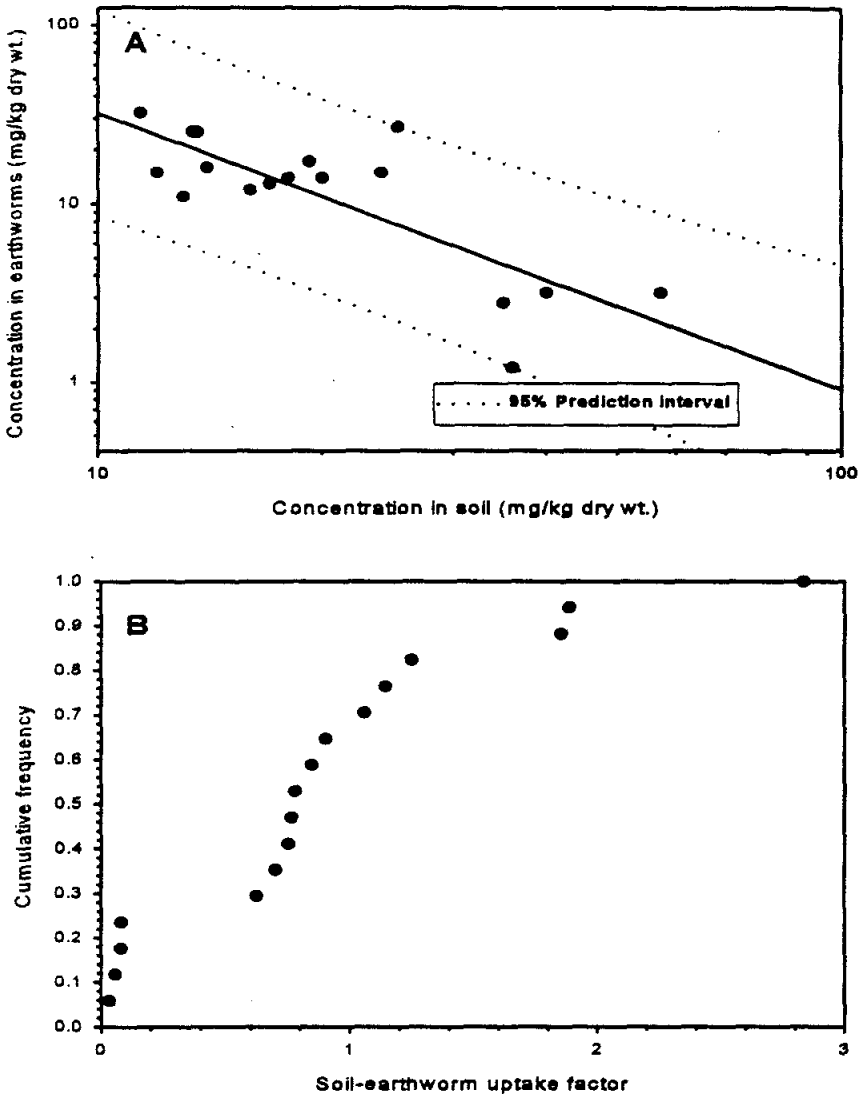

Figure 7. Literature-derived data on accumulation of Ni by earthworms. A) log-log scatterplot of Ni concentration in soil versus Ni concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

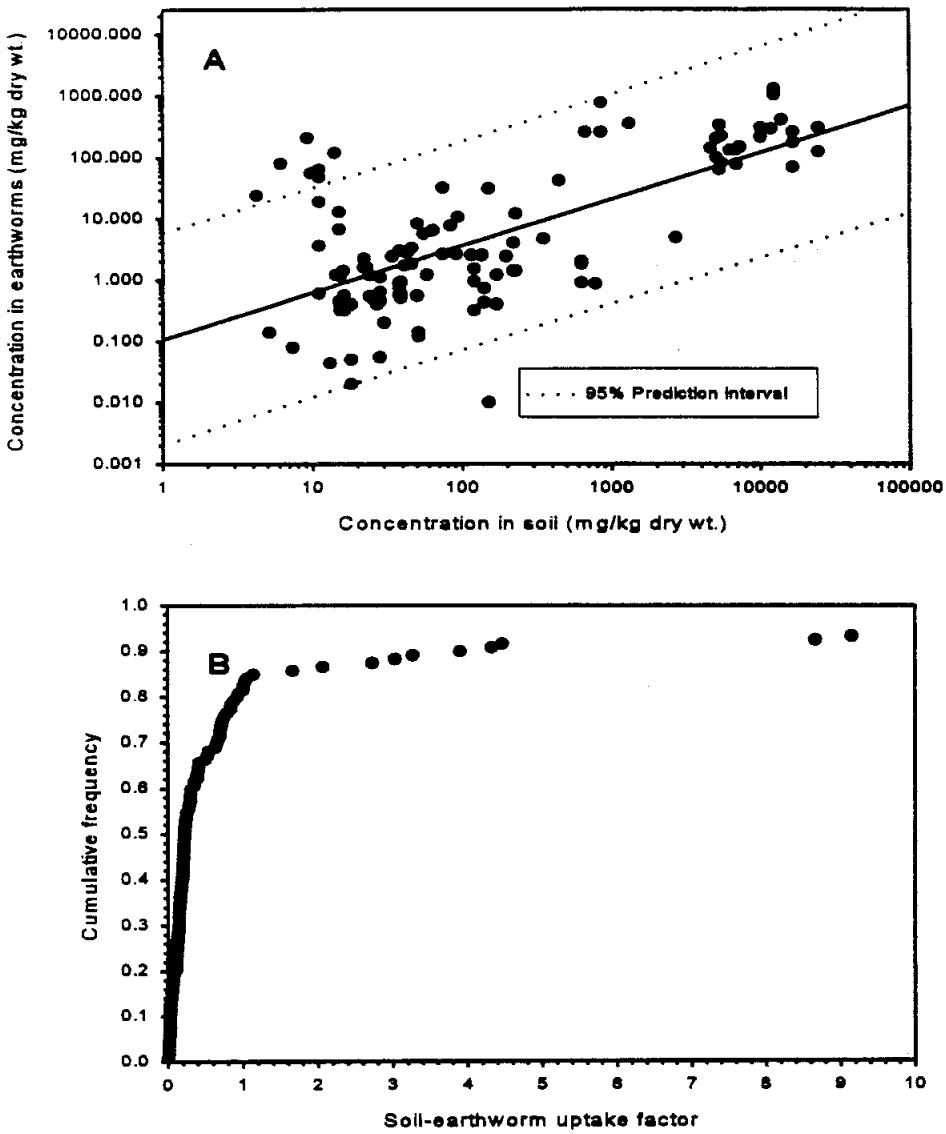

Figure 8. Literature-derived data on accumulation of Pb by earthworms. A) log-log scatterplot of Pb concentration in soil versus Pb concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Plot truncated at UF=10. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

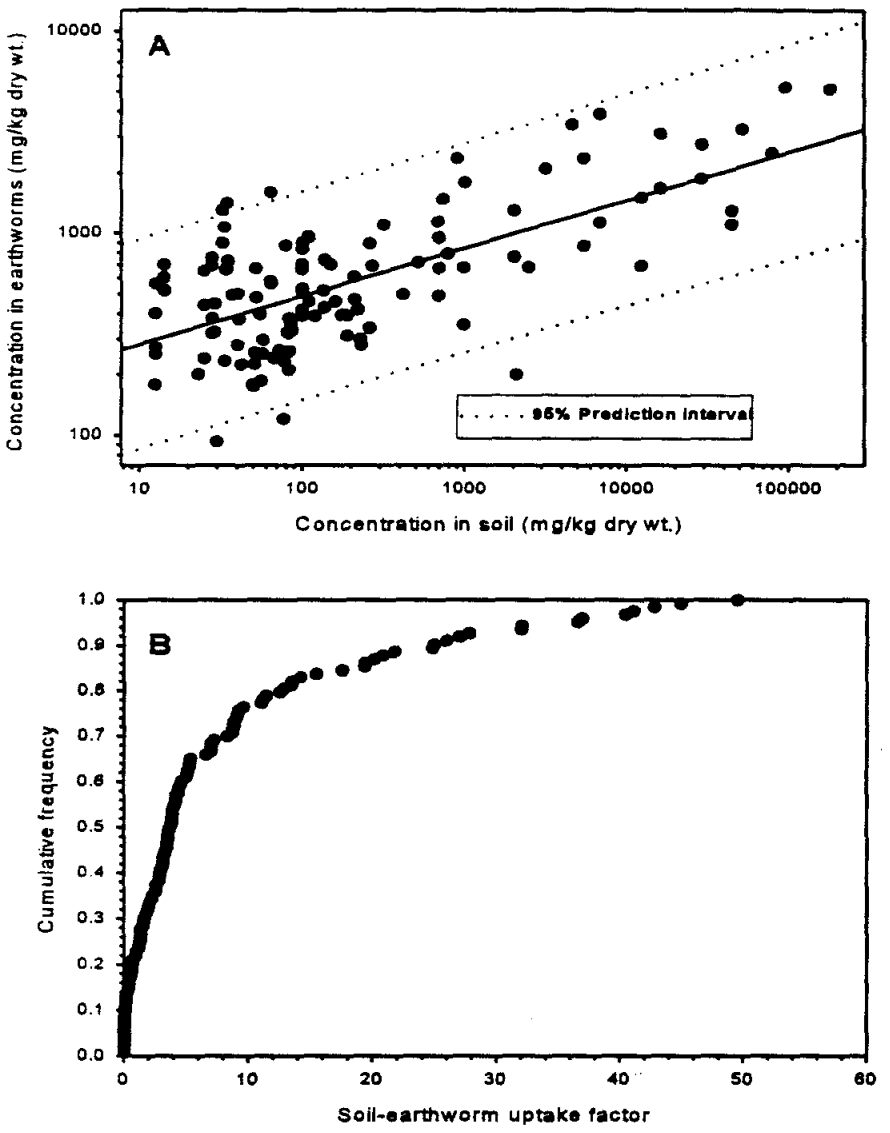

Figure 9. Literature-derived data on accumulation of Zn by earthworms. A) log-log scatterplot of Zn concentration in soil versus Zn concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

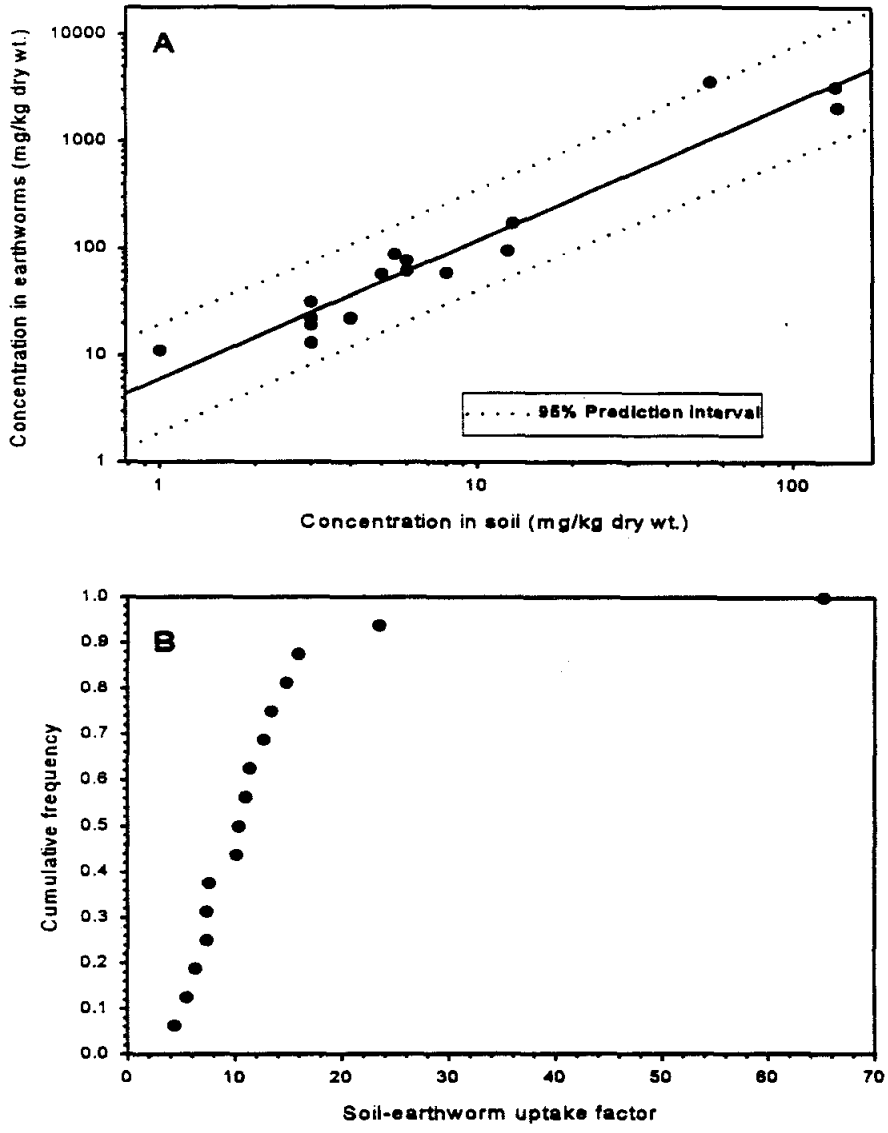

Figure 10. Literature-derived data on accumulation of PCBs by earthworms. A) log-log scatterplot of PCBs concentration in soil versus PCBs concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

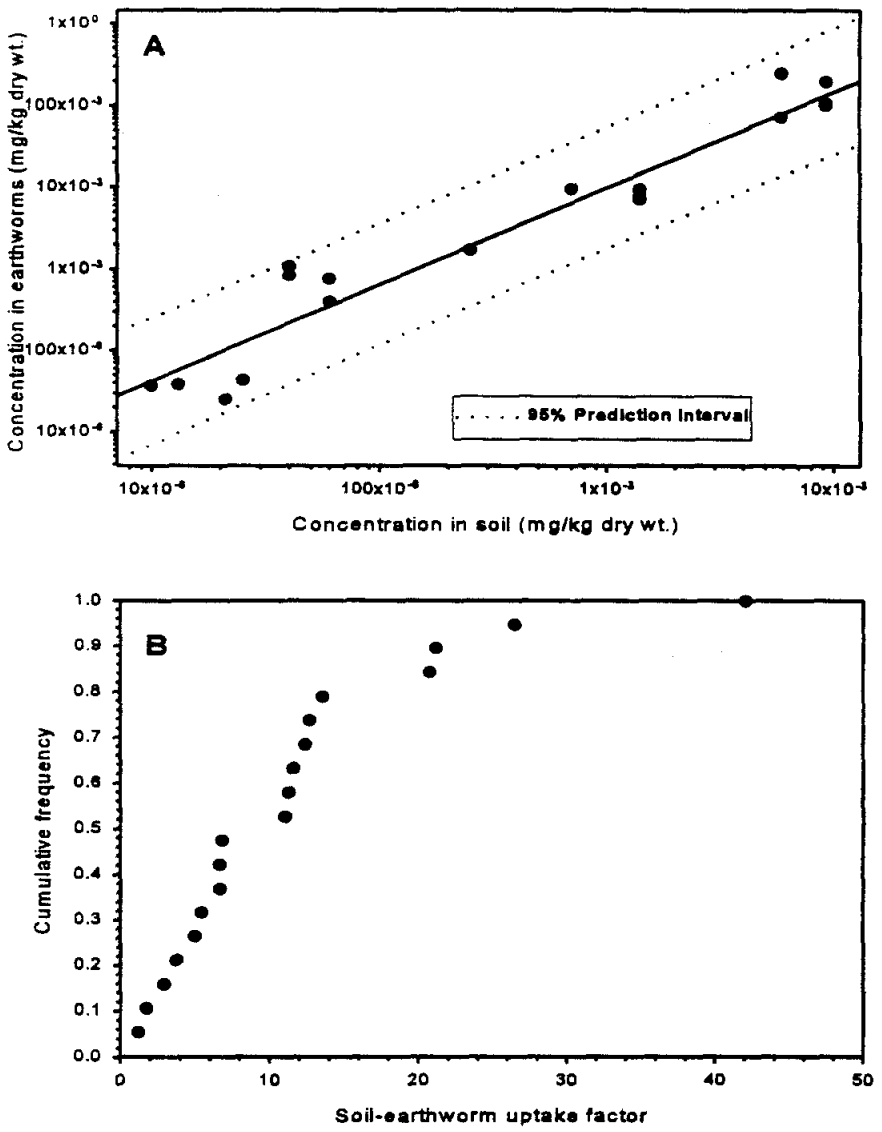

Figure 11. Literature-derived data on accumulation of 2,3,7,8 TCDD by earthworms. A) log-log scatterplot of 2,3,7,8 TCDD concentration in soil versus 2,3,7,8 TCDD concentration in depurated earthworms. Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix A.

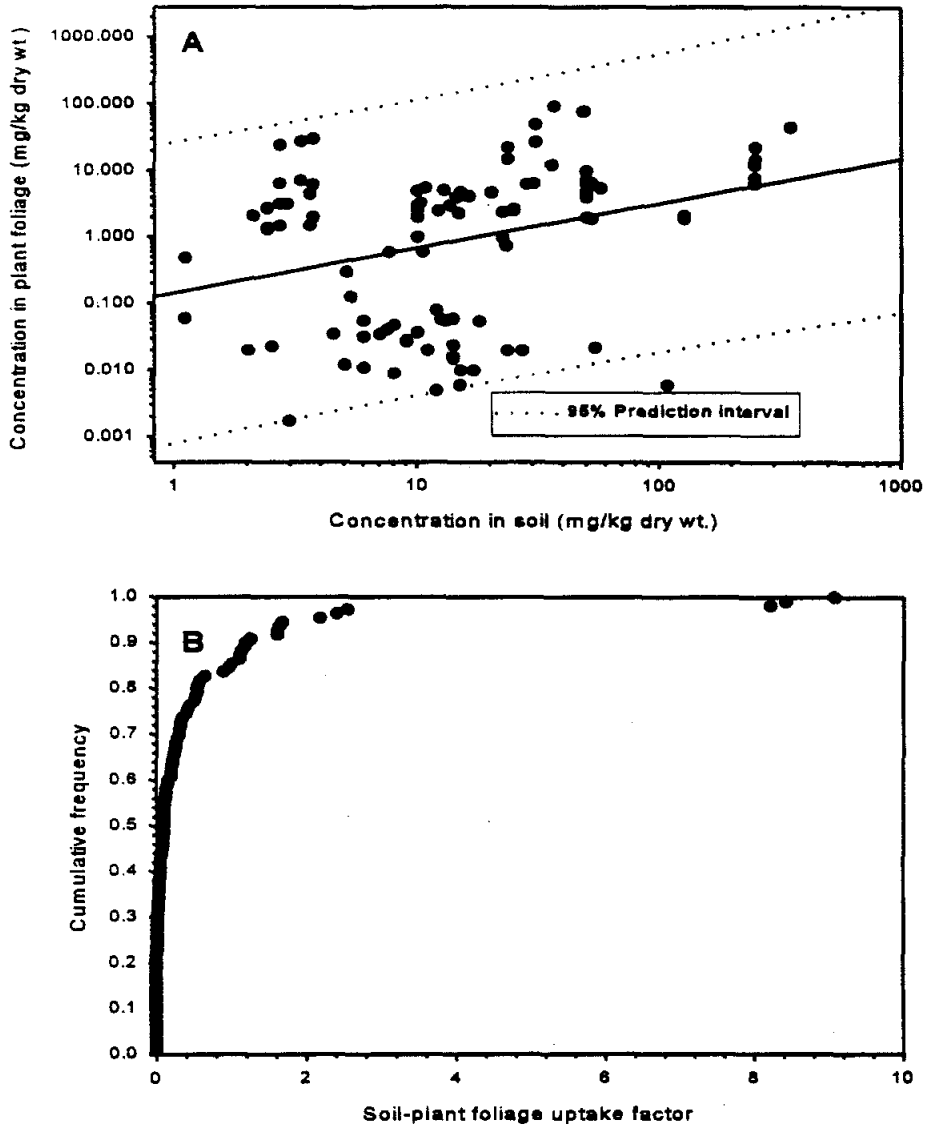

Figure 12. Literature-derived data on accumulation of As by terrestrial plants. A) log-log scatterplot of As concentration in soil versus As concentration in above ground tissues (excluding fruits or seeds). Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix B.

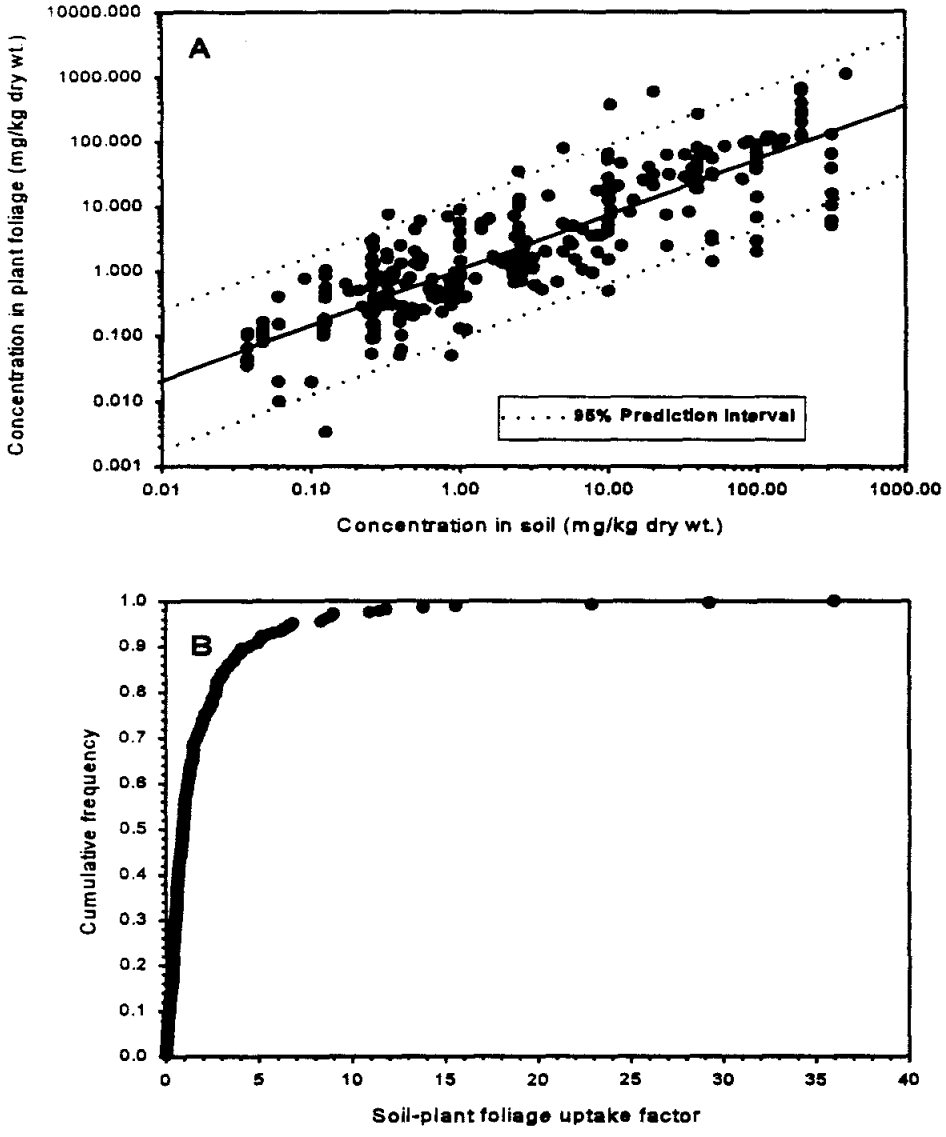

Figure 13. Literature-derived data on accumulation of Cd by terrestrial plants. A) log-log scatterplot of Cd concentration in soil versus Cd concentration in above ground tissues (excluding fruits or seeds). Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix B.

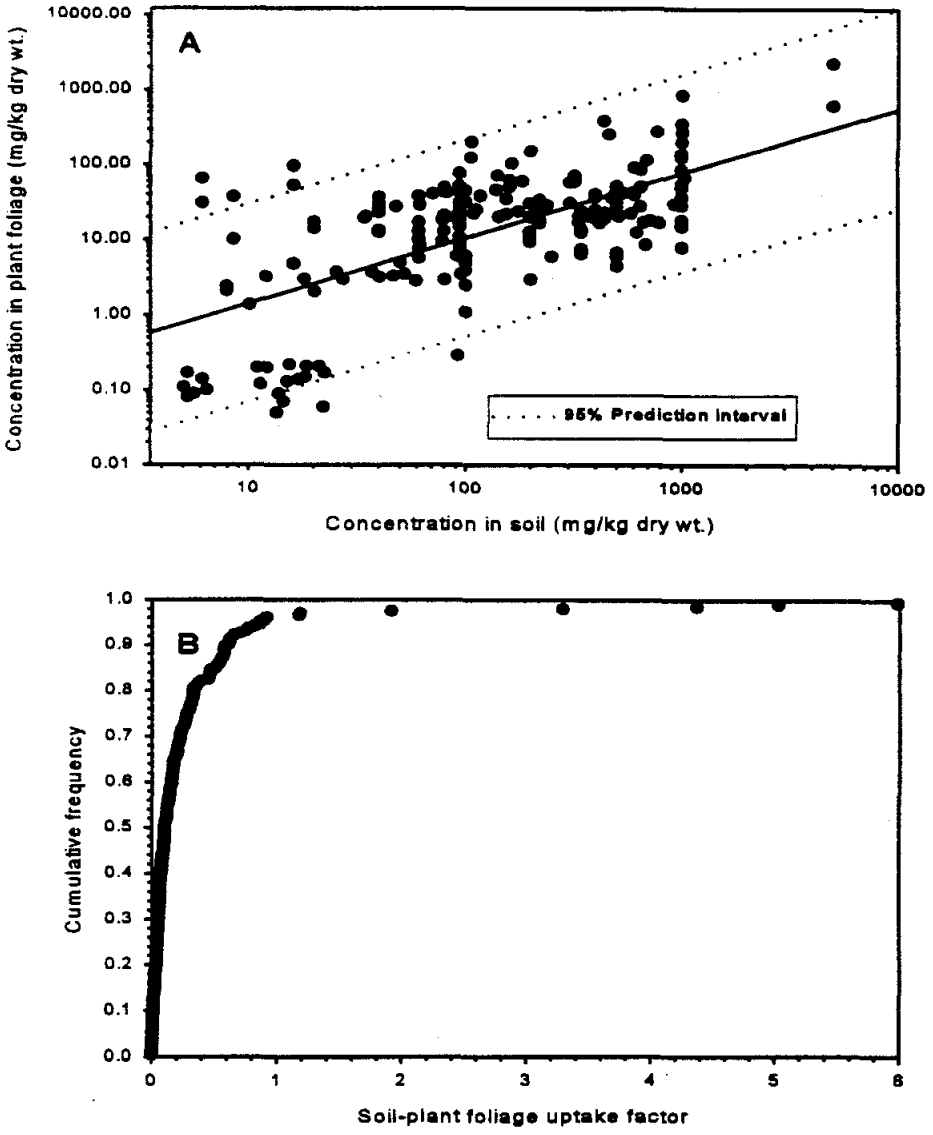

Figure 14. Literature-derived data on accumulation of Pb by terrestrial plants. A) log-log scatterplot of Pb concentration in soil versus Pb concentration in above ground tissues (excluding fruits or seeds). Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix B.

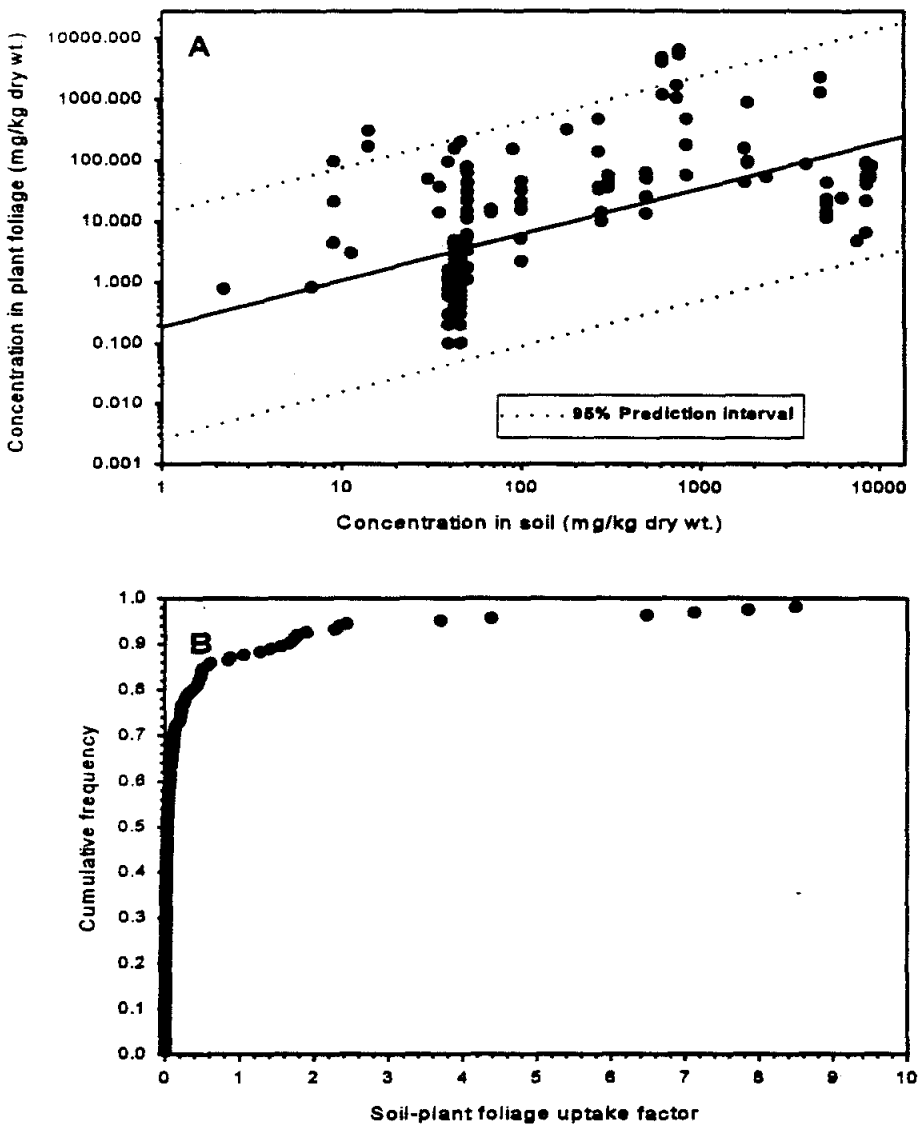

Figure 15. Literature-derived data on accumulation of Ni by terrestrial plants. A) log-log scatterplot of Ni concentration in soil versus Ni concentration in above ground tissues (excluding fruits or seeds). Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix B.

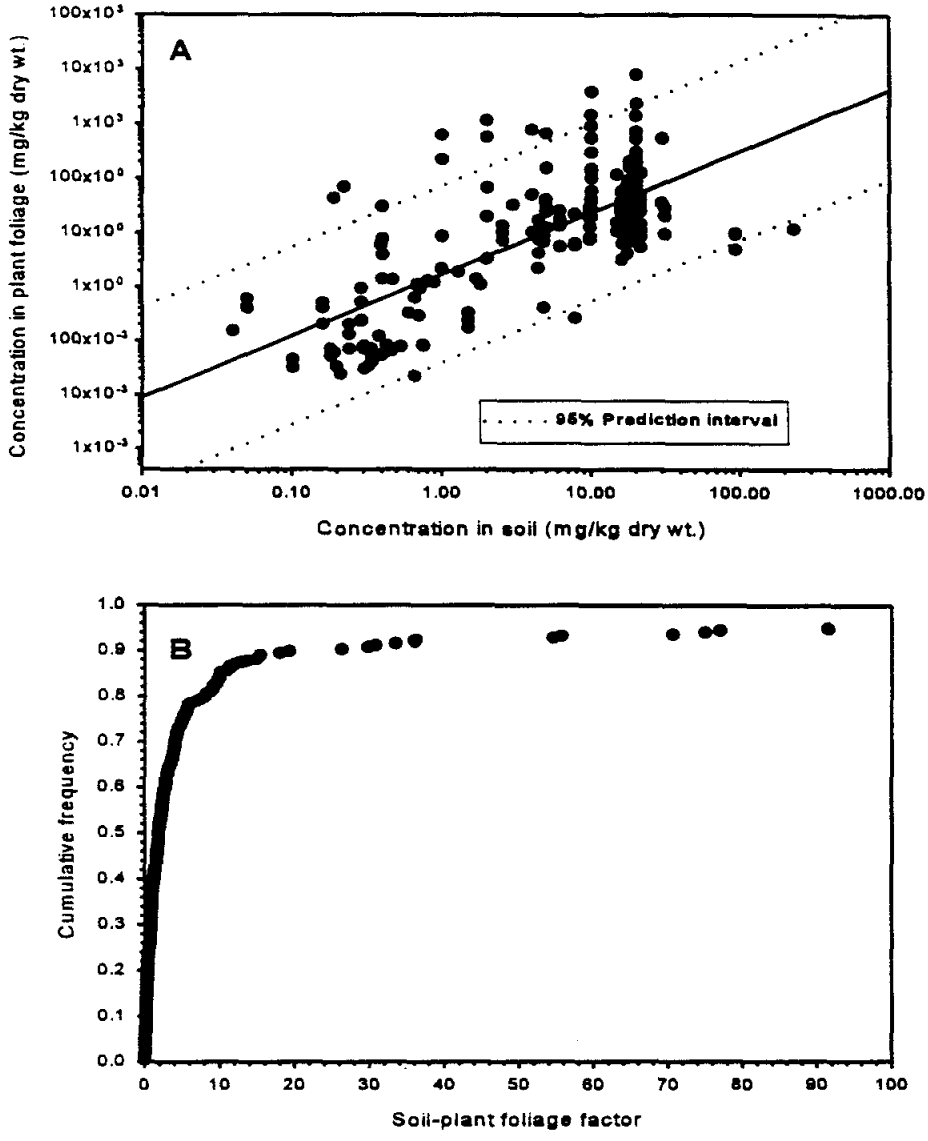

Figure 16. Literature-derived data on accumulation of Se by terrestrial plants. A) log-log scatterplot of Se concentration in soil versus Se concentration in above ground tissues (excluding fruits or seeds). Line represents regression fit to natural-log transformed data (see Table 8). B) Cumulative frequency distribution for UFs. Summary statistics for UFs are presented in Table 7. Summary of studies considered to evaluate accumulation are presented in Appendix B.

APPENDIX A.

SUMMARY OF CONTAMINANT UPTAKE LITERATURE FOR EARTHWORMS

Reference: Andersen 1979
Analytes considered: Pb and Cd
Species: *A. chlorotica, A. caliginosa, A. longa, A. rosea, and L. terrestris*
Geographic location of study: Denmark
Exposure duration: Resident
Worms depurated: yes
Analytical method: Atomic absorption (AA) spectroscopy
Soil extraction method: Nitric and perchloric acid
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH only
Purpose of study: To evaluate the population parameters and uptake of metals by earthworms in areas treated with municipal sewage sludge.
Study conclusions: Uptake of lead appears to be related to Ca content of soil.

Reference: Andersen and Laursen 1982
Analytes considered: Ca, Cd, Fe, Pb, Mn, and Zn
Species: *Lumbricus terrestris* and *Aporrectodea longa*
Geographic location of study: Denmark
Exposure duration: Resident
Worms depurated: yes
Analytical method: Atomic absorption/x-ray fluorescence
Soil extraction method: Nitric/perchloric acid digestions
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: no
Purpose of study: To evaluate heavy metal accumulation and distribution throughout earthworm body.
Study conclusions: Pb and Cd accumulate in the gut wall and are then transferred to the waste nodules. In *L. terrestris*, more Pb than Cd was transferred to waste nodules. Large amounts of Zn also accumulated in the gut wall. In *L. terrestris* the calciferous glands play a large role in regulation and excretion of heavy metals.

Reference: Beyer et al. 1982
Analytes considered: Pb, Cd, Cu, Ni, and Zn
Species: Not stated
Geographic location of study: Pennsylvania
Exposure duration: Resident
Worms depurated: Yes (mostly–authors estimate that ~75% soil removed from gut)
Analytical method: AA spectroscopy
Soil extraction method: Nnitric/hydrochloric acid
Soil characteristics (pH, CEC, % OM, % clay, etc.) presented: pH, CEC, and % OM
Purpose of Study: To evaluate the uptake of metals by earthworms in soils treated with sewage sludge
Study conclusions: Earthworm concentrations of Cd and Zn relative to soil, but not Cu, Pb, or Ni. High Zn concentrations in soil were negatively correlated with Cd concentrations in earthworms.

Reference: Beyer et al. 1985
Analytes considered: Pb, Cd, Cu, and Zn

Species: *Dendrobaena rubida and Eisenoides carolinensis*
Geographic location of study: Pennsylvania
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: Acid
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, % OM and CEC
Purpose of study: To evaluate the uptake and transfer of metals by biota in the vicinity of two zinc smelters.
Study conclusions: Metals are accumulated to higher levels by biota nearer the smelters.

Reference: Beyer and Cromartie 1987
Analytes considered: Pb, Cu, Zn, Cd, Cr, As, and Se
Species: Mixed
Geographic location of study: Maryland, Pennsylvania, and Virginia
Exposure duration: Resident
Worms depurated: Yes
Analytical method: Atomic absorption
Soil extraction method: Concentrated HCl and HNO_3
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: yes
Purpose of study: To determine how concentrations in earthworms compare to that in soils from diverse sites.
Study conclusions: Correlations between concentrations in soil and those in worms were low. The authors suggest that if worms are to be used as indicators of contamination, it is important to identify worm species, report soil characteristics, and collect similar worms from similar but uncontaminated locations.

Reference: Bull et al. 1977
Analytes considered: Mercury
Species: *Lumbricus terrestris*
Geographic location of study: Great Britain
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: Nitric/perchloric acid
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: no
Purpose of study: To evaluate the uptake and transfer of mercury in the foodweb near a chlor-alkali plant.
Study conclusions: Mercury was found at higher levels in worms near the plant. Methyl mercury concentrations in worms varied from 8 to 13%.

Reference: Carter 1983
Analytes considered: Cd, Cu, and Zn
Species: *A. chlorotica and L. rubellus*
Geographic location of study: British Columbia
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: Nitric/hydrochloric acid
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH only

Purpose of study: To evaluate the uptake and transfer of metals through a pasture food web.
Study conclusions: Cd was concentrated by earthworms, invertebrate predators, and herbivorous slugs over that in their foods. Millipedes concentrated Cu and Zn but not Cd.

Reference: Corp and Morgan 1991
Analytes considered: Cd, Cu, Pb, and Zn
Species: *Lumbricus rubellus*
Geographic location of study: Great Britain
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectrometry
Soil extraction method: HNO_3 digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: only pH
Purpose of study: compare patterns of metal accumulation in field-collected earthworms at nine contaminated field sites to that in "clean" worms added to soil from the sites. Only data from field-collected worms were used in development of an uptake factor in this report.
Study conclusions: (1) Pb, Zn, and Cd concentrations were higher in field-collected worms than in laboratory worms; (2) the relationship between tissue and soil metal concentrations was similar between the two groups of worms; and (3) high soil organic matter reduced Pb bioavailability while low pH increased bioavailability.

Reference: Czarnowska and Jopkiewicz 1978
Analytes considered: Cd, Cu, Pb, and Zn
Species: *Lumbricus terrestris*
Geographic location of study: Warsaw, Poland
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: Concentrated acid digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, % OM
Purpose of study: To study the uptake heavy metals in worms at increasing distances from Warsaw streets.
Study conclusions: Earthworms accumulated all four metals, Cd in particular.

Reference: Diercxsens et al. 1985
Analytes considered: Polychlorinated biphenyls (PCBs) and Cd, Cr, Cu, Pb, Mn, and Zn
Species: not stated
Geographic location of study: Germany
Exposure duration: Resident
Worms depurated: Yes
Analytical method:
Soil extraction method: Aqua regia digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH only
Purpose of study: To study metal accumulation in earthworms at a nature reserve and at site treated with sewage sludge.
Study conclusions: PCB concentrations in worm tissue and gut contents were greater than that in soil. Congener profiles in worm tissue and soil differed. Cd and Zn were also found to be accumulated.

Reference: ERT 1987
Analytes considered: 2,3,7,8-TCDD and 2,3,7,8-TCDF
Species: Not stated
Geographic location of study: Wisconsin
Exposure duration: Resident
Worms depurated: No
Analytical method: Mass spectroscopy
Soil extraction method:
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: none
Purpose of study: To evaluate the uptake of TCDD by earthworms in forests treated with paper mill sludge.
Study conclusions: Earthworm abundance was greater in sludge treated plots than in untreated plots. In treated plots, TCDD was accumulated to levels 3.3 times (on average) greater than that in soil.

Reference: Fisher and Koszorus 1992
Analytes considered: As, Hg, and Se
Species: *Eisnia fetida*
Geographic location of study: Laboratory
Exposure duration: 8 weeks
Worms depurated: Yes
Analytical method: X-ray spectrometry
Soil extraction method: Not stated
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: no
Purpose of study: To evaluate sublethal effects, uptake, and elimination of contaminants by earthworms.
Study conclusions: In general, accumulation rates decreased as soil concentrations increased.

Reference: Gish and Christensen 1973
Analytes considered: Cd, Ni, Pb, and Zn
Species: Mixed species, not differentiated
Geographic location of study: Maryland, near Washington DC
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectrometry
Soil extraction method: HCl digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: yes
Purpose of study: To determine whether earthworms near roads are accumulating heavy metals.
Study conclusions: Metal accumulations were higher where traffic volume was greatest. Metal residues in soils were positively correlated to soil organic matter. Accumulations of Pb and Zn were sufficiently high to be potentially toxic to worm predators.

Reference: Helmke et al. 1979
Analytes considered: 29 elements
Species: *Aporrectodea tuberculata*
Geographic location of study: Wisconsin
Exposure duration: Resident
Worms depurated: Yes
Analytical method: Neutron Activation
Soil extraction method: Not stated

Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: range of pHs in soil only
Purpose of study: To evaluate the uptake of contaminants by earthworms in sewage sludge amended soils.
Study conclusions: Concentrations of Cd, Cu, and Zn increase with increasing sludge application rate while Se concentrations decrease. Cd appeared to be readily accumulated. Concentrations of Hg and Cr in casts increased with increasing soil concentration while tissue concentrations did not, suggesting that these elements were not bioavailable.

Reference: Hendriks et al. 1995
Analytes considered: Cd, Cu, Mn, Ni, Pb, and Zn
Species: *Lumbricus rubellus*
Geographic location of study: Netherlands
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: Nitric acid digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: not stated
Purpose of study: To evaluate the uptake and transfer of contaminants in the Rhine river floodplain.
Study conclusions: Accumulation of metals by worms was comparable to that seen in other studies.

Reference: Ireland 1979
Analytes considered: Ca, Cd, Cu, Pb, Mn, and Zn
Species: *Lumbricus rubellus, Dendobaena veneta,* and *Eiseniella tetraedra*
Geographic location of study: Great Britain
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectrometry
Soil extraction method: HNO_3 digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) pPresented: none
Purpose of study: To study metal accumulation in earthworms at metal contaminated sites.
Study conclusions: *Lumbricus rubellus* accumulated and retained Pb. Cu, Zn, and Mn appeared to be regulated by the worms irrespective of soil concentrations.

Reference: Kreis et al. 1987
Analytes Considered: PCBs
Species: *Nicodrilus*
Geographic Location of Study: Switzerland
Exposure Duration: Resident
Worms Depurated: yes
Analytical Method: GC
Soil Extraction Method:
Soil Characteristics (pH, CEC, % OM, % Clay, etc.) Presented :
Purpose of Study: to evaluate PCB uptake at sites treated with sewage sludge.

Reference: Ma 1982
Analytes considered: Cd, Cr, Cu, Fe, Pb, Mn, Ni, and Zn
Species: *Allolobophora caliginosa*
Geographic location of study: Netherlands

Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: HCl digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, CEC, % OM
Purpose of study: To study the role of soil physicochemical properties on bioavailability of heavy metals to earthworms.
Study conclusions: The order in which *A. caliginosa* accumulated metals was Cr<Mn≤Fe<Ni<Pb≤Cu<Zn<Cd. The ratio of concentrations in worms to that in soil was negatively correlated to CEC for Cd, Cu, Fe, Mn, Ni, Pb, and Zn but not for Cr, which was poorly taken up. There was a negative pH effect on the uptake of Cd, Zn, and Pb. Soil pH was more important than CEC for Cd and Zn; pH and CEC were equally important for Pb. Cu uptake was affected by CEC but not by pH; soil Cu was the most important factor affecting the level of Cu in worms.

Reference: Ma 1987
Analytes considered: Cd, Cu, Pb, and Zn
Species: *Lumbricus rubellus*
Geographic location of study: Netherlands
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: HCl digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, CEC, % OM
Purpose of study: To study the uptake and transfer of heavy metals from soil through earthworms to moles.
Study conclusions: Accumulated levels in earthworms and moles do not consistently reflect metal levels in soil. In acidic, sandy soils, Cd may accumulate in worms, and critical levels to moles may be exceeded even when the soil levels are relatively low. Pb is also more readily accumulated by worms and moles associated with acidic soils than limed soils. There is no evidence to suggest that Cd, Pb, or Zn have any influence on Cu tissue levels in either worms or moles.

Reference: Martinucci et al. 1983
Analytes considered: 2,3,7,8-TCDD
Species: *A. rosea and A. caliginosa*
Geographic location of study: Seveso, Italy
Exposure duration: Resident
Worms depurated: Yes
Analytical method:
Soil extraction method: Not stated
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: no
Purpose of study: To evaluate the uptake of TCDD by earthworms.
Study conclusions: Earthworms accumulated TCDD to levels 14.5 times (on average) higher than that in soil. No interspecies differences in accumulation were observed. Earthworm activity may serve to bring TCDD back to the soil surface. Tissue levels generally do not appear to be toxic to the worms.

Reference: Morgan and Morgan 1991
Analytes considered: Ca, Cd, Pb, and Zn
Species: *Lumbricus rubellus*

Dendrodrilus rubidus
Geographic location of study: Great Britain (England and Wales)
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA
Soil extraction method: Concentrated HNO_3
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: no
Purpose of study: To determine if there are interspecies differences in accumulation in two sympatric, ecologically similar species at ten different locations.
Study conclusions: *L. rubellus* contained higher Zn and Ca and lower Pb and Cd than *D. rubidus*. Pb accumulation by both species was higher in soils with lower Ca.

Reference: Morgan and Morris 1982
Analytes considered: Ca, Cd, Pb, and Zn
Species: *Lumbricus rubellus and Dendrobaena rubidus*
Geographic location of study: Abandoned lead mine in Wales, Great Britain
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectrometry
Soil extraction method: HNO_3 digestion
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: only pH
Purpose of study: Determine internal distribution of heavy metals in the earthworm.
Study conclusions: The two worm species accumulate metals to different degrees.

Reference: Pietz et al. 1984
Analytes considered: Pb, Cd, Cr, Cu, Ni, and Zn
Species: Mix of species
Geographic location of study: Fulton Co., IL
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: Nitric/sulfuric acid
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH presented (but insufficient information to relate values to correct sample locations).
Purpose of study: To evaluate the uptake of metals by earthworms at mine sites amended with sewage sludge.
Study conclusions: Cd and Zn were accumulated to levels greater than that in soil; Cr, Cu, Ni, and Pb were not. Cr in earthworms was negatively related to soil pH.

Reference: Pizl and Josens 1995
Analytes considered: Pb, Cd, Cu, and Zn
Species: *A. chlorotica, A. caliginosa, A. icterica, A. rosea, L. castanea, L. rubellus, and L. terrestris*
Geographic location of study: Brussels Belgium
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: Nitric acid

Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, P, K, Mg, Na, and Ca
Purpose of study: To evaluate the population parameters and uptake of metals by earthworms along a gradient of urbanization.
Study conclusions: Earthworm density was negatively correlated to soil Cd and Mg concentrations. Biomass was negatively correlated with Pb, Cu, and Zn and positively with distance from city center. Intergeneric differences in accumulation were observed: *Aporrectodea* spp. accumulated Cd and Pb more readily than did *Lumbricus* spp.

Reference: Talmage and Walton 1993
Analytes considered: Hg
Species: Not stated
Geographic location of study: East Tennessee (Oak Ridge Reservation)
Exposure duration: Resident
Worms depurated: Yes
Analytical method: X-ray fluorescence, neutron activation, and AA spectroscopy, depending on concentration
Soil extraction method: Nitric/perchloric acid
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: no
Purpose of study: To evaluate the uptake, transfer, and toxicity of inorganic mercury at a contaminated site.
Study conclusions: Small amounts of mercury were taken up by earthworms. Hg accumulation by shrews approached nephrotoxic levels.

Reference: Van Hook 1974
Analytes considered: Cd, Pb, and Zn
Species: *Alabophera spp.*, *Lumbricus spp.*, and *Octalasium*. Species not differentiated.
Geographic location of study: East Tennessee (Oak Ridge Reservation)
Exposure duration: Resident
Worms depurated: Yes
Analytical method: Isotope dilution spark source mass spectrometry
Soil extraction method: Aqua regia
Soil characteristics (pH, CEC, % OM, % Clay, etc.) pPresented: no
Purpose of study: To evaluate the differential accumulation of Cd, Pb, and Zn from six soil types in East Tennessee.
Study conclusions: The earthworm species studied accumulated Cd and Zn to levels higher than that in soil but not Pb.

Reference: Van Rhee 1977
Analytes considered: Cu
Species: Mix of species
Geographic location of study: Netherlands
Exposure duration: Resident
Worms depurated: Yes
Analytical method: AA spectroscopy
Soil extraction method: Not stated
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: no
Purpose of study: To evaluate the uptake of Cu by earthworms in pastures treated with Cu-contaminated pig wastes.

Study conclusions: Earthworm density was not related to soil Cu, but Cu in worm tissue was highly correlated to that in soil.

Reference: Yeates et al. 1994
Analytes considered: As, Cr, and Cu
Species: *A. rosea and L. rubellus*
Geographic location of study: New Zealand
Exposure duration: Resident
Worms depurated: Yes
Analytical method: X-ray fluorescence spectroscopy
Soil extraction method: Not stated
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH only
Purpose of dtudy: To evaluate effects of surface runoff from a CCA wood-treatment plant on soil biota.
Study conclusions: Earthworms were absent from the site with the highest CCA concentrations. Bioconcentration of As, Cr, or Cu was not observed in either worm species (tissue levels were lower than soil levels).

APPENDIX B.

SUMMARY OF CONTAMINANT UPTAKE LITERATURE FOR PLANTS

Reference: Burton et al. 1984
Analytes considered: Cd, Cu, and Pb
Species: Sitka spruce (*Picea sitchensis*) seedlings
Category of species (Grass, Herb, Shrub, Tree): Tree
Geographic location of study: Greenhouse using acidic peaty gley soils from South Wales, with quartz sand added
Exposure duration: 0, 3, 17, 31, 59, 100 days
Soil extraction and analytical method: Perchloric-nitric acid mixture, and flame atomic absorption
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, C content, total N, extractable ammonium N, extractable nitrate N
Plant extraction and analytical method: Nnitric/perchloric acid and flame atomic absorption
Plant part analyzed: Roots and shoots
Purpose of study: To investigate uptake and toxicity of heavy metals on sitka spruce.
Study conclusions: Uptake of Cd and Pb by roots and shoots increased with increasing concentrations in soil, but copper uptake appeared to be independent of soil concentration. No metal interactions were observed.

Reference: Carlson and Bazzaz 1977
Analytes considered: Pb and Cd
Species: American sycamore (*Plantanus occidentalis* L.)
Category of species (Grass, Herb, Shrub, Tree): Tree
Geographic location of study: University of Illinois experimental farms at Urbana, 3-L pots containing Drummer silty clay loam with one part "Perlite" per six parts soil
Exposure duration: 90 days
Soil extraction and analytical method: Known metal additions
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: None (that could be obtained from the University of Illinois)
Plant extraction and analytical method: AA
Plant part analyzed: Foliage, new stems, and woody stems
Purpose of study: To investigate the accumulation and toxicity of Cd and Pb in sycamore, as well as the interaction of the metals.
Study conclusions: "Heavy metal accumulation by plant parts increased with soil treatment levels but was much lower than values previously reported in the literature."

Reference: Carlson and Rolfe 1979
Analytes considered: Cd, Pb
Species: Rye grass (*Lolium perenne* L.) and red fescue (*Festuca rubra* L.)
Category of species (Grass, Herb, Shrub, Tree): Grass
Geographic location of study: Pot study in glass house in Illinois
Exposure duration: 10, 20, 30 days
Soil extraction and analytical method: Known treatment additions
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH and CEC
Plant extraction and analytical method: HCL and AA methods

Plant part analyzed: Clippings 2.5 cm aboveground
Purpose of study: To investigate the accumulation and effects of Cd and Pb on growth of rye grass and red fescue, the interaction of the metals, and the effects of fertilization.
Study conclusions: The accumulation of Cd in plants treated with two metals was greater than that of plants to which Cd only was added, while the addition of Cd did not increase uptake of Pb by plants. Fertilization increased uptake of Cd in both plant species but reduced the uptake of Pb in rye.

Reference: Gildon and Tinker 1983
Analytes considered: Cd and Zn
Species: White clover, [onion (*Allium cepa* F_1 hybrid var. Hygro) in paper but not uptake statistics]
Category of species (Grass, Herb, Shrub, Tree): Grass and root vegetable
Geographic location of study: Pots at Woburn farm of Rothamsted Experimental Station, UK
Exposure duration: 5 or 10 weeks
Soil extraction and analytical method: Known additions, also 0.5 M acetic acid extractions, and AA spectroscopy
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented:
Plant extraction and analytical method: AA spectroscopy
Plant part analyzed: Shoots and roots
Purpose of study: To examine the effects of heavy metals on infection of onions with vesicular-arbuscular mycorrhizal fungus. Secondarily, uptake of cadmium by white clover was measured in the absence of the fungi.
Study conclusions: Uptake of Cd increased with Cd concentration in soil, but not linearly.

Reference: He and Singh 1994
Analytes considered: Cd
Species: Oat (*Avena sativa L.*), ryegrass (*Lolium multiflorum L.*), carrot (*Daucus carota L.*), and spinach (*Spinacia oleracea L.*)
Category of species (Grass, Herb, Shrub, Tree): Grass and herb
Geographic location of study: Pots containing soil collected from Aas and Elverum, southeastern Norway
Exposure duration: 55 to 73 days
Soil extraction and analytical method:
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH range created by liming, % C, % clay, % silt, % sand, P
Plant extraction and analytical method:
Plant part analyzed: Oat straw and grain, ryegrass, carrot tubers and leaves, and spinach tops
Purpose of study: To investigate the uptake of Cd by various crops in the presence of different P fertilizers.
Study conclusions: Cd concentrations in crops generally increased with decreasing pH. Cd-containing NPK fertilizers increased the Cd concentration in plants. Crop yield was not affected much by fertilization or liming.

Reference: Haghiri, F. 1973
Analytes considered: Cd
Species: Lettuce (*Latuca sativa* L.), celery (*Apium graveolens* L.), green pepper (*Capsicum frutescens* L.), radish (*Raphanus sativus* L.), soybean (*Glycine max* L.), and wheat (*Triticum aestivum* L.)
Category of species (Grass, Herb, Shrub, Tree): Herb and grass
Geographic location of study: Pots containing Marengo silty clay loam, probably from Ohio
Exposure duration: 5 weeks

Soil extraction and analytical method: Known quantity of Cd added
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, organic matter content and CEC
Plant extraction and analytical method: $HClO_4/HNO_3$, AA spectroscopy
Plant part analyzed: Soybean and wheat tops
Purpose of study: To investigate the uptake of Cd by agricultural plants.
Study conclusions: Cd accumulation by plant tops increased and yield decreased with increasing concentrations of Cd in soil. In the vegetables tested, Cd uptake was highest for lettuce, then radish tops, celery stalks, celery leaves, green pepper, and radish roots. In soybean tops and beans, Cd accumulation was highest in the stem, followed by leaves, pods, and beans.

Reference: Heggo et al. 1990
Analytes considered: Zn, Cu, Zn, Mn, and Fe
Species: Soybean (*Glycine max* L. Merr. "Essex")
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Pots containing silt loam soils in greenhouse near Zn smelter at Palmerton, PA
Exposure duration: 6 weeks
Soil extraction and analytical Method: Nitric/perchloric acid and AA (DTPA extractions also available)
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH
Plant extraction and analytical method: Sulfuric/perchloric acid
Plant part analyzed: Leaves, stems, and roots
Purpose of study: To study the effect of mycorrhizal fungi on the uptake of metals from soils near a zinc smelter by soybeans. Treatments included sterilization with fungi added, with soil bacteria added, or with no additions. Data used for uptake factor calculations were from sterilized soil.
Study conclusions: The mycorrhizae treatment led to decreased concentrations of Zn, Cd, and Mn in plant leaves at high soil concentrations. The fungi increased foliar concentrations of the metals in low-metal soils. The presence of mycorrhizae increased plant biomass and concentrations of P and N in leaves.

Reference: Hutchinson et al. 1974
Analytes considered: Pb, Cd, Zn, Cu, and Ni
Species: Lettuce, radish, corn, oat, onion, carrot, and parsnip
Category of species (Grass, Herb, Shrub, Tree): Herb and grass
Geographic location of study: Holland Marsh muck soil area, Ontario
Exposure duration: Harvest maturity
Soil extraction and analytical method: Nitric/perchloric acid, AA
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented:
Plant extraction and analytical meNhod: nitric/perchloric acid, AA
Plant part analyzed: Shoot and root
Purpose of study: To determine the uptake of several metals from a muck soil by crops and human growers.
Study conclusions: Copper was less bioavailable than other metals, with the order being Cu, Ni, Pb, Zn, and Cd. The foliage of salad and leaf crops accumulated the most Cd and Pb.

Reference: Jiang and Singh 1994
Analytes considered: As (sodium arsenite, disodium hydrogen arsenate)
Species: Ryegrass (*Lolium perenne L.*) And barley (*Hordeum vulgare L.*)
Category of species (Grass, Herb, Shrub, Tree): Agricultural herb and grass
Geographic location of study: Greenhouse using soils collected from Aas, Norway

Exposure duration: < 1 year
Soil extraction and analytical method: no As extracted, backgound below detection
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, clay organic carbon, Fe, Al, and P
Plant analytical method: Gutzeit method: reaction of H and As (with Zn as catalyst) to produce arsine
Plant part analyzed: Straw and grains
Purpose of study: As the authors state, "this work was undertaken to study: (1) the effect of arsenite and arsenate forms of As on crop yield and the As uptake by ryegrass . . . and barley . . . grown in two soils of different texture; (2) the residual effect of the As forms initially applied; (3) As uptake by these crops from a NPK fertilizer containing varying levels of As as influenced by liming, soils texture, and P application."
Study conclusions: Arsenic uptake by crops was much higher in sand than in loam. Arsenic concentration in barley straw was much higher **than** in barley grain. The availability of As to crops tended to decrease with aging of the chemical in years two and three. Under some conditions, As uptake by barley was higher when arsenite was the source rather than arsenate. Added P significantly increased uptake of As by ryegrass but not by barley. High rates of As application caused a yield reduction in crops, especially of barley in the sand.

Reference: John 1973
Analytes considered: Cd
Species: Broccoli/cauliflower (*Brassica oleracea* L.), oat (*Avena sativa* L.), leaf lettuce (*Lactuca sativa* L., cv. crispa), spinach (*Spinacia oleracea* L.), pea (*Pisum sativum* L.), radish (*Raphanus sativus* L.), and carrot (*Daucus carota* L., cv. *sativa)*
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Pots containing soil from the Ap horizon of Hazelwood silt loam from Lower Fraser Valley of British Columbia, Canada
Exposure duration: 35 to 130 days, depending on species maturity
Soil extraction and analytical method: Known quantity added
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, % oxidizable organic matter, CEC, and base saturation
Plant extraction and analytical method: Nitric-perchloric acid mixture AA spectroscopy
Plant part analyzed: broccoli/cauliflower leaf; oat stalk, leaf, and husk; lettuce, leaf; spinach, leaf; pea, vine; radish, top; and carrot, top
Purpose of study: To investigate Cd uptake by various parts of food crops.
Study conclusions: In edible plant parts, the highest Cd accumulation was in lettuce and spinach leaves, followed by uptake in brassica tops, radish and carrot tubers, pea seeds, and oat grains. Yields of some parts were reduced by Cd phytotoxicity.

Reference: Khan and Frankland 1983
Analytes considered: Cd, Pb, and Zn
Species: Radish (*Raphanus sativa* L. cv Cherry Belle)
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Columns of brown earth soils from Dytchleys Field Station near Brentwood and from Weald Country Park, Essex, UK
Exposure duration: 42 days
Soil extraction and analytical method: Hot HNO_3 followed by aqua regia (HCl/HNO_3), EDTA extractions also available; and AA spectrophotometry
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH and soil horizon
Plant extraction and analytical method: 2M H_2SO4 followed by 6M HCl, followed by conc. Hcl; AA spectrophotometry

Plant part analyzed: Roots and shoots
Purpose of study: To determine the toxic effects of cadmium and lead on radish plants and their movement through the soil profile and uptake by the plant.
Study conclusions: Lead was less mobile than Cd. Cd was accumulated more by the plant shoot and Pb by the plant root. When Cd or Pb caused phytotoxicity, Zn levels in the plant were close to deficient values. Cd toxicity caused chlorosis, while Pb toxicity caused reduced growth.

Reference: Lagerwerff 1971
Analytes considered: Cd, Pb, Zn
Species: Radish
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Pots containing soil sampled from three locations near roads, probably in Maryland
Exposure duration: Maturity
Soil extraction and analytical method: 1 N HCl (provide correction factor for estimating total concentrations of cadmium and lead), and AA spectroscopy
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH (altered)
Plant extraction and analytical method: Acid digestion and AA spectroscopy
Plant part analyzed: Tops and roots
Purpose of study: To investigate the uptake of the three metals by radish and the yield in the presence of metals. Treatments included exposure to light aerial contamination (200 m from a road) and indoor separation from air pollution.
Study conclusions: In general, yield increased with metal addition to the soil, perhaps because of zinc deficiency. "The content of each metal in the plants increased by only a fraction of the increase in the soil metal content." Increasing the pH from 5.9 to 7.2 led to a decrease in metal content and yield of radish. The accumulation of metals by the plants grown outside (in air contamination) was significantly higher than that of plants grown inside. The authors estimate contributions of air contamination to accumulation.

Reference: Lamersdorf et al. 1991
Analytes considered: Pb and Hg
Species: Norway spruce (*Picea abies*)
Category of species (Grass, Herb, Shrub, Tree): Tree
Geographic location of study: Northwest Germany
Exposure duration: Indefinite
Soil extraction and analytical method: HNO_3 and AA spectroscopy
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: CEC and pH
Plant extraction and analytical method: HNO_3 and AA spectroscopy
Plant part analyzed: Needle
Purpose of study: To calculate a mass balance of metals at a forest site. Uptake was measured in needles, branches (bark/cortex), stem (bark/cortex/wood), fine roots (vital/subvital), and roots (cortex/wood). Only needles were used to calculate the uptake factor for this report.
Study conclusions: The concentration of cadmium in needles was similar to that in the humus layer. Lead in needles was significantly lower.

Reference: MacPhee et al. 1960
Analytes considered: As, DDT, BHC, chlordane, and parathion
Species: Carrot, bean, and pea

Category of species (Grass, Herb, Shrub, Tree): Herb and root crop
Geographic location of study: Experimental plots, Kentville, Nova Scotia
Exposure duration: < 1 year
Soil extraction and analytical method: Stated in Chisolm et al. 1955, Can. J. Agr. Sci. 35:433-439
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: None
Plant extraction and analytical method: Stated in Chisolm et al. 1955, Can. J. Agr. Sci. 35:433-439
Plant part analyzed: Seeds, pods, vines, and leaves
Purpose of study: To study the persistence and effects of pesticides in soil.
Study conclusions: Uptake of arsenic increased with concentration in soil.

Reference: Miles and Parker 1979.
Analytes considered: Cd, Pb, Zn, and Cu
Species: Little bluestem (*Andropogon scoparius*) and black-eyed Susan (*Rudbeckia hirta*)
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic ;ocation of study: Ggreenhouse experiment using soils from rural and urban locations, probably in or near Indiana
Exposure duration: 12 weeks
Soil extraction and analytical method: Nitric acid digestion (also DTPA) and AA spectroscopy
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, organic matter, CEC, N, K, and P
Plant extraction and analytical method: Nitric acid digestion (also DTPA) and AA spectroscopy
Plant part analyzed: Top and root
Purpose of study: To study toxicity (germination and growth) and uptake of heavy metals in two plants.
Study conclusions: Increases in soil concentrations led to increases in plant concentrations (tops and roots). Uptake was usually greater for black-eyed Susans than for little bluestems. Accumulation was usually higher when rural soils were used than when urban soils were used. Cadmium was readily translocated from root to shoot in black-eyed Susans.

Reference: Miller et al. 1976
Analytes considered: Cd
Species: Soybean (*Glycine max* L. Merr. var. Amsoy)
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Pots containing the surface horizon of nine agricultural soils from Illinois
Exposure duration: 4 weeks
Soil extraction and analytical method: Known addition of Cd
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, CEC, and P
Plant extraction and analytical method: Ashed, boiling 3N Hcl, and AA spectroscopy
Plant part analyzed: Shoots
Purpose of study: To investigate the uptake of Cd and the effects of the metal on yields of soybean. Soils with a range of cation exchange capacities, pHs, and available soil P were used.
Study conclusions: Cd uptake and growth inhibition was highest in low-pH soils. The concentration of Cd in the shoots was significantly correlated with soil CEC, Bray P_1 test for available soil P, and the interaction of CEC X pH, the interaction of the P_1 test X pH, the soil Cd concentration, and the dry weight of the shoots. No correlation was observed between soil pH and accumulation.

Reference: Miller et al. 1977
Analytes considered: Cd and Pb
Species: Corn (*Zea mays* L., Wf9 x M14)

Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Pots containing soil that was probably from Illinois
Exposure duration: 10, 17, 24, and 31 days
Soil extraction and analytical method: Known chemical addition
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented:
Plant extraction and analytical method: Ashed, 3N Hcl and AA
Plant part analyzed: Shoots
Purpose of study: To investigate the uptake of Pb and Cd in corn and the interactive effect of the two chemicals.
Study conclusions: Accumulation of Cd was increased by the addition of Pb. Cadium in soil reduced the uptake of Pb.

Reference: Otte et al. 1990
Analytes considered: As
Species: Common reed (*Phragmites australis* (Cav.) Trin. ex. Steud.) and stinging nettle
Category of species (Grass, Herb, Shrub, Tree): Grass and herb
Geographic location of study: Flood plains of Dordtsche Biesbosch, Netherlands
Exposure duration: Unknown, maybe life of plant
Soil extraction and analytical method: 6M HCl or aqua regia (HNO_3/HCl)
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: P only
Plant extraction and analytical method: HNO_3/$HClO_4$ and AAS and MHS hydride system
Plant part analyzed: Roots and shoots
Purpose of study: To study the uptake of arsenic in the field and under experimental conditions with varying concentrations of phosphate.
Study conclusions: Plants took up increasing levels of As with increasing concentrations in the soil. Reeds accumulated more As in the roots, and nettles accumulated more in the shoots. Arsenic concentrations in the roots of the nettle were positively correlated with P but negatively correlated with As in the soil. Concentrations of As in the roots of the reed were not correlated with the concentrations of As and P in the soil.

Reference: Sadana and Singh 1987
Analytes considered: Cd, Pb, and Zn
Species: Wheat (*Triticum aestivum* L. var. WL 711)
Category of species (Grass, Herb, Shrub, Tree): Grass
Geographic location of study: Pots containing loamy soil from Punjab Agricultural University farm at Ludhiana, India
Exposure duration: Maturity
Soil extraction and analytical method: Known amount added and background metals extracted with 1 N HCl
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, organic carbon content electrical conductance, and calcium carbonate
Plant extraction and analytical method: HNO_3:$HClO_4$:$H2SO_4$ = 9:3:1 and AA spectrometry
Plant part analyzed: Straw and grain
Purpose of study: To investigate the uptake and yield reduction of wheat in metal-polluted soil.
Study conclusions: The Cd and Pb accumulation in wheat straw was much higher than in grain; however, the uptake of Zn in grain was higher than in straw.

Reference: Sadiq 1985
Analytes considered Cd, Pb, Ni, Mn, Cu, Zn, and Fe
Species: Corn
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Saudi Arabia
Exposure duration: 1 month
Soil extraction and analytical method: Nitric-perchloric acid (also DTPA extraction)
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH
Plant extraction and analytical method: Nitric-perchloric acid
Plant part analyzed: Whole plant
Purpose of study: To study the uptake of 3 metals by corn in 16 calcareous soils.
Study conclusions: Cd was accumulated to higher levels than Pb or Ni. Concentrations of Cd, Mn, Cu, and Zn in corn (but not Ni or Pb) were significantly correlated to the DTPA-extractable metal in soil.

Reference: Sadiq 1986
Analytes considered: As
Species: Corn
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Saudi Arabia
Exposure duration: 25 days
Soil extraction and analytical method: Known quantity of As added to soil (also DTPA extraction)
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: Electrical conductivity, calcium carbonate equiv., chloride, and bicarbonate-extractable P
Plant extraction and analytical method: Nitric-perchloric acid
Plant part analyzed: Whole plant
Purpose of study: To study the uptake of As by corn in 19 soils.
Study conclusions: Concentrations of As in corn plants were significantly correlated to water-extractable As and P in the soil but not to DTPA-extractable or total As.

Reference: Severson et al. 1992
Analytes considered: As, Ba, Cd, Ce, Co, Cr, Cu, Hg, La, Li, Mn, Ni, Pb, Se, Sr, V, Y, and Zn
Species: Dune grass (*Ammophilia arenaria*), dune willow (*Salix repens*), and feather moss (*Hylocomium splendens*)
Category of species (Grass, Herb, Shrub, Tree): Grass and shrub
Geographic location of study: Frisian Islands, Germany
Exposure duration: Indefinite, lifetime of plant
Soil extraction and analytical method: Extraction method not stated. ICP-AES used for all elements except for As and Se, determined using hydridegeneration AA spectroscopy and Hg, determined using AA spectroscopy.
Soil characteristics (pH, CEC, % OM, % Clay, etc.) pPresented: pH, %C, Al, and Fe
Plant extraction and analytical method: Extraction method not stated. ICP-AES used for all elements except for As and Se, determined using hydride generation AA spectroscopy and Hg, determined using AA spectroscopy.
Plant part analyzed: Stems and leaves at 10 cm above surface (dune grass) and leaves from willow shrubs
Purpose of study: To measure element concentrations in soil and vegetation of the Frisian Islands.
Study conclusions: Concentrations permit the calculation of uptake factors. Most element concentrations (except possibly Hg and Pb in soil) do not suggest anthropogenic sources of contamination.

Reference: Wallace et al. 1977
Analytes considered: Cd, Mn, Fe, Cu, and Zn
Species: Bush bean (*Phaseolus vulgaris* L. var. "Improved Tendergreen") and corn (*Zea mays* L. var, "Golden Bantam")
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Pots of Yolo loam soil, probably from California
Exposure duration: 30 days (beans), and 24 days (corn)
Soil extraction and analytical method: Known addition of Cd
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented:
Plant extraction and analytical method: 1/10 N HCl and emission spectrography
Plant part analyzed: Leaves and stems or shoots
Purpose of study: To investigate Cd uptake in the presence of other metals and chelating agents was investigated. Studies were done in soil or soil solution.
Study cnclusions: In solution, Cd always decreased the concentration of Mn in leaves, stems, and roots. In soil, added Cd decreased concentrations of Mn and Cu in foliage. Without the added Cd, chelating agents increased concentrations of Mn, Cu, and Fe in plants. Decreased yields are also discussed.

Reference: Xian 1989
Analytes considered: Cd, Zn, and Pb
Species: Cabbage
Category of species (Grass, Herb, Shrub, Tree): Herb
Geographic location of study: Greenhouse, Tokyo
Exposure duration: 90 days
Soil extraction and analytical method: HNO_3-$HClO_4$ (total), and other fractions, including exchangeable, carbonate, Fe-Mn oxide, organic, and residual
Soil characteristics (pH, CEC, % OM, % Clay, etc.) presented: pH, CEC, organic C, inorganic C, Fe, Mn, and texture
Plant extraction and analytical method: HNO_3-$HClO_4$ digestion, flame AA spectrophotometer
Plant part analyzed: Roots and shoots
Purpose of study: To determine uptake of the metals from different soil fractions.
Study conclusions: The uptake was related to soil concentration. Metals in exchangeable and organic fractions of soil determined accumulation more than total concentrations of the metals in soil. Uptake factors were higher for Cd than for Zn or Pb, which is notably the order of the solubility of the metals.

Attachment 4

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Effects of Ionizing Radiation on Terrestrial Plants and Animals: A Workshop Report

Lawrence W. Barnthouse

Environmental Sciences Division
Publication No. 4494

MANAGED BY
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FOR THE UNITED STATES
DEPARTMENT OF ENERGY

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Environmental Sciences Division

EFFECTS OF IONIZING RADIATION ON TERRESTRIAL PLANTS AND ANIMALS: A WORKSHOP REPORT

Lawrence W. Barnthouse

Environmental Sciences Division
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EXECUTIVE SUMMARY

The U.S. Department of Energy (DOE) Air, Water, and Radiation Division (EH-412) is preparing to issue protective radiological standards for aquatic and terrestrial organisms. To support this effort, DOE sponsored a workshop to evaluate the adequacy of current approaches to radiological protection. Workshop participants reviewed and discussed a 1992 International Atomic Energy Agency (IAEA) report on radiological protection of biota for its adequacy and completeness in answering the following questions: Can DOE use these data and conclusions for promulgating radiological standards for the protection of terrestrial organisms? Are the conclusions given in this report still valid or have they been superseded by more recent data?

The consensus of the workshop participants was that the dose limits for animals and plants recommended by the IAEA are adequately supported by the available scientific information. Participants agreed, however, that better guidance on application of those dose limits is needed. Participants further agreed with the IAEA that dose limits designed to protect humans generally protect biota as well, except when (1) human access is restricted without restricting access by biota, (2) unique exposure pathways exist, (3) rare or endangered species are present, or (4) other stresses are significant. To deal with these exceptions, site-specific exposures should be considered in developing secondary standards.

Existing exposure models were found to be sufficient in principle for developing secondary standards. Workshop participants concluded, however, that (1) site-specific transfer coefficients are needed for some important species and exposure routes and (2) improved methods of dosimetry for reference biota are needed to eliminate unnecessary conservatism and provide a practical approach for implementating the standards.

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1. INTRODUCTION

Radiological protection of plants and animals is currently a subject of regulatory concern. On the basis of a recent published report on this topic by the International Atomic Energy Agency (IAEA 1992), the National Council on Radiation Protection is planning to reevaluate the existing information on effects of radiation on biota, and the U.S. Department of Energy (DOE) is preparing guidelines for the protection of terrestrial biota. Scientific information relevant to guideline development includes information on the movement and bioavailability of radionuclides in terrestrial environments, transfer of radionuclides in terrestrial food chains, dose calculation methods for terrestrial plants and animals, and dose-response relationships for exposed biota.

To support its guideline development effort, the DOE Air, Water, and Radiation Division (EH-412) sponsored a workshop to evaluate the adequacy of current approaches to radiological protection, as exemplified by the IAEA report. Workshop participants reviewed and discussed the 1992 IAEA report for its adequacy and completeness in answering the following questions: Can DOE use these data and conclusions for promulgating radiological standards for the protection of terrestrial organisms? Are the conclusions given in this report still valid or have they been superseded by more recent data?

The workshop, held in Oak Ridge, Tennessee, on June 14–15, 1995, was attended by 12 experts in radioecology and ecological risk assessment. The attendees heard presentations on (1) DOE's perspective and regulatory responsibilities, presented by Mr. Andrew Wallo III, Director of the DOE Air, Water, and Radiation Division; (2) the rationale underlying the conclusions contained in IAEA 73, presented by Dr. Gordon Blaylock of the SENES Oak Ridge Center for Risk Analysis; and (3) a summary of data available from the former Soviet Union, presented by Dr. John Trabalka of Oak Ridge National Laboratory. Following these presentations, the participants discussed the adequacy of the data and models available for setting radiological protection standards for terrestrial biota. In evaluating the selection and interpretation of data on biological effects of ionizing radiation, the attendees considered

- study selection criteria,
- the adequacy of support for assumptions employed by the IAEA working group,
- the adequacy of data available for various taxonomic groups,
- the relevance of the biological endpoints included in the IAEA evaluation,
- alternative methods of analysis, and
- the potential existence of new laboratory or field data not considered by the IAEA.

In evaluating the methods for calculating radiological doses to biota, the attendees considered

- the generality of the available models,
- the adequacy of support for assumptions and parameter values used by the IAEA,
- the adequacy of validation for the models used,
- alternative methods of analysis, and
- the potential existence of new data for model parameterization or validation.

Section 2 contains summaries of the presentations given at the workshop. Section 3 summarizes the findings and conclusions regarding both the adequacy of the existing standards and the research and development activities needed to support implementation of the standards.

2. SUMMARY OF PRESENTATIONS

2.1 PRESENTATION BY ANDREW WALLO: DOE'S REGULATORY PERSPECTIVE

The DOE Office of Environmental Policy and Assistance is responsible for the interpretation of environmental regulations and for the development and issuance of DOE requirements and guidance for radiological protection of the public and the environment.

DOE's requirements for radiological protection of the public and the environment are found in DOE Order No. 5400.1, *General Environmental Protection Program,* and in DOE Order 5400.5, which will eventually be promulgated as 10 CFR Pt. 834, *Radiation Protection of the Public and Environment*, by the DOE Air, Water, and Radiation Division. Feedback on the draft of 10 CFR Pt. 834 indicates a need for a holistic approach to radiological protection of the environment. The need to integrate ecological protection into these radiation programs has also been identified in various federal interagency efforts in which DOE participates. To accomplish this integration, we need to evaluate exposure pathways for aquatic and terrestrial organisms and ecosystems and to develop protective radiological standards for aquatic and terrestrial organisms.

The current order and the proposed rule include guidelines for protection of aquatic organisms. By the end of the calendar year 1995, the goal is to identify or develop radiation protection standards for the terrestrial biota that could be incorporated into DOE regulations. The objective is to have guidelines that will be useful to DOE as well as other agencies in implementing environmental protection and restoration programs. DOE believes that the recent IAEA guidance is a reasonable template for these guidelines.

The 1992 IAEA report endorses the 1977 International Commission on Radiological rotection (ICRP) report and states that chronic radiation dose rates below 1 mGy/d (0.1 rad/d) will not harm plant and animal populations and that radiation standards for human protection will also protect populations of nonhuman biota. The IAEA report concludes that specific radiation protection standards for nonhuman biota are not needed where there are protective standards for humans in place. However, DOE needs to define how broadly this guidance can be applied. Clearly, the guidance applies in situations where properties are being released without control because the stringent protection requirements for the public, which are set at a level low enough to protect individuals who reside on the site and obtain all sustenance from the site, will ensure that ecosystems are protected. In some situations, however, the public are protected by restricting access or use of property; these restrictions are not necessarily effective in controlling the movement or access of plants and animals. We must determine if special ecological guidelines are needed for these situations.

The workshop should provide DOE answers to the following questions:

- Is the recommendation by the IAEA sound?
- What, if any, guidelines are needed to protect nonhuman biota?
- How can this concept be implemented in radiation protection guidance such as DOE's 10 CFR Pt. 834?

Ideally, radiation protection requirements should be flexible enough to allow each facility to develop its own approaches on protection of the environment (i.e., aquatic and terrestrial biota). In addition, guidance and methodology that can be used to demonstrate compliance with ecological radiation protection requirements need to be identified or developed. The workshop participants should determine if guidance or technical documents are available that would provide sufficient information on how to protect aquatic and terrestrial organisms.

The participants should address three issues:

- Is the 1992 IAEA document the only document available for protecting plants and animals from radiation, and is this document sufficient in its recommendations regarding protective radiation standards for nonhuman biota?
- Do we have methods available to demonstrate compliance with dose limits for nonhuman biota?
- A critical aspect of protecting plants and animals from radiation is the concept of protecting a population rather than individuals of a given species; the exception is endangered species, where individuals must be protected. Is the population concept appropriate for protecting an ecosystem?

The scope of the applicability of radiation protection guidelines must also be defined. The guidelines can be stated as (1) general levels of operations or cleanup, (2) site-specific levels, (3) screening values, (4) levels requiring no detailed studies, or (5) a combination of several of these. Methods of implementing the guidelines must be clear.

In summary, we need to know if ecological radiation protection standards are needed, and, if so, we need to know the appropriate level of these standards and how they should be implemented.

2.2 PRESENTATION BY GORDON BLAYLOCK: SUMMARY OF THE IAEA REPORT

The IAEA Technical Report No. 332 had two objectives: (1) to determine whether the statements of the ICRP about the protection of nonhuman organisms and populations are consistent with current knowledge and (2) to determine whether or not radiation

protection standards for aquatic and terrestrial biota are warranted. In 1977, the ICRP reached the following conclusion:

> Although the principal objective of radiation protection is the achievement and maintenance of appropriately safe conditions for activities involving human exposure, the level of safety required for the protection of human individuals is thought likely to be adequate to protect other species, although not necessarily individual members of those species. The Commission therefore believes that if man is adequately protected then other living things are also likely to be sufficiently protected. (ICRP 1977)

The IAEA noted that, although this assumption has generally been accepted and adopted for standard setting, it had not been rigorously evaluated. The IAEA Report No. 332 was intended to be a rigorous review of the scientific information relevant to the ICRP's earlier conclusions. The report dealt primarily with potential effects on natural plant and animal populations exposed to routine, chronic releases of radionuclides that are controlled to limit exposure of humans to specified safety standards. Accidental releases and releases to areas where human access would be controlled were not specifically considered. The IAEA report specifically evaluated situations in which (1) environmental releases are limited to levels that protect the most highly exposed humans, and (2) the biota of the natural environment share the same environment as the most exposed humans.

Underlying the report is a basic assumption concerning the difference between the way society views risks to people and the way it views risks to other organisms. Our values are strongly focused upon individual humans, and standards are designed to protect the most exposed or most sensitive individuals. In contrast, we view and value most other species as populations rather than as identifiable individuals. Hence, the focus of the IAEA was on defining standards that would protect the viability of populations of organisms, even though some individuals might be adversely affected. The IAEA report adopted the following definition of a population:

> A population is a biological unit for study, with a number of varying statistics (e.g., number, density, birth rate, death rate, sex ratio, age distribution), and which derives a biological meaning from the fact that some direct or indirect interaction among its members are more important than those between its members and members of other populations. (1992)

The report considered two types of exposures to populations. An acute exposure is one that is delivered in a time period that is short compared with the time over which any obvious biological response develops. A chronic exposure is one that could continue over a large fraction of the natural life of the organism. The IAEA evaluated data relating to terrestrial plants, mammals, birds, reptiles, amphibians, and invertebrates. The objective of the data review was to identify acute doses and chronic dose rates "below which the likelihood of observing population level effects is remote." Both experimental (laboratory

and field) and observational (involving areas of high natural background radiation and areas with significant anthropogenic contamination) studies were evaluated. A detailed evaluation of data relating to effects of ionizing radiation on aquatic biota was not performed because the IAEA believed that existing comprehensive reviews of these data were adequate.

For acute effects, the IAEA found that reproduction is likely to be the most limiting endpoint in terms of survival of populations. Lethal doses were judged to vary widely among different populations, with birds, mammals, and a few tree species being the most sensitive among those considered by the working group. Acute doses of 0.1 Gy/d (10 rad/d) or less were judged very unlikely to produce persistent, measurable deleterious changes in populations or communities of terrestrial plants or animals. For chronic effects, the working group found again that reproduction is likely the most limiting process. Sensitivity varies markedly among different taxa; certain mammals, birds, reptiles, and a few tree species appear to be most sensitive. For invertebrates, indirect responses caused by radiation-induced changes in vegetation appear to be more critical than direct effects of radiation on the organisms themselves. The working group concluded that irradiation at chronic dose rates of 1 mGy/d (100 mrad/d or 0.1 rad/d) to even the most radiosensitive species does not appear likely to cause observable changes in terrestrial animal populations. For aquatic biota, the working group found that a chronic dose rate of 10 mGy/d (1 rad/d) would be unlikely to adversely affect populations. The IAEA cautioned, however, that reproductive effects in long-lived species with low reproductive capacity might require further consideration.

In addition to estimating a "safe" dose for biota, the IAEA evaluated whether application of current radiological protection standards for human exposure would result in maintenance of doses to biota below the "safe" level. Three exposure scenarios were evaluated:

- controlled releases of radionuclides from the atmosphere,
- controlled releases of radionuclides to a freshwater aquatic system,
- and uncontrolled constant releases of radionuclides from a shallow-land nuclear waste repository.

For each scenario, steady-state environmental concentrations of selected radionuclides were calculated that would yield a radiation dose to man equal to the annual dose limit for members of the public (1 mSv/year). These concentrations were used to calculate equilibrium dose rates to reproductive or growth tissues of aquatic and terrestrial biota. The resulting doses were compared with the "safe" dose rates (1 rad/d for aquatic biota and 0.1 rad/d for terrestrial biota) recommended by the group. Fifteen isotopes were considered in at least one of the three release scenarios: ^{3}H, ^{14}C, ^{32}P, ^{60}Co, ^{90}Sr, ^{95}Zr, ^{99}Tc, ^{129}I, ^{131}I, ^{137}Cs, ^{226}Ra, ^{235}U, ^{238}U, ^{239}U, and ^{241}Am. Three approaches were employed in estimating radiological doses: the published results of the PATHWAY model (Whicker

and Kirchner 1987), limits on air concentrations and annual intakes derived by the ICRP (1979–81), and computer simulations conducted in 1987 by a class in radionuclide kinetics at Colorado State University. Parameter selection emphasized situations that would yield maximum environmental concentrations and therefore maximum doses to biota.

Evaluations of doses to plant tissue considered foliar depositions, root uptake, and external exposure. Evaluations of doses to animal tissue considered external exposure, inhalation, and ingestion. Upper-estimate dose rates to soil organisms were calculated to be ≤5 mGy/d (0.5 rad/d) for all radionuclides. According to the IAEA, available scientific literature supports a conclusion that this dose rate would not cause measurable perturbations in populations of soil microorganisms or soil invertebrates. Upper estimate dose rates for terrestrial plants and animals were in all cases <1 mGy/d (0.1 rad/d).

On the basis of these results, the IAEA endorsed the ICRP's assertion that regulation of radionuclide releases to levels that protect man will also protect biota. IAEA qualified this endorsement by noting that there may be circumstances, such as the presence of rare or endangered species, in which the generic dose calculations presented in the IAEA report may be insufficient and site-specific analyses may be required.

2.3 PRESENTATION BY J. R. TRABALKA: OVERVIEW OF RUSSIAN/ CONFEDERATION OF INDEPENDENT STATES INFORMATION SOURCES, WITH EMPHASIS ON STUDIES AT THE 1957 EXPLOSION SITE IN THE URALS

A number of recent published reports have summarized data collected following three major accidental radionuclide releases in the former Soviet Union. The releases came from the Mayak reprocessing facility into the Techa River (1950–51), the 1957 explosion of stored radioactive waste at Kyshtym, and the 1986 accident at the Chernobyl Nuclear Power Plant. The reports include

- the proceedings of the 1990 Luxembourg seminar entitled *Comparative Assessment of the Environmental Impact of Radionuclides Released During Three Major Nuclear Accidents: Kyshtym, Windscale, Chernobyl*, EUR 13574, Commission of the European Communities, 1991 (2 volumes);
- "Radiobiology and Radioecology in the Vicinity of Chernobyl," *Science of the Total Environment* **112,** 1992;
- *Ecological After-Effects of Radioactive Contamination at South Ural,* V. E. Solkov and D. A. Krivolutskii (eds.), Nauka Publishers, Moscow, 1992 (in Russian); and
- the report of an International Union of Radioecology working group (Task Force 5), chaired by Dr. Dennis Woodhead, on effects of enhanced radiation exposure to wild organisms in their natural environment (unpublished draft).

In addition, a workshop entitled *Radioecology: Advances and Perspectives* was held on October 3–7, 1994, at Sebastopol, Ukraine. This workshop focused on the damaging effects of radionuclide contamination from accidents and waste disposal, especially from the incidents at Chernobyl and Mayak. Examined effects included extensive tree mortality in pine forests, changes in thyroid activity and reduced viability of cattle near Chernobyl, impacts on small rodents near Chernobyl, and impacts on aquatic biota in water bodies near Mayak. The principal conclusions from the workshop were as follows:

- No deleterious effects of radiation could be observed in locations where radiological doses were less than or equal to 5 rad/year.
- Where doses between 5 and 400 rad/year were received, radiation effects were "ecologically masked," meaning that adverse effects on individual organisms were observed but no changes in populations or ecosystems occurred.
- Where doses were >400 rad/year, damaging effects on populations and communities occurred.
- Total destruction of ecosystems occurred where doses exceeded 10,000 rad/year.

Dr. Trabalka has performed a detailed evaluation of scientific literature published by Russian radioecologists following the Kyshtym disaster. He found a number of uncertainties and limitations that affect the interpretation of these data. For example, high spatial variability in deposition following the September, 1957 explosion led to major uncertainties concerning the true levels of radiation to which biota were exposed. Deposition of atmospherically transported particles dropped off rapidly both longitudinally and in cross section along the main deposition axis. Local effects of surface features (e.g., vegetation type and topography) caused substantial fine-scale variability in deposition. Moreover, following initial deposition, the action of wind and precipitation resulted in substantial redeposition of particles. Radionuclides initially deposited on tree crowns were rapidly washed off and deposited on the litter surface, where further migration through leaching and plant root uptake occurred. The release consisted predominantly of ^{90}Sr, decay products of ^{90}Sr, and short-lived (half-life: <1 year) isotopes. The radiation that remained five years after the accident was due almost entirely to ^{90}Sr.

These uncertainties are significant because studies conducted immediately following the accident were of relatively poor quality by today's standards. Large errors existed in original measurements of radionuclide deposition; current values are based on reconstructions rather than on actual measurements. Dosimetry (measurements of the actual radiation doses received by organisms) was not attempted. All reported effects were related to levels of initial surface deposition (e.g., ^{90}Sr activity in Ci/km^2). Other concerns raised by Dr. Trabalka include the methodology and timing of the ecological studies and the failure to distinguish between direct effects of radiation and indirect effects such as successional changes in plant communities following forest die-off or altered predator-prey relationships. Potential confounding factors such as immigration of

organisms from uncontaminated areas and toxicity from chemical pollutants released from nearby industrial facilities were not obtained.

Despite these uncertainties, approximate dose-response relationships can be derived from studies of "acute" effects of radiation at Kyshtym (i.e., effects resulting from the first 1 to 1.5 years of exposure following the accident):

- Where dose rates exceeded 300–500 rad/d at the soil surface (deposition 4000 Ci/km^2 ^{90}Sr), complete mortality of even the most tolerant plant species (e.g., species with renewal buds below the soil surface) occurred.
- Where dose rates exceeded 40–50 rad/d at the soil surface, complete mortality of grasses and herbs with renewal buds at or near the surface occurred. These plants were replaced by plants with renewal buds buried in the soil. Slow recovery occurred after 3–4 years,
- Where dose rates to the meristem buds of birch trees exceeded 40–50 rad/d (^{90}Sr deposition 4000 Ci/km^2), mortality to trees was complete. At lower dose rates, withered crowns, underdeveloped leaves, and phenological shifts (delay in opening of leaves and flowers, premature leaf fall) were observed over 4 years.
- Where dose rates at the soil surface were ≤30 rad/d, seed germination was reduced in plants with renewal buds near the soil surface; morphological changes (gigantism, chlorosis, blued and contorted leaves, lower numbers of seeds in spikes) were observed in some species over 2–3 years.
- A 1958 study demonstrated reduced nesting of birds in contaminated forests where estimated dose rates in tree crowns exceeded 20 rad/d (^{90}Sr deposition 2000 Ci/km^2). Lethal doses to resident birds and mammals would have been expected where dose rates exceeded 10 rad/d, but effects of the Kyshtym accident on birds and mammals were not evaluated until 1964.
- Pine trees were the most sensitive of the biota examined. Where dose rates to needles were 5–10 rad/d or higher, complete mortality of pines occurred within 2 years. At lower dose rates, a variety of sublethal effects were observed.

Dr. Trabalka's overall evaluation is that, although a substantial quantity of radioecological data has been collected in the former Soviet Union, Western scientists have probably not evaluated much of the data. Those studies that have been evaluated, however, are generally consistent with other published literature. The 1994 workshop in Sebastopol included participants knowledgeable about data collected at Chernobyl and Mayak; participants at this workshop reached conclusions similar to those presented in the IAEA Report No. 332.

3. SUMMARY OF WORKSHOP DISCUSSION

Following the presentations, workshop participants developed an independent evaluation of the information relevant to setting radiological protection standards for terrestrial biota. The discussion paralleled the format of the IAEA Report No. 332. First, the laboratory and field data on biological effects of ionizing radiation were discussed. Then, methods and assumptions involved in radiological dose calculations were discussed.

3.1 BIOLOGICAL EFFECTS DATA

3.1.1 Laboratory Studies

Participants first identified the types of laboratory data that could be used in setting radiological protection standards. The following types of effects were discussed:

- chromosomal aberrations, defined as visually observable morphological changes in chromosome structure;
- DNA damage, defined as damage to DNA molecules, detectable through biochemical assays;
- cancer, defined as the development of tumors or other benign or malignant lesions analogous to those that occur in humans;
- growth reduction, defined as a reduction in the rate of growth of organisms, including both animals and plants;
- reproduction effects, including sterility, reduction in fecundity, and occurrence of developmental abnormalities or reduction in viability of offspring;
- reduced seed germination in plants; and
- mortality, including both acute lethality and long-term reduction in life span.

Participants identified a number of methodological problems limiting the value of laboratory studies. First, techniques employed for both external and internal dosimetry in early experimental studies were much less accurate than are those used today. Second, the species tested were, for the most part, selected either because of ease of handling or relevance to human health research. Rodents, beagles, chickens, and *Drosophila* have been the most common animals studied. Most laboratory research on radiation effects on plants has been performed with seeds and seedlings. Third, the range of sensitivities of species and life stages in nature is undoubtedly much greater than the range of sensitivities of species and life stages for which laboratory data are available. Nutritional status is known to affect responses of animals to stress; because of parasitism, disease, or variations in food availability, animals in nature are probably often more vulnerable to added stresses such as ionizing radiation than are well-fed laboratory animals. In addition to these difficulties, most of the emphasis in laboratory research has been on acute

exposures, and, even for these, changes in methodology through time make it difficult to compare results of different studies.

Workshop participants compiled a list of criteria for evaluating published laboratory studies:

- Duration. Chronic studies, defined as studies in which organisms are exposed throughout most or all of their life spans, are preferred over acute studies.
- Replication. Studies should include sufficient replication for confidence limits around test endpoints, such as LD50s, to be reliably calculated. (Participants did not specify a minimally acceptable statistical power or other criterion for determining a required number of replicates.)
- Presence of dose-response relationship. Participants agreed that studies in which the magnitude of the measured response did not increase with increasing radiation dose should not be used for setting protection standards.
- Taxonomic distribution. Data used for standard setting should include tests on mammals, birds, reptiles, invertebrates, and plants; studies covering a wide range of taxa are preferable to those covering a narrow range.
- Ecological relevance of endpoints. Only test endpoints that have clear consequences for the abundance and persistence of populations should be considered. These endpoints include growth, reproduction, and survival. Genetic changes and morphological changes, such as tumors, do not have unambiguous population-level consequences and should not be used.
- Accuracy of dosimetry. Studies that use the most modern dosimetric methods are preferred; studies using less accurate methods may be used with appropriate qualifications.

3.1.2 Field Studies

The general consensus of participants was that field data are usually more valuable than laboratory data for assessing ecological effects of ionizing radiation. Two kinds of field studies have been performed: experimental studies, in which natural ecosystems have been exposed to radiation under controlled conditions, and monitoring studies, in which measurements of radiation exposures and effects have been made in contaminated environments.

The conditions in experimental studies are much more natural than in laboratory studies. The full array of natural biota are potentially available for study although, in practice, data on birds and large, mobile animals are difficult to collect. For plants, soil-dwelling invertebrates, and small mammals, population-level effects can be directly observed. Moreover, highly accurate dosimetry is possible, at least for external exposures (participants noted that early field experiments, conducted prior to the development of thermoluminescent dosimetry techniques, must be interpreted with caution). Indirect

effects, notably changes in plant and animal community composition caused by reduced abundance of sensitive plant species, can also be observed.

Monitoring of contaminated ecosystems has additional advantages. Many such sites involve exposures over a much larger spatial scale than is possible in a field experiment. Sites where data relevant to radiological protection standards have been collected include Mayak, Kyshtym, Chernobyl, Windscale, the DOE reservations, nuclear-weapons testing sites throughout the world, uranium-mining sites, and regions with high natural background radiation. Because of the larger spatial extent of exposures associated with many of these sites, mobile animals can, at least in principle, be included. Moreover, the range of ecosystem types in which monitoring studies have been performed is far greater than the range for which experimental data are available. Whereas most experimental studies have used primarily acute external exposures, monitoring studies involve both internal and external exposures and, in many cases, chronic exposures.

Field studies are also subject to a variety of important limitations. Almost all experimental studies, particularly those in which doses are high enough to produce detectable biological effects, have been limited to acute external exposures. In the small number of field experiments involving direct application of isotopes to plants or soils, dose rates have been below biological effects thresholds; the results, therefore, are useful primarily for estimating transfer coefficients. Experiments involving applied isotopes are also subject to significant uncertainties owing to spatial variations in isotope application and the difficulty of obtaining accurate dose measurements for internal exposures. Background environmental variability is inevitably higher in field experiments than in controlled laboratory settings, and sample sizes and numbers of replicates are usually small. Thus, the minimum detectable biological effect is much larger in field experiments than in laboratory experiments. Moreover, for practical reasons, experimental studies have emphasized effects on sedentary species, especially plants, because observed dose-response relationships are generally poor except for plants.

Monitoring studies are subject to additional uncertainties relating to the high spatial heterogeneity of radionuclide deposition rates. For some of the most important monitoring studies (e.g., Kyshtym), direct measurements of deposition rates are unavailable; for others (e.g., Chernobyl), spatial patterns in deposition must be interpolated from limited field sampling. Dose rates for monitoring studies conducted outside the former Soviet Union have generally been too low to produce measurable ecological effects. Because good principles of experimental design (e.g., replication, controls) cannot be applied in monitoring studies, it is often impossible to unambiguously distinguish between radiological effects and effects caused by chemical contamination, habitat disturbance, or natural climatic variation. This is especially true for birds, mobile mammals, and any situations in which doses were less than catastrophic. Moreover, primary effects (i.e., direct effects of radionuclides on organisms) are difficult to distinguish from secondary or indirect effects such as those resulting from plant

community succession following death of sensitive species. Finally, methods used have differed greatly among studies, making comparisons difficult.

With these limitations in mind, workshop participants developed a list of criteria for selection of field studies for use in standard setting:

- Duration. Long-term studies are preferable to short-term; this criterion applies both to the duration of the dose and the duration of the study.
- Accuracy of dosimetry. Studies that use the most modern dosimetric methods are preferred; studies using less accurate methods may be used with appropriate qualifications.
- Presence of dose-response relationship. The magnitude of the measured response should increase with increasing radiation dose; intermediate responses as well as high responses and negative responses should be observed.
- Type of effect observed. Demonstrations of impacts on populations and communities are more useful than measurements of impacts on individual organisms.
- Statistical design. Studies with good statistical designs (site selection, sample replication) for estimating doses and effects are preferred over studies with poor or no statistical designs.
- Quality of documentation. Methods used should be fully documented so that the reliability of the data can be effectively evaluated.

3.1.3 Evaluation of Data Selection Criteria Used by the IAEA

Participants found that, in general, the data selection criteria used by the IAEA were similar to those developed at the workshop. Field studies were used in preference to laboratory studies when good quality studies of both kinds were available. Chronic studies were preferred over acute studies. Like the workshop participants, the IAEA excluded biological endpoints that are not clearly indicative of potential population-level effects. The excluded endpoints include genetic effects (chromosomal aberrations and DNA damage) and tumor induction.

3.2 DOSE CALCULATIONS

Two aspects of dose calculation were discussed at the workshop: (1) the use of environmental transport models to estimate the environmental partitioning and transport of radionuclides and (2) the use of direct measurements and dosimetric models to calculate the radiation doses received by whole organisms and specific target tissues.

3.2.1 Environmental Transport Calculations

Participants agreed that environmental transport modeling will always be necessary in radiological risk assessment, especially for assessing risks associated with new facilities for which actual monitoring data do not yet exist or for assessing doses to humans and biota from existing environmental contamination. The participants did not attempt critical evaluation of any existing environmental transport models. Recent model comparison studies have shown that the choices of assumptions and data sets for parameterization have much more influence on the accuracy of model predictions than do differences between the various models available for performing dose calculations. Hence, it is unlikely that the use by the IAEA of a model other than PATHWAY would have altered the conclusions reached by the IAEA.

A fundamental principal of environmental pathways modeling is that the fewer steps in model extrapolation required, the more accurate the predictions. Hence, measurements should be made as close to the target organism as possible. For example, in estimating internal radiological doses to herbivorous wildlife such as deer, measurements of radiological activity in vegetation browsed by deer are preferable to estimates of vegetation activity extrapolated from measured activity in soil. However, in the IAEA study, all dose calculations were made on the basis of assumed deposition rates on soil. The IAEA attempted to minimize the impact of uncertainties in soil-to-plant-to-herbivore transfer coefficients by using the same set of assumptions to estimate both doses to humans and doses to biota. The IAEA argued that the ratio of these two doses, which was the quality of interest, would be insensitive to uncertainties in transfer coefficients.

Workshop participants agreed in general with the IAEA's argument. Participants noted, however, that the validity of the argument is dependent on high similarity of transfer processes in human vs biotic food chains and that circumstances exist in which these processes may be substantially different, resulting in unusually efficient transfer to biota. Some ecosystems contain unique environmental pathways, such as the lichen-to-reindeer pathway, that differ qualitatively from the soil-to-plant-to-animal transfers simulated by PATHWAY and other similar models. Moreover, high soil acidity and low soil clay content promote uptake of radionuclides and other contaminants by plants. Such soils are poor sites for agriculture but can support diverse natural ecosystems. In addition, some organisms may have unusual life histories that lead to anomalously high exposures to environmental radionuclides. Participants suggested that site-specific assessments should evaluate the potential existence of these kinds of circumstances.

3.2.2 Dosimetry for Biota

Workshop participants found that the greatest single uncertainty affecting radiological dose calculations for biota is in dosimetry; the calculation of the total radiation dose

received by an organism from both external and internal sources. Three types of doses must be considered:

1. radiated external dose, the dose received from radioactive particles not in direct contact with the organism itself;
2. deposited external dose, the dose received from radioactive particles deposited directly on the surface of the organism; and
3. internal dose, the dose received from radionuclides ingested or inhaled by the organism.

Only one of these three doses, the radiated external dose, can be reliably measured in the field. For the other two sources, dosimetric models are needed to relate measurements of radionuclide activity to the dose absorbed by target tissue. The geometry of the organism, the ability of different radioactive particles to penetrate various tissues, and the partitioning of radionuclides within the organism all influence the dose received by target tissues. Through years of research, reliable dosimetric models have been developed for man and for well-studies laboratory animals such as mice and rats. However, similar models do not exist for most kinds of terrestrial biota. The importance of uncertainty in dosimetry for terrestrial organisms is currently unknown. Although the participants briefly discussed the issue of using weighting factors to account for differences in the damage-causing potential of different types of radiation, they reached no conclusion. They did agree that models are needed for the following kinds of terrestrial biota:

- mammals (large and small),
- birds (large and small),
- plants (evergreens, deciduous trees, shrubs/grass), and
- soil invertebrates (earthworms).

4. CONCLUSIONS AND RECOMMENDATIONS

The consensus of workshop participants was that the 0.1 rad/d limit for animals and the 1 rad/d limit for plants recommended by the IAEA are adequately supported by the available scientific information. However, they concluded that

- guidance on implementing the limits is needed, and
- the existing data support the application of the 0.1 rad/d limit for populations of terrestrial and aquatic fauna to *representative* rather than *maximally exposed* individuals. A dose not exceeding 0.1 rad/d to representative members of a population of terrestrial or aquatic fauna would not cause adverse effects at the population level. Therefore, application of such a screening criterion would ensure protection of terrestrial and aquatic fauna and would be consistent with the NCRP and IAEA recommendations.

Participants further agreed with the IAEA that protecting humans generally protects biota except when (1) human access is restricted but access by biota is not restricted, (2) unique exposure pathways exist, (3) rare or endangered species are present, or (4) other stresses are significant. To deal with these exceptions, site-specific exposures should be considered in developing secondary standards. The participants concluded that existing exposure models are sufficient in principle for developing secondary standards. However, transfer coefficients must be developed for some important species and exposure routes that have not been adequately studied, and improved dosimetric models for reference biota are needed to eliminate unnecessary conservatism and provide a practical approach to implementation of the standards.

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APPENDIX

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Ecological Screening Benchmarks Database

Available Through the World-Wide Web

The Ecological Screening Benchmarks Database was developed and refined with funding received through an Interagency Agreement between the Department of Energy and the Environmental Protection Agency National Center for Environmental Assessment, under the auspices of Strategic Environmental Research and Development Program (SERDP) project number 70.

The Ecological Screening Benchmarks Database is a tool for application in ecological risk assessments. One of the initial stages in ecological risk assessments is the screening of contaminants to determine if any are of concern. Screening is performed by comparing concentrations in ambient media to benchmark concentrations that indicate either a high likelihood of significant effects (upper screening benchmarks) or a low likelihood of significant effects (lower screening benchmarks).

Overview -

The Ecological Screening Benchmarks Database is a stand-alone, Fox-Pro-based program designed to facilitate searching for and acquisition of ecotoxicological screening benchmarks. The database has five sections, containing information derived from the Oak Ridge National Laboratory (ORNL) ecotoxicological benchmark documents. These sections are for aquatic biota (Suter and Tsao 1996), sediment-associated biota (Jones 1996), avian and mammalian wildlife (Sample et al. 1996), terrestrial plants (Will and Suter 1995a), and soil or litter invertebrates and microbial processes (Will and Suter 1995b). Each section contains information summarizing the derivation and application of the benchmarks and is searchable by chemical name or CAS number. Search results may be printed directly or saved as Lotus, Excel, DIF, Symphony, or ASCII files.

The sediment-associated biota section contains up to 15 different benchmarks for approximately 120 chemicals; the aquatic biota section contains up to 13 different benchmarks for 115 chemicals. In addition to containing no observed adverse effects levels (NOAELs) and/or lowest observed adverse effects levels (LOAELs) for 85 chemicals for 9 mammalian and 11 avian species, the wildlife section contains NOAEL- and LOAEL-based food, water, and piscivore benchmarks. The terrestrial plant section reports soil and/or soil solution benchmarks for 38 chemicals. The soil and litter invertebrate section contains benchmarks for 35 chemicals for earthworms and 32 chemicals for microbial processes.

Ecological Screening Benchmarks Database

Available Through the World-Wide Web

Access to Database -

The database has been updated regularly as revisions are made to the benchmark documents. The last revision (database version 1.6) was completed in November 1996. The Database is available in a down-loadable DOS-based version, which is located at the *Ecological Risk Analysis: Tools and Applications Web Site* (Oak Ridge National Laboratory) at the following URL:

http://www.hsrd.ornl.gov/ecorisk/ecorisk.html

The Database can also be accessed through the *Office of Environmental Policy and Assistance (OEPA) web site on the Dose and Risk Assessment hub*. The address is:

http://tis-nt.eh.doe.gov/oepa/risk

Ongoing Improvements -

The Office of Environmental Policy and Assistance is making the Ecological Benchmark Database accessible *interactively*, without the need for downloading files. To review this feature, go to the OEPA web site Dose and Risk Assessment hub address listed above, go to the "Databases of DOE Complex-wide Contaminants of Interest" menu, click on the "Ecological Benchmarks" title, and follow the search instructions. This feature is in the final stages of development.

Ecological Screening Benchmarks Database

Available Through the World-Wide Web

References -

Jones, D. S., R. N. Hull, and G. W. Suter, II 1996. *Toxicological Benchmarks for Screening of Potential Contaminants of Concern for Effects on Sediment-Associated Biota: 1996 Revision,* ES/ER/TM-95/R2, Lockheed Martin Energy Systems, Inc., Oak Ridge, Tenn.

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Attachment 5

ESTIMATING THE ABSORBED DOSE FROM DERMAL EXPOSURE TO ENVIRONMENTAL POLLUTANTS:

Development of Guidelines for Acquisition, Interpretation and Use of *In vivo* and *In vitro* Data

EPA Cooperative Agreement No.: CR822757

SUMMARY OF ACCOMPLISHMENTS

Principal Investigators:

Dr. Annette L. Bunge, Colorado School of Mines, Golden, CO

Dr. Richard H. Guy, University of California, San Francisco, CA

EPA Project Officer:

Dr. Richard Hertzberg, National Center for Environmental Assessment (mailing address: U.S. EPA-Region 4, Waste Management Division, Atlanta Federal Center, 100 Alabama St., SW, Atlanta, GA 30303-3104)

ESTIMATING THE ABSORBED DOSE FROM DERMAL EXPOSURE TO ENVIRONMENTAL POLLUTANTS:

Development of Guidelines for Acquisition, Interpretation and Use of *In vivo* and *In vitro* Data

SUMMARY OF ACCOMPLISHMENTS

Annette L. Bunge, Colorado School of Mines, Golden, CO
Richard H. Guy, University of California, San Francisco, CA
Richard Hertzberg, U.S. EPA Project Officer

Summary

This cooperative agreement spans three years, of which roughly half was supported by SERDP resources. In Years 1 and 2, the investigators acquired, evaluated, and qualified dermal absorption data for inclusion in several databases of permeability coefficients and partition coefficients from human and animal skins. In Year 3, the investigators will use these databases to develop methods for predicting dermal absorption parameters (i.e., permeability and partition coefficients) from molecular property information or from measurements made on other animal skins. It is this product - a validated, empirically supported method for estimating dermal absorption - that is required by risk assessors to assess human health risks from dermal exposure. The databases assembled during the first two years of this project are substantial for scientific research, but have a limited value for risk-based regulation without the effort of the final year to transfer information into a form suitable for implementation. In addition, external validation of our databases and analyses through refereed publication is essential before EPA or other agencies can accept our results and recommend them for wide spread use. These publications remain to be completed during Year 3.

Importantly, this research will provide greatly needed information for dermal risk assessment. Stated briefly, EPA and other agencies are making estimates about dermal exposure risk using data, which, in some cases, have been incorrectly obtain, incorrectly analyzed, or incorrectly used. The investigators are correcting as many of those results as possible during the database creation, documenting other data problems which are not correctable, and identifying the critical data needs (and how to correctly obtain and analyze those data to obtain quality results). The results of this research are fully qualified databases for developing and testing predictive correlations of dermal absorption parameters, lists of critical data requirements, and a list of fully documented guidelines for acquisition, interpretation and use of dermal absorption data. Altogether then, the results from the first two years combined with those from Year 3 will assist EPA and SERDP to put their limited research dollars to the most effective use.

1. PROJECT DESCRIPTION

The goal of this research is to develop a consistent, scientifically and experimentally based, procedure for computing realistic estimates of the dermally absorbed dose from an environmental exposure. The computation should ensure protection of human health and be simple enough for routine risk assessments. This strategy is based on the premise that the diverse nature and number of environmental pollutants, and the variety of exposure scenarios mandate development of predictive algorithms. It is infeasible experimentally to determine dermal absorption for all chemicals of environmental concern from all possible exposure scenarios.

Of the exposure routes, dermal absorption is unquestionably the least understood. While dermal exposure data for environmental pollutants are limited, there is a reasonably large data base of *in vitro* and *in vivo* dermal absorption measurements of primarily pharmaceutical chemicals into human and animal skin. A primary goal is to utilize these data to develop preliminary predictive algorithms of the dermally absorbed dose for *in vivo* human exposures. Further experiments will be needed. The investigators will use information from algorithms developed in this project to identify the key experiments that are needed and specify the proper experimental protocols and analyses that will provide unambiguous results. This approach most efficiently meets the project goal by utilizing, as much as possible, the data that already exist and identifying where future experimental resources are most critically needed.

Overall, this project aims to address an important and unresolved issue of significant environmental concern, namely: To what extent does dermal absorption contribute to the overall human health risk associated with exposure to environmental pollutants? The most important deliverable from the completed project is guidelines: for acquisition of *in vivo* or *in vitro* dermal absorption data, for analyzing dermal absorption data to obtain descriptive parameters (such as permeability and partition coefficients), and for using dermal absorption data.

2. SUMMARY OF WORK COMPLETED

This 3-year, interdisciplinary and inter-institutional project utilizes the unique perspective and strengths of the Principal Investigator (PI), a chemical engineering professor at the Colorado School of Mines (CSM), and the Co-PI, a professor of pharmacy and pharmaceutical chemistry at the University of California, San Francisco (UCSF). In the two years since the project began on June 20, 1994, the co-PI's have worked on eight tasks, which are discussed below.

TASK 1

The investigators constructed and continue to maintain and add data to the comprehensive bibliographic skin database of the percutaneous/penetration literature. The database, which operates under the software, Endnote2 Plus™, currently contains almost 6,000 citations. In the past year, they developed file transfer protocols for making file transfers and updates between UCSF and CSM regular and routine. These protocols can also be used to transfer the database to interested users at EPA and elsewhere. Indeed, they have supplied this extensive database to EPA personnel, including investigators in the Office of Pesticide Programs and the

Environmental Monitoring Systems Laboratory (Las Vegas).

TASK 2

The researchers extracted from the skin database *in vitro* and *in vivo* penetration (permeability) data, and partition coefficient and diffusion coefficient values to be used in the development of structure-activity algorithms. The permeability database for human skin, which now contains about 200 chemicals, is almost twice as large as previously reported databases. More importantly, all data included in the database were taken from the primary reference, each data point has been evaluated for certain quality criteria (e.g., measurements obtained at steady-state, no more than 90% in an ionized form, etc.), and the justification of quality judgments have been fully documented. Similar databases have been assembled for several animals including rats, mice, snakes and guinea pigs. The partition coefficient databases for human and animal skins are being developed presently.

TASK 3

The investigators have acquired several chemical parameters that are required for the development of structure-activity algorithms including: octanol water partition coefficients, acid dissociation constants, molecular volumes, hydrogen bond donor activity, hydrogen bond accepting activity, polarizability, and molar refractivity. As for the permeability and partition coefficient databases, data quality is essential. In this case, data quality was ensured by utilizing qualified and validated databases (e.g., "star" values of the octanol-water partition coefficients from the Pomona College data base).

TASK 4

The investigators developed a new algorithm for predicting stratum corneum permeability from parameters representing the nature of the penetrating chemical (i.e., molecular volume, hydrogen bond donor activity, hydrogen bond accepting activity, polarizability, and molar refractivity). They also developed several additional correlations of human skin permeability coefficients as functions of octanol-water partitioning and molecular weight. The investigators are continuing to evaluate these correlations for reliability.

TASK 5

Several correlations for predicting skin permeability coefficients from water have been published (for example, Potts and Guy, 1993; McKone, 1993; Bogen et al., 1995 among others). Risk assessors, usually with minimal or no information, are inevitably left to choose which is the most appropriate for their particular scenario. During Year 2, the researchers quantitatively examined these correlations with respect to the data included in the Flynn database. Some of the published correlations were developed from a small or non-diverse data sets, and the investigators have shown that these represent poorly the entire set of data. In addition, they identified that some of the data variability is due to variations in the temperature at which the measurement was made. That is, permeability coefficient values measured at 25°C are

statistically lower than values measured at 30° or 37°C.

TASK 6

The researchers started developing a new algorithm for predicting stratum corneum-water partition coefficients from parameters representing the nature of the penetrating chemical (i.e., molecular volume, hydrogen bond donor activity, hydrogen bond accepting activity, polarizability, and molar refractivity). This algorithm will be redeveloped with additional data acquired during the end of Year 2 and the beginning of Year 3.

TASK 7

The investigators have examined several procedures for analyzing *in vivo* and *in vitro* experimental data to obtain unambiguous values of permeability coefficients and partition coefficients. Specifically, they have reanalyzed several *in vivo* and *in vitro* experiments to obtain values for permeability coefficients and partition coefficients that are not confounded by unsteady-state absorption or by lag times in urinary or fecal elimination rates. These calculations have been detailed in the masters thesis of Mr. Brent Vecchia (a student of Dr. Bunge), which is nearly complete. In addition, during the past year the investigators have identified a new experimental method for unambiguously determining permeability and partition coefficients from human *in vivo* experiments. The experimental evidence for this procedure, developed under a companion research project, is provided in the attached manuscript ("Measurement, analysis and prediction of molecular transport through human skin *in vivo*," F. Pirot, Y.N. Kalia, A.L. Stinchcomb, G. Keating, A.L. Bunge, and R.H. Guy, submitted for publication).

TASK 8

The investigators have continued to develop methods for estimating uncertainty in parameters derived from experimental data and the resulting uncertainty in the experimentally determined and predicted absorbed dose. Among the questions addressed in this task are: (1) How large must be the difference between predicted and experimental permeability values for the two to be statistically different? and (2) How large must be the difference between *in vitro* and *in vivo* data for the two to be statistically different?

3. WORK PLANNED FOR YEAR 3 (JUNE 20, 1996 - JUNE 19, 1997)

In Years 1 and 2, the researchers supported under this cooperative agreement acquired, evaluated, and qualified dermal absorption data for inclusion in several databases of permeability coefficients and partition coefficients from human and animal skins. In Year 3, they will use these databases to develop methods for predicting dermal absorption parameters (i.e., permeability and partition coefficients) from molecular property information or from measurements made on other animal skins. Several publications will also be completed during Year 3.

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From: RICK HERTZBERG
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Date: 9/20/96 5:26pm
Subject: Colorado progress report for SERDP

The attached WP5.1 file is the Progress Report for the SERDP-funded portion of the co-op with the Colorado School of Mines. There is no breakdown of what part of year 2 was actually funded by SERDP, just a summary of year 1 and 2 accomplishments.

If there is any more you would like me to do, just ask.
-Rick

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Attachment 5

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October 10, 1997

Ms. Nancy Bauer
U.S. Environmental Protection Agency
ECAO-CIN
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Cincinnati, OH 45268

RE: Cooperative No. CR-822757

Dear Ms. Bauer:

I am submitting one copy of Brent Vecchia's master's thesis to you as a final report for EPA Cooperative Agreement Number CR-822757 entitled "Estimating the absorbed dose from dermal exposure to environmental pollutants." This was a joint project between the Colorado School of Mines (prime contractor) and the University of California, San Francisco (subcontractor). The research described in Mr. Vecchia's thesis was also partially supported by funding from the National Institute of Environmental Health Sciences (R01-ES06825) and the US Air Force (Office of Scientific Research F49620-95-1-021). Nevertheless, the majority of work summarized in this thesis were obtained as a direct result of EPA project 822757.

I recently presented many of the results described in Mr. Vecchia's thesis at an EPA workshop organized by Kim Hoang at ORD. There was considerable interest in Mr. Vecchia's results and his thesis.

Please let me know if you need further information. You can reach me by phone at (303) 273-3722, by FAX at (303) 273-3730, or by internet at abunge@mines.edu.

Sincerely,

Annette L. Bunge
Professor

copy to: Richard Hertzberg

Chemical Engineering and
Petroleum Refining Department

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October 10, 1997

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Dear Ms. Bauer:

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absorbed dose from dermal exposure to environmental p
project between the Colorado School of Mines (prime co
California, San Francisco (subcontractor). The research
was also partially supported by funding from the Nationa
Health Sciences (R01-ES06825) and the US Air Force (C
F49620-95-1-021). Nevertheless, the majority of work s
obtained as a direct result of EPA project 822757.

I recently presented many of the results described
EPA workshop organized by Kim Hoang at ORD. There
Vecchia's results and his thesis.

Please let me know if you need further informatio
at (303) 273-3722, by FAX at (303) 273-3730, or by interne

Sincerely,

Annette L. Bunge
Professor

copy to: Richard Hertzberg

Attachment 5

Estimating the Dermally Absorbed Dose from Chemical Exposure: Data Analysis, Parameter Estimation, and Sensitivity to Parameter Uncertainties.

by

Brent E. Vecchia

A thesis submitted to the Faculty and Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Master of Science (Chemical Engineering and Petroleum Refining).

Golden, Colorado

Date 9/29/97

Signed: Brent E. Vecchia
Brent E. Vecchia

Approved: Annette L. Bunge
Dr. Annette L. Bunge

Golden, Colorado

Date 9/30/97

Dr. Robert M. Baldwin
Professor and Head,
Department of Chemical
Engineering and
Petroleum Refining

ABSTRACT

Quantifying the absorption of pharmaceutical or toxic chemicals into skin has many direct applications in transdermal (through the skin) delivery of pharmaceutical medications and in estimating the human health risk from dermal exposure to environmental contamination. Despite its importance, dermal absorption remains a poorly understood form of chemical exposure. One contributing reason is that dermal absorption measurements are dramatically underutilized. This thesis documents and analyzes more of the available data (primarily for waterborne organic compounds). The focus is on the collection and analysis of important datasets to determine useful parameters (e.g., permeability coefficients and stratum corneum-vehicle partition coefficients) and correlation of these parameters. The databases documented here (1) are significantly larger than previous databases, (2) distinguish meaningful measurements from those which cannot be interpreted, and (3) contain relevant information that was not originally reported (either obtained through communication with the authors or calculated). The analysis we employ requires that certain quality criteria be satisfied by the data (e.g., steady state, constant vehicle concentration), and better accounts for chemistry of skin penetrants (chemical ionization, validated octanol-water partition coefficients). Simple predictive correlations are presented, which reasonably describe a wide body of the validated data.

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1. PERCUTANEOUS ABSORPTION AND RISK ASSESSMENT

1.1. Why Study Dermal Absorption?

Research in dermal absorption is aimed at better understanding and quantifying the absorption of chemicals into the human body by passage through the skin barrier. In clinical dermatology and pharmaceutical science, quantifying dermal absorption is useful for designing treatments of skin disorders and transdermal (through the skin) delivery systems for some drugs. In another sense, the susceptibility of the skin to absorption of contaminants in water (terrestrial or treated drinking water), soil, and industrial vapors has been acknowledged as important. Dermal absorption may be the dominant mechanism of exposure for some contaminants (McKone, 1987; Morgan *et al.*, 1991; Shatkin and Brown, 1991). Recently, extensive investigations are underway to better quantify environmental exposure risks.

To elaborate on this last issue, health risk assessments require a sensible estimate (experimental data, or empirical predictions) of the amount of material that has entered the skin (the conservative assumption is that all dermally absorbed chemical could eventually find its way into the living body). The importance of accurately estimating the extent of exposure is evident; improper estimates would cause excessive and costly environmental remediation, or worse, might place human health at risk. Despite the importance, dermal absorption remains one of the least well understood forms of chemical exposure (US EPA, 1992).

1.2. Morphology of the Skin

An important starting point for the analysis and utilization of dermal penetration data is an understanding of the structure and function of the skin. Figure 1.1 is a schematic showing some morphological features of the skin (US EPA, 1992). The skin of humans and many animals is anatomically a membrane laminate. The outermost two layers of skin form a thin sheet, called the epidermis, layered on a much thicker layer of tissue called the dermis (Cleek and Bunge, 1993; Scheuplein and Blank, 1971). The epidermis provides the primary barrier to permeation of water and most other chemicals, and some abrasion resistance, while the interior dermis provides most of the bulk and toughness of the skin (Downing, 1992).

The outer two layers, the stratum corneum (SC) and the viable epidermis (VE), are an unvascularized region approximately 100 μm in thickness, while the interior dermis is several hundred microns in thickness and well perfused with capillaries (Scheuplein and Blank, 1971). Because the epidermis is devoid of capillaries it must receive its nutrients and dispose of its wastes by diffusional transport with the dermis (Downing, 1992). Exchange of chemicals with the blood, in the dermis, is generally considered pseudo-instantaneous relative to rates of diffusion through the epidermis (Scheuplein and Blank, 1971). This is less true when vascular blood flow has been limited with vasodilators, or stopped altogether by removing the skin from the body (i.e., performing experiments *in vitro* on excised skin).

Hair follicles and sweat ducts originate deep within the dermis and interrupt the three skin layers, cutting through the diffusional barrier of the skin. However, these structures occupy a small fraction of the skin, generally less than 1% (dependent upon anatomical location), and, for most chemicals, appendageal pathways do not contribute significantly to the total permeability (Scheuplein and Blank, 1971; Siddiqui *et al.*, 1989). For slowly penetrating macromolecules and larger hydrophilic (water loving) or ionized

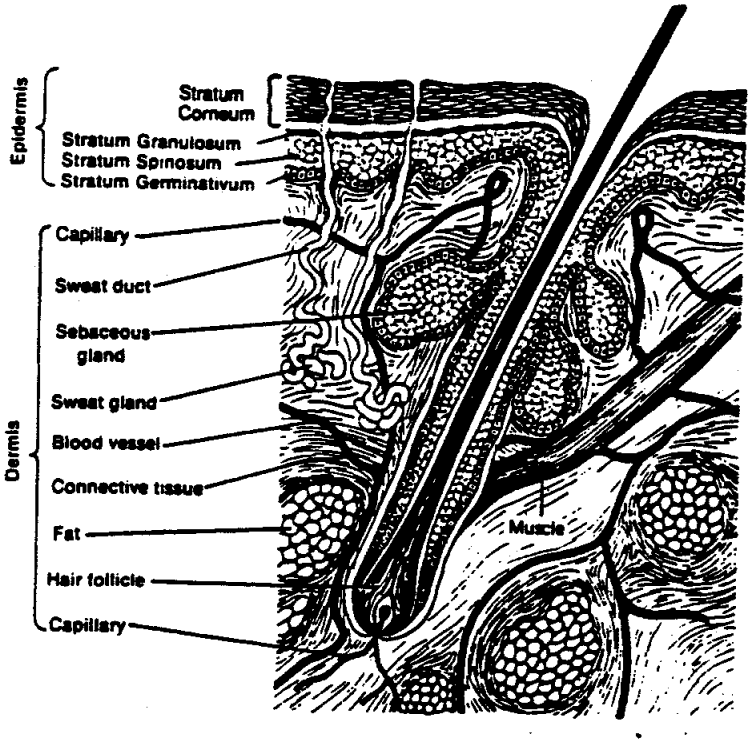

Figure 1.1 Morphological features of the skin.

compounds these pathways become significant.

The outermost layer of skin, the SC, is 10-50 μm thick and is composed of dead, partially desiccated, keratinized epidermal cells, and lipids (US EPA, 1992). The SC contains about 40% protein (mainly keratin), about 40% water, and on the order of 15-20% lipids (triglycerides, fatty acids, cholesterol, and phospholipids) (Downing, 1992; Michaels *et al.*, 1975). Structurally, it is a close packed array of dense and flattened cells surrounded by intercellular layers of liquid-crystalline lipids and protein (Forslind, 1994). The SC is structurally heterogeneous. Accordingly, the SC is often depicted as dense proteinaceous bricks mortared together by intercellular lipids (Michaels *et al.*, 1975). A broader discussion, particularly focusing on the chemical aspects of penetration, can be found in several excellent references (Downing, 1992; Forslind, 1994).

The SC generally presents the dominant resistive barrier to absorption (Scheuplein and Blank, 1971). This is particularly true for hydrophilic chemicals (water and chemicals which have an affinity for water), which permeate rapidly through the VE but slowly permeate through the SC (Cleek and Bunge, 1993). Survival in a terrestrial environment requires that the SC prevent water penetration leading to desiccation of the interior skin and body (Forslind, 1994). This function provides a simple, but very useful, explanation of the rate of dermal absorption of many other compounds. In the SC roughly twice as much fibrous matter occupies roughly one tenth the space it does in the VE, so it is not unexpected that the SC is a more formidable barrier to penetration.

Below the SC lies a thicker layer, 50 to 100 μm, of living viable epidermis (VE), containing at its base the germinative or basal cell layer whose cells move outward to replace he outer epidermis as it wears away (US EPA, 1992). New cells cause existing cells to move outward, where they undergo structural and chemical changes. Ultimately the cells cornify, producing the dead, dehydrated, polymerized network of flattened cells that is the SC (Scheuplein and Blank, 1971). Outer layers of the SC continually exfoliate,

in a process known as desquamation, maintaining the epidermis at a constant thickness. The entire SC is shed, or desquamated, once every 7 to 21 days (US EPA, 1992).

The deepest layer of skin, the dermis, is virtually noncellular and collagenous, and is the most aqueous of the three layers (US EPA, 1992). The dermis provides the least resistance to transport, because it is less dense and is highly vascularized by capillary networks throughout (Scheuplein and Blank, 1971).

1.3. Models of the Skin

Due to the structural heterogeneity of the SC, two diffusion pathways have been proposed, intercellular and intracellular. In diffusion by the intercellular pathway, a compound penetrates the SC through both keratinized cells and the intercellular lipids. In diffusion by the intracellular pathway, a compound penetrates the SC through a tortuous pathway around the cells (i.e., diffusion is restricted to the lipid phase which surrounds the keratinized cells). Experimental evidence suggests that lipophilic chemicals follow the intracellular route (Albery and Hadgraft, 1979; Scheuplein and Blank, 1971). The penetration of hydrophilic compounds is more difficult to measure and is less well understood. The effects of SC heterogeneity on transport properties through skin are not satisfactorily resolved.

Mathematical models have been developed which provide a semi-theoretical basis for interpreting experimental data and extrapolating measurements to alternative exposure scenarios. Most frequently, transport through skin is assumed to occur by passive Fickian (gradient driven) diffusion with constant diffusion coefficients, skin thickness, and SC-vehicle partition coefficients. The two-layer membrane model of skin used throughout this work accounts for larger absorption rates during the initial exposure period as well as the hydrophilic barrier which the VE presents to lipophilic (fat soluble) compounds (Cleek and Bunge, 1993). This model is developed around several common assumptions: (a) constant vehicle concentration, (b) sink conditions maintained on the interior surface

of the skin, (c) instantaneous equilibration of the penetrant at the skin-vehicle interface and at the interface between the SC and VE, and (d) the vehicle and the penetrant are assumed not to alter the skin barrier.

1.4. Topics of Thesis

The 10 chapters of this thesis pertain to estimation of the dermally absorbed dose from chemical exposure. Emphasis is placed on data analysis, parameter estimation, and sensitivity to parameter uncertainties. This thesis documents, critically evaluates, and utilizes measurements of percutaneous absorption, some measurements being reviewed in this way for the first time. The primary focus is on calculating, interpreting, and predicting transport parameters that are necessary for estimating the dermally absorbed dose. Generally, two transport parameters are required: (1) the permeability coefficient - which measures how easily a chemical penetrates skin, and (2) the skin-water partition coefficient - which measures the affinity of skin for a chemical relative to water's affinity for that chemical.

The next two chapters consider the analysis of different types of *in vivo* (i.e., in the living body) dermal absorption experiments which require more sophisticated analysis than *in vitro* (i.e., not occurring in the living body) experiments. *In vivo* dermal absorption usually is not measured directly and the dermally absorbed dose must be inferred from other measurements. Chapters 2 and 3 contain analysis of *in vivo* experimental data when two different methods of indirectly measuring the dermally absorbed dose are used. In Chapter 2 total dermal absorption and permeability coefficients are determined by inference from the experimentally measured urinary elimination of a portion of the dermally absorbed dose. In Chapter 3 total dermal absorption and permeability coefficients are determined by inference from the experimentally measured depletion of chemical from a beaker containing a hairless guinea pig submersed in an aqueous exposure solution. Dermal absorption initially

occurs rapidly during the unsteady-state period of absorption, but, only the later and slower rates of absorption during steady-state should be used to calculate permeability coefficients. The analysis in Chapter 3 illustrates a method that aids in the difficult determination of the steady-state measurements of absorption to be used to calculate permeability coefficients.

The prediction of permeability coefficients for aqueous chemicals is considered in Chapters 4 and 5. In Chapter 4 we compare 16 published correlations for predicting the SC permeability coefficient for aqueous chemicals with a well known and sizable database of experimentally measured permeability coefficients (Flynn, 1990). The idea of data quality is formally introduced into the analysis of permeability coefficients in Chapter 5 by the presentation of a set of validation criteria based on the experimental conditions of permeability coefficient experiments and the chemistry of the penetrating compound. Finally, in Chapter 5 simple predictive correlations are presented which reasonably describe a wide body of the permeability coefficient data and can be used to make predictive estimates.

The prediction of SC-water partition coefficients is considered in Chapter 6. A set of validation criteria is developed based on the experimental conditions of partition coefficient experiments and the chemistry of partitioning compounds. Finally in Chapter 6 simple equations are presented which reasonable describe a wide body of SC-water partition coefficient data and can be used to make predictive estimates.

Chapter 7 considers the relationship between permeability coefficients measured with human skin and the skin of common laboratory animals. Although dermal absorption data has been collected for several common laboratory animals the information for extrapolating these results to human exposure has been inconsistent. In Chapter 7 those extrapolations are clarified through empirical comparisons of

permeability coefficients measured in human skin and animal skin from several different species.

Chapter 8 investigates the relevancy of predictions generated using dermal absorption models and empirical predictive correlations for dermal transport parameters. Experimentally measured uptake of aqueous organic compounds is compared with predicted uptake.

The ratio of permeability coefficients in the stratum corneum (a lipophilic membrane) and the viable epidermis (a hydrophilic membrane), B, is a dermal transport parameter that is required to estimate dermal absorption using models of dermal absorption developed for highly non-polar chemicals. In Chapter 9, a simple correlation is proposed for estimation of B that is consistent with a limited database of experimentally measured B-values.

Finally, in Chapter 10 a mathematical model of damaged skin is developed. The model is used to predict the effect that skin damage has on the lag time (a time representative of the transition between unsteady-state and steady-state absorption).

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2. APPLICATION OF PHARMACOKINETIC MODELS TO *IN VIVO* EXCRETION DATA

2.1. Abstract

One of the common methods for collecting *in vivo* dermal absorption data is through monitoring elimination of the dermally absorbed dose in the urine (and/or feces). To calculate the dermally absorbed dose from excretion data mathematical models of skin penetration are combined with a pharmacokinetic representation of the body's physiology. This chapter examines methods for analysis of *in vivo* urinary and fecal elimination data reported previously for TCDD and pentachlorophenol (PCP). Calculated from the pharmacokinetic analysis discussed here, the amount of TCDD absorbed is about 40% larger than previously estimated. A previous estimate of the amount of PCP absorbed is consistent with pharmacokinetic analysis, but, the advantage of pharmacokinetic analysis is illustrated by the much shorter monitoring period required to make this estimate.

2.2. Introduction

Experimenters are often concerned that *in vitro* experiments alter the biological aspects of dermal absorption and *in vivo* experiments are more relevant than *in vitro* experiments. However, *in vivo* experiments require more complicated forms of experimentation and analysis, and the results are generally more uncertain.

In vivo dermal absorption experiments involve animal or human exposure to a chemically contaminated vehicle (e.g., water, soil, or a topical ointment) and the

measurement of a resulting consequence of dermal absorption, from which dermal uptake is inferred. In certain cases, absorption can be quantified by measuring a physiological response to dermally absorbed chemical, but such cases are naturally infrequent. Alternatively, absorption can be determined by summing the contamination found in assays of the tissues, the site of application, and major excretory products. The disadvantage of this approach is that human subjects often can not be tested and the methodology is usually time consuming. Additionally, since elimination pathways (e.g., breath, urine, feces, sweat, hair, and nails) and storage pathways (e.g., fat, fatty tissues, and bone (for heavy metals)) are diverse, it is difficult to account for all of the contamination. A better approach is to monitor the contaminant in the blood plasma, or detect it as unaltered chemical and metabolites in excreta, and then compare this with a well-calibrated exposure. Chemical concentration in plasma responds directly to absorption, but changes rapidly subject to metabolism and tissue distribution each with different half-lives. Urinary concentrations however, tend to integrate the chemical absorption over time. Thus, analysis of urine provides one of the principle methods for determining *in vivo* dermal absorption.

To assess dermal risk it is the percent dose absorbed into the stratum corneum that is of practical interest. Researchers must infer the amount of dermal absorption from the collected elimination data. The method pioneered by Feldmann and Maibach (Feldmann and Maibach, 1974) assumes that the fate of an intravenously injected dose is essentially identical to the fate of a dermally absorbed dose. The researchers monitor the amount of chemical excreted after both intravenous bolus and dermal exposure until the amount being excreted becomes undetectable by the instrumentation. The total amount of chemical appearing in the urine following dermal exposure for a finite period is adjusted for other modes of storage and excretion with the percentage of the intravenous dose which was eliminated. Adequate time intervals for collecting excreta following the

dermal exposure are necessary. Three week time periods were required for DDT, hexachlorophene, and PCB's (Shaw and Guthrie, 1983).

Cooper pioneered a more sophisticated method (Cooper, 1976). The method predicted the transdermal urinary elimination profile assuming diffusion across the skin and a one-compartment pharmacokinetic model for describing drug elimination. The systemic model chosen was a body compartment with interacting substrate, subject to metabolic, biliary, and urinary elimination. In later publications, Siegel, (Siegel, 1986a; Siegel, 1986b) developed more general lag times incorporating penetration across the skin and elimination in the urine. These have only occasionally been used by experimenters who have more often used the simpler method proposed by Feldmann and Maibach (Feldmann and Maibach, 1974) or slight variations on this method (Andersen *et al.*, 1980; Roy *et al.*, 1990; Shaw and Guthrie, 1983; Wester *et al.*, 1993); to name only a few).

2.3. Theory

2.3.1. The Model

A pharmacokinetically-based mathematical model was developed to describe transport of a chemical across skin and its elimination in the urine. The model combines a two-compartment model for systemic distribution and elimination with a percutaneous absorption model (either a membrane model or a compartment model of the stratum corneum).

2.3.2. The Animal Compartments

Figure 2.1 shows a systemic two-compartment model for the *in vivo* animal. This model consists of two compartments with irreversible storage in compartment 2 and excretionary elimination from the compartment 1. This model differs from the one used

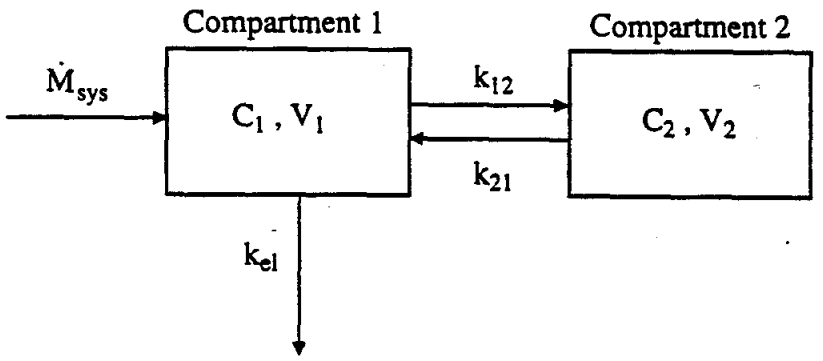

Figure 2.1 Pharmacokinetic representation of the *in vivo* animal showing the rate of mass entering from the skin ($\dot{M}_{sys}$), the two systemic compartments, and the pathways of storage (k_{12}) and elimination (k_{el}).

by Silcox and coworkers (Silcox *et al.*, 1990), in that, the partitioning into fatty tissues is essentially irreversible over the period of data collection. Although there is no quantitative physiological relationship in the model, physiological function can be assigned to the two compartments. Compartment 1 represents the rapidly perfused tissues, which do not have a significant affinity for the chemical. Compartment 2 represents the slowly perfused tissues, into which the chemical partitions more strongly. It is sometimes useful to consider that compartment 1 represents the blood while compartment 2 represents the fatty, organ tissues.

2.3.3. Elimination of the Bolus

Differential mass balance equations have been solved to describe the rate of elimination of a bolus injection from the systemic compartment model in Fig. 2.1 (see Appendix A). The initial concentration in the rapidly perfused tissue compartment is the contaminant mass in the bolus dose (δ_{IV}) divided by the apparent volume of this compartment (V_1). The cumulative mass fraction of dose eliminated is,

$$\frac{M_{el}}{\delta_{IV}} = \beta\left(1 - e^{-bt}\right) \tag{2.1}$$

where β and b have the following definitions,

$$\beta = \frac{k_{el}}{(k_{el} + k_{12})} \tag{2.2}$$

$$b = (k_{el} + k_{12}) \tag{2.3}$$

in terms of the kinetic constants, k_{12} and k_{el}, shown in Fig. 2.1.

2.3.4. Models of the Skin

Membrane (Cleek and Bunge, 1993) and compartment (stirred tank) models (McCarley and Bunge, 1997) can be used to model chemical penetration across the skin.

The cumulative mass permeating a single membrane to enter the blood (modified from equations presented in Cleek and Bunge (Cleek and Bunge, 1993)) is:

$$\left(\frac{M_{sys}^{m}}{\delta_d}\right)_B = \frac{\gamma}{\beta}\left(\lambda\tau - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n e^{-n^2\pi^2\lambda\tau}}{n^2}\right) \tag{2.4}$$

and the cumulative mass entering the stratum corneum (modified from equations presented in Cleek and Bunge (Cleek and Bunge, 1993)) is:

$$\left(\frac{M_{in}^{m}}{\delta_d}\right)_B = \frac{\gamma}{\beta}\left(\lambda\tau + \frac{1}{3} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{e^{-n^2\pi^2\lambda\tau}}{n^2}\right) \tag{2.5}$$

In these expressions τ is the dimensionless time ($\tau = b\,t$), and λ is the ratio of the rate of diffusion to the rate of elimination, defined as:

$$\lambda = \frac{D_c}{L_c^2(k_{el} + k_{12})} \tag{2.6}$$

where D_c and L_c are the diffusivity of chemical in and thickness of the stratum corneum, respectively. In the experiments to be analyzed, chemically loaded soil was applied to skin and, consequently, the parameter γ is defined as:

$$\gamma = \frac{\beta f_{ac} A L_c \underline{K}_{cs}}{M_{soil}} \tag{2.7}$$

where M_{soil} is the mass of soil on the skin (i.e., the soil loading), $\underline{K}_{cs}$ is the skin to soil partitioning coefficient (with units of [(mass contaminant)/(volume stratum corneum)]/[(mass contaminant)/(mass soil)], A is the exposure area, and f_{ac} is the fractional coverage of soil on the skin. When adhering soil particles completely cover the surface of the skin, this is referred to as monolayer coverage, and for this and larger amounts of soil applied to the skin, $f_{ac} = 1$ (Bunge and Parks, 1997). When the soil particles do not completely cover the surface of the skin, areas of skin do not directly

contact the soil and this incomplete soil coverage is represented by $f_{ac} < 1$ (Bunge and Parks, 1997).

Alternatively, a compartment model can be used to describe transport across the stratum corneum. In this case the cumulative mass entering the rapidly perfused tissues (the mass leaving the skin) is:

$$\left(\frac{M_{sys}^{c}}{\delta_d}\right)_B = \frac{2\alpha_c\gamma}{\beta(1+\alpha_c)}\left(\lambda\tau + \frac{e^{-2(1+\alpha_c)\lambda\tau}}{2(1+\alpha_c)} - \frac{1}{2(1+\alpha_c)}\right) \tag{2.8}$$

and the cumulative mass entering the skin is:

$$\left(\frac{M_{in}^{c}}{\delta_d}\right)_B = \frac{2\alpha_c\gamma}{\beta(1+\alpha_c)}\left(\lambda\tau - \frac{e^{-2(1+\alpha_c)\lambda\tau}}{2\alpha_c(1+\alpha_c)} + \frac{1}{2\alpha_c(1+\alpha_c)}\right) \tag{2.9}$$

The parameter α_c measures the relative rates of chemical removal by blood to chemical diffusion through the skin. That is,

$$\alpha_c = \frac{k_{rc} L_c}{2 A D_c} \tag{2.10}$$

where

$$k_{rc} = \frac{1}{\frac{K_{cb}}{A q_b} + \frac{L_c}{2 D_c A}} \tag{2.11}$$

In equations (2.10) and (2.11), q_b is the cutaneous blood flow per unit area of skin (q_b), and K_{cb} is the partition coefficient between the stratum corneum and blood. Defined this way, $\alpha_c = 1$ when the blood flow is very large providing infinite sink conditions on the inside of the skin, and $\alpha_c = 0$ when there is no blood flow. The compartment model of skin is mathematically simpler than the membrane model representation and can be more easily combined with pharmacokinetic models of systemic distribution and elimination.

Models of the skin are combined with the systemic pharmacokinetic model of the laboratory animal, shown in Fig. 2.1, to describe the dermal absorption and elimination

processes of *in vivo* experiments. The kinetic response of the animal to the dermally-absorbed chemical is determined from the rate of excretion of a known bolus (administered directly to the blood), which deliberately bypasses the dermal barrier. The pharmacokinetic rate constants that describe this data are then used in a combined dermal absorption-pharmacokinetic model to determine the dermal dose required to produce the measured elimination profile. In the next few sections, the combined equations are developed for two cases: (1) excretion is monitored while the chemical exposure continues, and (2) excretion is monitored during and after the chemical exposure.

2.3.5. Case 1: Cumulative Mass Eliminated Before Exposure Ends

The differential mass balances for the system of compartments shown in Figure 2.1 can be solved by Laplace transforms to describe the rate of elimination from the rapidly perfused compartment into the urine and feces (see Appendix A). The mass eliminated, based on the single compartment model of skin, is given by

$$\left(\frac{M_{el}^{c}}{\delta_d}\right)_B = \frac{2\alpha_c\gamma}{1+\alpha_c}\left(\lambda\tau+\lambda\left(e^{-\tau}-1\right)+\frac{e^{-\tau}-1}{2(1+\alpha_c)} + \frac{e^{-2\lambda\tau(1+\alpha_c)}-e^{-\tau}}{2(1+\alpha_c)(1-2\lambda-2\alpha_c\lambda)}\right) \tag{2.12}$$

where the subscript B designates the mass eliminated before chemical has been removed from the skin. A similar expression developed for the single membrane model of skin is

$$\left(\frac{M_{el}^{m}}{\delta_d}\right)_B = \gamma\left(\lambda\tau+\frac{1+6\lambda}{6}\left(e^{-\tau}-1\right)+\frac{2}{\pi}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\left(\frac{e^{-\tau}-e^{-n^2\pi^2\lambda\tau}}{1-n^2\pi^2\lambda}\right)\right) \tag{2.13}$$

In the long-time limit (i.e., steady-state designated with superscript ∞), the normalized mass eliminated in the urine is related to the normalized mass absorbed into the SC and

the systemic compartment concentration according to the relationship: $(M_{el}^{c} / \delta_d)_B^{\infty} = \beta [(M_{sys}^{c} / \delta_d)_B^{\infty} + C_l^{\infty} V_l]$ where $C_l^{\infty} = 2\alpha_c \gamma \delta_d \lambda / [\beta V_l (1+\alpha_c)]$.

2.3.6. Case 2: Cumulative Mass Eliminated After Exposure Ends

Different equations describe elimination after the chemical exposure has ended. Until the time of chemical removal, the cumulative mass leaving the skin is correctly described by Eqs. (2.8) or (2.4). After removal of the vehicle the mass entering the body from the skin, for the single compartment model of skin, is given by:

$$\left(\frac{M_{sys}^{c}}{\delta_d}\right)_A = \frac{\gamma}{\beta}\left(\frac{\left(e^{-2\lambda\tau_{exp}(1+\alpha_c)}-1\right)\left(e^{2\alpha_c\lambda(\tau_{exp}-\tau)}-1\right)}{1+\alpha_c}\right) + \frac{2\alpha_c\gamma}{\beta(1+\alpha_c)}\left(\frac{e^{-2\lambda\tau_{exp}(1+\alpha_c)}-1}{2(1+\alpha_c)}+\lambda\tau_{exp}\right) \tag{2.14}$$

where the subscript A is used to denote times after removal of the chemical. In Eq. (2.14) τ_{exp} is the dimensionless time at which the contaminant was removed. The normalized mass out of the SC evaluated with Eq. (2.14) (after removal) and Eq. (2.8) (before removal) are identical at the time of removal of the vehicle (i.e., $(M_{sys}^{c} / \delta_d)_A = (M_{sys}^{c} / \delta_d)_B$ at $\tau = \tau_{exp}$). Also, at long times, the mass out of the SC calculated with Eq. (2.14) (after removal) should be identical to the mass absorbed into the SC with Eq. (2.9) (before removal) evaluated at the time of vehicle removal (i.e., $\lim_{\tau\to\infty}(M_{sys}^{c} / \delta_d)_A = (M_{in}^{c} / \delta_d)_B$ at $\tau = \tau_{exp}$). Equivalent expressions, based on a single membrane model of skin, are considerably more complex and were not developed.

For the case where blood effectively removes the penetrating compound as it leaves the skin (i.e., $\alpha_c = 1.0$), the cumulative mass eliminated in the time after the chemical is removed is:

$$\left(\frac{M_{el}^{c}}{\delta_d}\right)_A = \gamma\left(\lambda\tau_{exp} - \frac{4\lambda^2}{1-4\lambda}e^{-\tau} + \frac{2\lambda^2}{1-2\lambda}e^{\tau_{exp}-\tau} + \frac{2\lambda^2}{(1-2\lambda)(1-4\lambda)}e^{-4\lambda\tau_{exp}+\tau_{exp}-\tau} + \frac{1}{2(1-2\lambda)}e^{-2\lambda(\tau_{exp}+\tau)} - \frac{1}{2(1-2\lambda)}e^{2\lambda(\tau_{exp}-\tau)} - \frac{e^{-4\lambda\tau_{exp}}}{4} + \frac{1}{4}\right) \quad (2.15)$$

The long-time limit of the normalized mass eliminated in the urine evaluated with Eq. (2.15) should be a fraction β of the normalized mass absorbed into the SC up to the time the vehicle was removed (Eq. (2.9)) (i.e., $\lim_{\tau\to\infty}(M_{el}^{c}/\delta_d)_A = \beta(M_{in}^{c}/\delta_d)_B$ at $\tau = \tau_{exp}$).

2.4. Results and Discussion

Researchers use at least three different methods for interpreting *in vivo* excretion data. Two widely cited datasets from the literature are reanalyzed to demonstrate these methods, and, to obtain more and better information from these datasets. The two examples are soil sorbed 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) absorption in female Sprague-Dawley rats (Roy *et al.*, 1990; US EPA, 1992), and pentachlorophenol (PCP) absorption in rhesus monkeys exposed to contaminated soils (Wester *et al.*, 1993). In both the Sprague-Dawley rat and rhesus monkey experiments, excreta were collected and reported following exposure to an intravenous bolus and following topical application of the contaminant. Permeability coefficients were not reported for the TCDD or PCP data but can be determined using pharmacokinetic models.

2.4.1. Case 1: Analysis of Excretion Data Before Chemical was Removed from Skin

In a poster study cited by the interim report on dermal absorption (US EPA, 1992), Roy and colleagues investigated the dermal absorption of TCDD from low organic (0.45% organic carbon content) soil vehicles in the female Sprague-Dawley rat. The composition of the whole soil was 15.1% sand, 68.2% silt, and 16.7% clay. However, only those particles less than 150 microns were used in the dermal exposure experiments. The chemical concentration in soil was 1 ppm and the soil loading was 10 mg of soil/cm^2 of skin. For soil with this range of particle sizes, we expect that 10 (mg soil)/(cm^2 skin) is an adequate amount of soil to cover the surface of the skin with at least one layer of soil particles (i.e., monolayer coverage) and $f_{ac} = 1$ (Bunge and Parks, 1997).

Table 2.1 reports the elimination of TCDD (measured by liquid scintillation) in urine and feces following intravenous (IV) dosing and a topical exposure at times of 24, 48, 72, and 96 hours after IV administration or the dermal exposure began (US EPA, 1992). The TCDD contaminated soil remained in contact with the skin throughout the entire 96 hour period in which data was collected. There was no negligible change in the TCDD concentration on the soil during the 96-hour exposure.

Systemic pharmacokinetic parameters (k_{12} and k_{el}) were determined by nonlinear regression (using the Gauss-Newton method with stephalving to search for the least-squares estimate in JMP (SAS Institute, 1995)) of Eq. (2.1) to the IV elimination data in Table 2.1. The IV bolus elimination data and the regression fit (solid curve) are shown in Fig. 2.2. The optimal values and approximate standard errors were $\beta = 0.241(\pm 0.008)$ (written for the fraction eliminated) and $b = 0.023(\pm 0.002)$ hrs^{-1}. For this fit r^2 was 0.99, the sum of the squared errors (SSE) between the prediction and the observations was 4.71×10^{-5}, the mean square error (MSE) was 1.57×10^{-5}, and the root mean square error (RMSE) was 3.96×10^{-3}. Applying the pharmacokinetic definitions (i.e., Eqs. (2.2) and (2.3)), we calculate: $k_{12} = 0.0173$ hrs^{-1} and $k_{el} = 0.00549$ hrs^{-1}.

Table 2.1 TCDD Urinary and Fecal Elimination Following Intravenous Dosing and Topical Application (Roy *et al*., 1990)

Time (Hours)	$\tau = bt$	Fraction Eliminated	
		IV Bolus[a]	Dermal[b]
0	0	0	0
24	0.55	0.097	0.008
48	1.09	0.165	0.018
72	1.64	0.195	0.029
96	2.19	0.212	0.037

[a] Expressed as fraction of the bolus dose eliminated.

[b] Expressed as fraction of the vehicle dose eliminated.

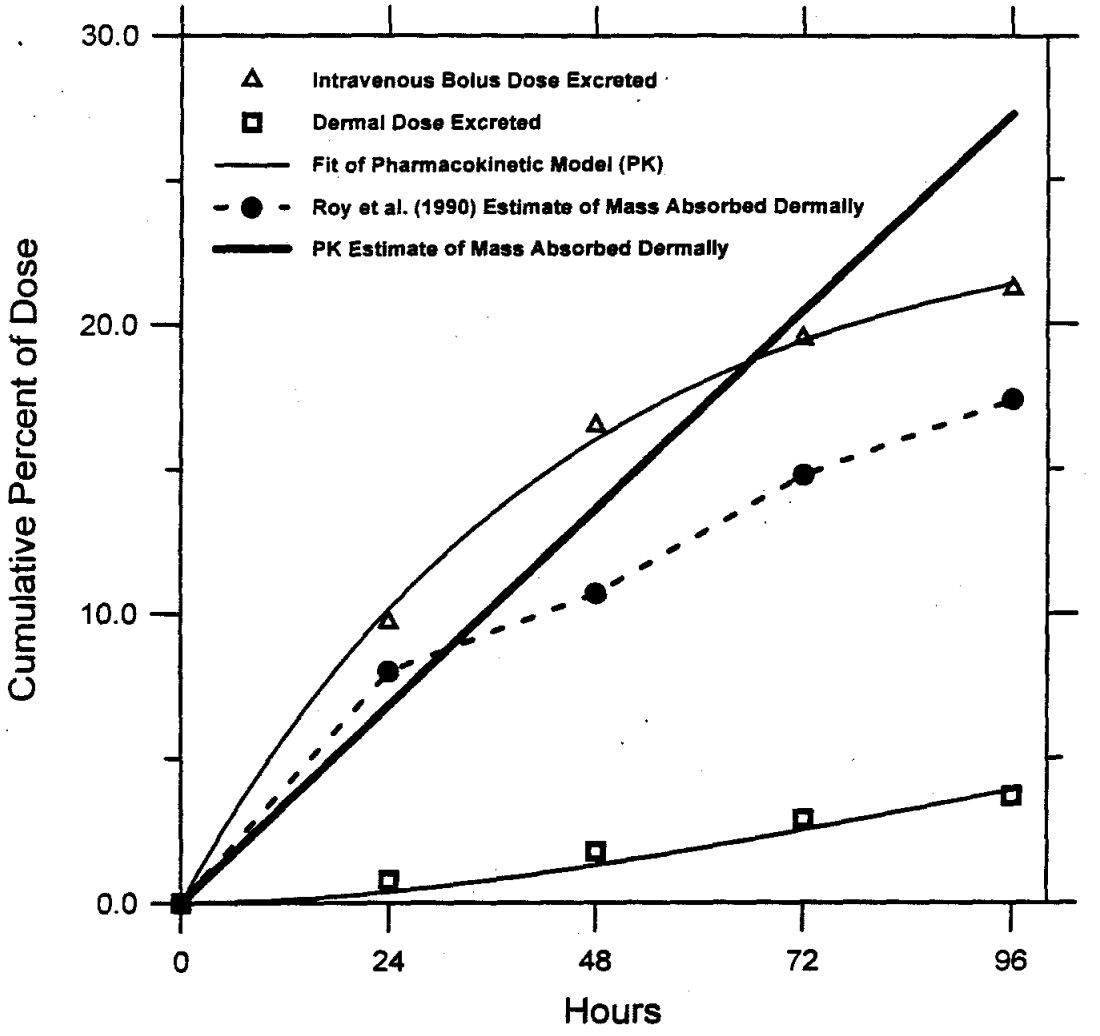

Figure 2.2 Analysis of TCDD urinary and fecal data following intravenous and topical application.

For the case where blood effectively removes the penetrating compound as it leaves the skin (i.e., $\alpha_c = 1.0$), Eq. (2.12) reduces to a nonlinear function of λ and γ:

$$\left(\frac{M_{el}^{c}}{\delta_d}\right)_B = \gamma\lambda\tau + \gamma e^{-\tau}\left[\lambda + \frac{1}{4} - \frac{1}{4(1-4\lambda)}\right] - \gamma\left[\lambda + \frac{1}{4} - \frac{e^{-4\lambda\tau}}{4(1-4\lambda)}\right] \tag{2.16}$$

Although, the nonlinear regression of the dermal exposure data listed in Table 2.1 to Eq. (2.16) did not converge, the regressions did indicate that λ was much larger than γ.

Simplifying Eq. (2.16) for large λ relative to γ:

$$\left(\frac{M_{el}^{c}}{\delta_d}\right)_B = \gamma\lambda\left(\tau + e^{-\tau} - 1\right) \tag{2.17}$$

Equation (2.17) shows that for rapid dermal absorption coupled with relatively slow elimination it is not possible to separately know the skin diffusion coefficient the partition coefficient to the skin. Only the product $\lambda\gamma$,

$$\lambda\gamma = \frac{\beta}{b}\left(\frac{f_{ac}A}{M_{soil}}\right)\underline{P}_{cs} \tag{2.18}$$

which contains the permeability coefficient, $\underline{P}_{cs} = \underline{K}_{cs}D_c/L_c$, can be determined. The product $\gamma\lambda$ was uniquely determined by least squares regression for the dermal exposure data listed in Table 2.1.

When linear regression was performed and a zero intercept was forced $\gamma\lambda$ = 0.0313(±0.0024) with a high degree of correlation ($r^2 = 0.934$, $r^2_{adj} = 0.684$, RMSE = 0.00385 and model F-ratio = 171.2). Uncertainties in parentheses are standard errors of the regression coefficients. The r^2(adj.) statistic is analogous to r^2 but allows for more relevant comparisons between models with different numbers of fitted parameters (JMP User's Guide (SAS Institute, 1995)). Specifically, $(1 - r^2)$ = error sum of squares/total sum of squares and $(1 - r^2(\text{adj.}) = (1 - r^2)(n - 1)/(n - p)$ where n = # of data points and p = # of parameters. RMSE is the root mean square error of the model, which is zero when

the model perfectly correlates the data. Model F-Ratio is the (sum of squares for the model divided by the degrees of freedom for the model) / (sum of squares for the error divided by the degrees of freedom for the error). The model F-ratio = 1 when there is zero correlation with the parameters and is large for correlations with good predictive power. Because the number of fitted parameters is in the denominator of the F-Ratio, changes in the model F-Ratio with an increase in the number of parameters should reflect the effect on predictive power relative to the number of fitted parameters. Thus, a correlation with a larger number of parameters might give a higher r^2 (or r^2(adj.)) but a lower F-Ratio than a correlation with fewer parameters. This would indicate that the improvement in predictive power (as indicated by a larger r^2) was not as large per parameter as for the equation with fewer parameters.

When linear regression with a variable intercept was performed the intercept was found to be 0.0035(±0.0022) and $\gamma\lambda$ =0.0276(±0.0031) with a high degrees of correlation (r^2 = 0.965, r^2_{adj} = 0.953, RMSE = 0.0033, model F-ratio = 81.6). The intercept is not very significantly different from zero in this regression. Both the regression with a zero intercept and a non-zero intercept show that the product $\gamma\lambda$ is not significantly different from 0.030. Figure 2.2 shows that Eq. (2.17) closely describes the data when $\gamma\lambda$ = 0.030. The permeability coefficient of TCDD can be calculated from $\gamma\lambda$ (0.030). Based on a soil loading (M_{soil} /(f_{ac} A)) = 10 mg/cm^2 and β/b = 0.24/0.023 = 10, $\underline{P}_{cs}$ = 2.84x10^{-5} g/(cm^2 hr).

Since there are only five data points, certain individual datapoints are particularly influential on the regressions. When the elimination measured at the first time (i.e., at 24 hours) was excluded from the linear regression, with zeroed intercept, the optimal parameter $\lambda\gamma$ = 0.0311(±0.0024) for which the RMSE = 0.0038 and the model F-ratio = 175.0. This value does not differ significantly from the fit obtained when all datapoints are included. However, when the last (i.e., 96-hour) datapoint was excluded from the

regression, the optimal parameter $\lambda\gamma = 0.0367(\pm 0.0027)$ is more different from 0.030 (for which the RMSE = 0.0025 and the model F-ratio = 188.6). The dose eliminated at 96 hours is lower than expected by a model which optimally represents the other data points. This last datapoint may be lower because of some change in the rate of elimination or uptake from the soil (e.g., soil is in less intimate contact with the skin).

Figure 2.2 shows the mass absorbed into the stratum corneum as predicted by Eq. (2.9) with parameters determined from regression of the urinary elimination data. For λ large relative to γ and effective removal of the penetrating compound by the cutaneous blood (i.e., $\alpha_c = 1$) Eq. (2.9) simplifies to:

$$\left(\frac{M_{in}^{c}}{\delta_d}\right)_B = \frac{\gamma\,\lambda\,\tau}{\beta} \tag{2.19}$$

Based on this calculation with $\lambda\gamma = 0.030$, at the 96 hour termination of the experiment 27.3% of the dose applied in the soil had been absorbed into the SC.

In a previous analysis of the TCDD data, (in Table 6.4 of US EPA (US EPA, 1992)) the cumulative percentage of the dermally absorbed dose eliminated over the reported collection times (i.e., at 24, 48, 72, and 96 hours) were divided by the cumulative fraction of the IV bolus administration eliminated at the same time. This approach assumed that the entire dermal dose has spent the same length of time in the animal as the intravenous dose. In fact, dermal absorption continued over the entire exposure period and, consequently, most of the dermally absorbed dose has had a shorter residence time. Consequently, the fraction of the dermal dose eliminated over a given collection period is necessarily less than that observed for elimination of the intravenous dose collected over the same period. Thus, the estimate of dermal absorption at 96 hours calculated by adjusting with the IV eliminated dose at 96 hours was 0.037/0.212 = 0.175 which is about 36% less than estimated by pharmacokinetic analysis.

2.4.2. Case 2: Analysis of Excretion Data After Chemical was Removed from Skin

Wester *et al.* (Wester *et al.*, 1993) investigated the dermal uptake of PCP from soil (48 - 80 mesh sieve fraction) into the abdominal skin of female rhesus monkeys (n = 4). Exposure to the soil lasted for 24 hours, after which time the soil vehicle was removed by surface washing. The concentration of PCP in the soil was 17 ppm and the soil loading was 40 mg soil/cm^2 (approximately monolayer coverage for this sized particle) which resulted in a chemical concentration on skin of 0.7 μg/cm^2. The area of exposure was 12 cm^2 of abdominal skin but this entire area may not have been covered by a monolayer of soil due to soil settling to the bottom of the cover when the animal was seated. The soil used in these studies, designated as Yolo County soil 65-California-57-8, was 26% sand, 26% clay, 48% silt and 0.9% organic carbon (all fractions apply to soil before sieving).

Results from a bolus IV injection of PCP (Wester and Maibach, 1975b) were used to adjust the dermal elimination data for other modes of elimination and storage. Table 2.2 gives the percentages of the dose eliminated in the urine following PCP administration by a bolus IV injection and by dermal exposure to PCP contaminated soil. From the cumulative mass appearing in urine over 14 days, measured with liquid scintillation counting, they calculated that 45.2 ± 4.5% of the IV dose was ultimately eliminated. Assuming that the same mass fraction of the dermally absorbed dose would be eliminated to the urine after 14-days, the total cumulative percent of topical dose eliminated [11.1 ± 2.9 (s.d.) %] was divided by 0.452 to obtain an estimate for the cumulative dermal absorption over a 24-hour exposure of 24.4 ± 6.4 (s.d.) %. The confidence intervals reported are based on a standard deviation over the four animals.

Nonlinear regression (using the Gauss-Newton method with stephalving to search for the least-squares estimate in JMP (SAS Institute, 1995)) was used to optimally fit Eq. (2.1) to the IV data from Table 2.2. The pharmacokinetic parameters obtained with

Table 2.2 PCP Urinary Elimination Following Intraveneous Dosing and Topical Application (Wester *et al*., 1993)

Time (Hours)	$\tau = bt$	Fraction Eliminated	
		IV Bolus[a]	Dermal[b]
0	0	0	0
24	0.10	0.048	0.018
48	0.19	0.106	0.035
72	0.29	0.159	0.046
96	0.39	0.197	0.054
120	0.48	0.239	0.061
144	0.58	0.266	0.067
168	0.68	0.289	0.073
192	0.78	0.312	0.080
216	0.87	0.333	0.087
240	0.97	0.360	0.093
264	1.07	0.379	0.099
288	1.16	0.409	0.103
312	1.26	0.431	0.107
336	1.36	0.452	0.111

[a] Expressed as fraction of the bolus dose eliminated.

[b] Expressed as fraction of the topical dose eliminated.

nonlinear regression were $\beta = 0.593$ and $b = 0.00404\ hr^{-1}$. The r^2 was 0.997, the sum of the squared errors (SSE) was 8.81×10^{-4}, the mean square error (MSE) was 6.8×10^{-5}, and the root mean square error (RMSE) was 8.2×10^{-3}. Figure 2.3 shows that this fit accurately describes the data. Applying the pharmacokinetic definitions to these parameters $k_{el} = 0.00239\ hr^{-1}$ and $k_{12} = 0.00164\ hr^{-1}$.

For PCP metabolized in the rhesus monkey, the elimination rate is slow relative to diffusion across skin (i.e., λ is large). In the limit of large λ and large blood flow (i.e., $\alpha_c = 1$), Eq. (2.15) simplifies to,

$$\left(\frac{M_{el}^{c}}{\delta_d}\right)_A = \gamma\lambda\left(e^{-\tau} + \tau_{exp} - e^{(\tau_{exp}-\tau)}\right) \tag{2.20}$$

This equation shows that for rapidly-absorbed and slowly-eliminated chemicals, the separate contributions of the diffusive coefficient and partition coefficient can not be determined, although the product $\lambda\gamma$ containing the permeability, can be unambiguously calculated.

For PCP, $\gamma\lambda$ was determined by regressing Eq. (2.17) to the urinary elimination data collected before removal of the vehicle ($\tau < \tau_{exp}$) and Eq. (2.20) to the data collected after removal of the vehicle ($\tau \geq \tau_{exp}$). This regression determined that $\gamma\lambda = 1.619$ ($r^2 = 0.960$, $r^2_{adj} = 0.866$, RMSE = 0.0068, model F-ratio = 1860). Figure 2.3 shows that the elimination data are well represented by this fit. Using Eq. (2.19) with $\gamma\lambda = 1.619$ and $\beta = 0.593$ we calculate that 26.5% of the applied dose was absorbed during the 24 hours exposure.

Figure 2.3 shows a plot of Eq. (2.9) with these parameters, compared to the method of Wester *et al* (Wester *et al.*, 1993). As expected, the amount of dermal absorption calculated by the pharmacokinetic analysis (26.5%) is similar to that calculated by Wester *et al.* (24.4%). (Wester *et al.*, 1993). This difference is small because of the large time over which elimination of PCP was monitored (14 days).

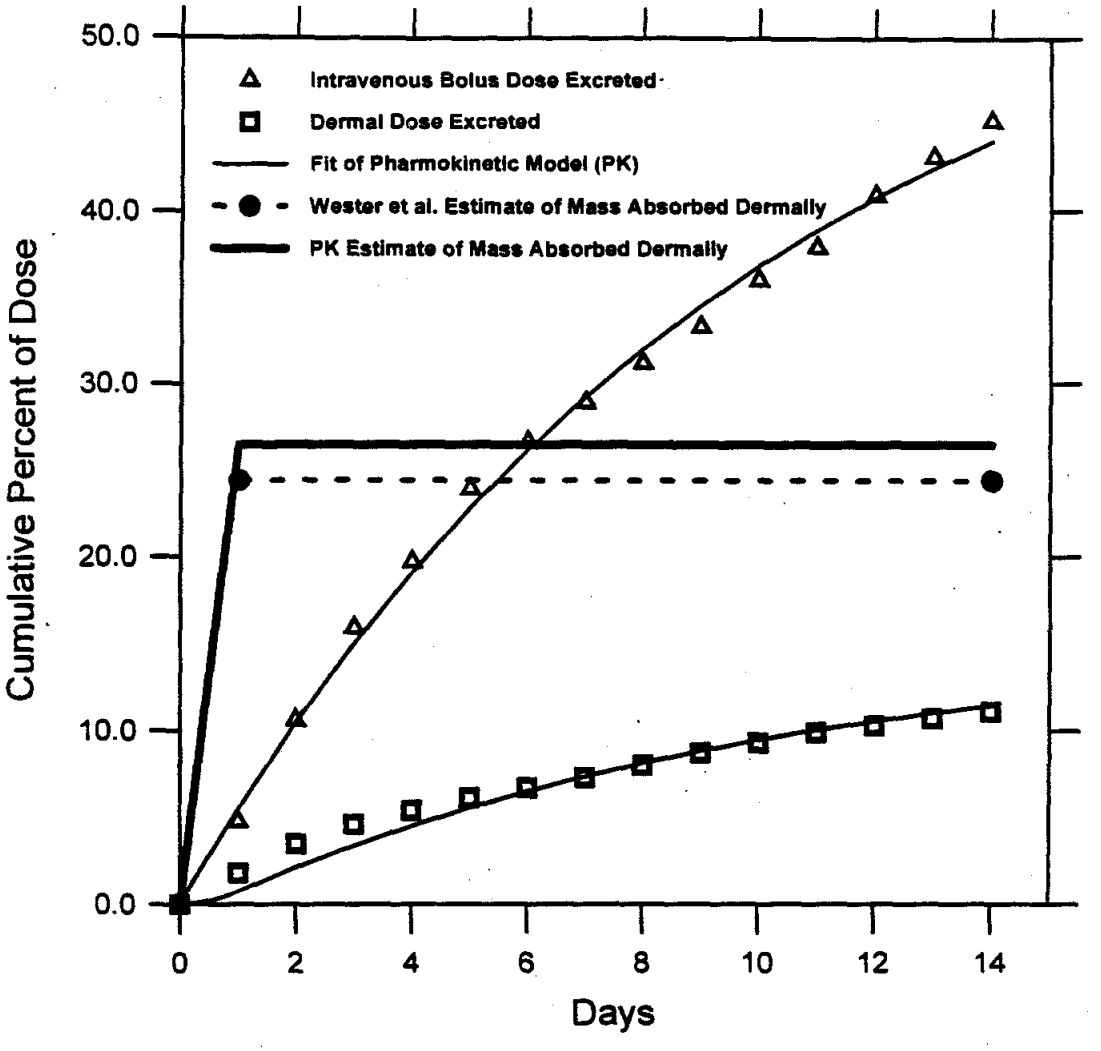

Figure 2.3 Analysis of PCP urinary elimination data following intravenous dosing and topical application.

A permeability coefficient was not previously reported for PCP but one can be calculated from the data. Using Eq. (2.18) with $\gamma\lambda = 1.619$, $M_{soil}/(f_{ac} A) = 40$ mg/cm^2, and $\beta/b = 0.593/0.00404$hr $= 146.8$ hr^{-1} we calculate a permeability coefficient, $\underline{P}_{cs} = 4.41\text{x}10^{-4}$ g/(cm^2 hr), for PCP.

An important advantage of the pharmacokinetic analysis is that it eliminates the need to collect urine data for such a long period. For the adjustment approach used by Wester *et al.* (Wester *et al.*, 1993) to be correct, elimination must be monitored until further elimination is insignificant. However, the pharmacokinetic analysis is meaningful even when the cumulative elimination is incomplete. This is better for the laboratory animals and generally reduces the cost of the experiment. We have performed the same pharmacokinetic analysis on the first half of the Wester *et al.* data and determined that $\gamma\lambda = 1.776$ ($r^2 = 0.815$, $r^2_{adj} = 0.648$, RMSE = 0.0083, model F-ratio = 288.4). Using Eq. (2.19) and $\gamma\lambda = 1.776$ we calculate that 29% of the applied dose was absorbed during the 24 hours exposure. Using $\gamma\lambda = 1.776$ and the procedure for calculating the permeability coefficient described above, we calculate $\underline{P}_{cs} = 4.84\text{x}10^{-4}$ g/(cm^2 hr), for PCP. The similarity between this permeability coefficient and the one calculated using data collected over a period twice as long illustrate the usefulness of the pharmacokinetic approach.

2.4.3. Special Considerations

A key assumption of the pharmacokinetic analysis is that chemical delivery by IV bolus or dermal absorption does not effect the mechanisms of elimination. Provided that the bolus and the dermally absorbed dose eliminate by the same mechanism, estimates can be improved if the bolus delivery is made to mimic dermal delivery as closely as possible. One approach to test that this is the case would be to compare elimination kinetics for IV doses administered at a constant rate over an extended period.

When elimination kinetics are slow relative to the rate of dermal penetration (i.e., large λ), the lag time for crossing the skin membrane is much slower than the lag time for elimination in urine or feces. Because the rate of dermal absorption is initially faster than at steady-state, permeability estimates calculated for large λ should be larger than the steady-state permeability coefficients. To infer information about the unsteady-state absorption process λ should be small. The quantitative effect of λ on analysis of unsteady-state dermal absorption requires more investigation.

Single membrane or compartment models are relevant for low to moderate lipophilicity compounds ($\log K_{ow} \leq 3$) where the barrier of the skin is essentially that of the stratum corneum. Both PCP ($\log K_{ow} = 5.86$; (Hansch *et al.*, 1995)) and TCDD ($\log K_{ow} = 6.80$; (Hansch *et al.*, 1995)) are highly lipophilic and both the stratum corneum and the viable epidermis may contribute a significant resistance to penetration. Therefore, the transport parameters calculated are effective values assuming all resistance resulted from one homogeneous skin layer. Nevertheless, since the total barrier function was accounted for, the previous analyses are true to the calculation of total mass absorbed.

2.5. Conclusions

Dermal absorption can be underestimated by an analysis that does not consider the temporal effects of storage and elimination of a compound (i.e., the pharmacokinetics of that compound). This was illustrated by a reanalysis of data for dermal absorption of (1) TCDD in rats from soil (Roy *et al.*, 1990; US EPA, 1992), and (2) PCP in rhesus monkeys from soil (Wester *et al.*, 1993). A pharmacokinetic analysis provides useful information (i.e., a permeability coefficient) and is a more accurate representation of the data than either of the two alternative methods. Finally, the model which we derived to

analyze the data has revealed the quantitatively limitations of calculating the dermal absorption from excretion data; analysis is best for rapidly eliminated penetrants.

2.6. Notation

A = Surface area of chemical exposure
b = Pharmacokinetic parameter ($k_{el}+k_{12}$)
C_1 = Chemical concentration in first compartment
C_2 = Chemical concentration in second compartment
D_c = Effective diffusivity of the absorbing chemical in the SC
f_{ac} = Fraction of skin covered by vehicle
IV = Intravenous injection
k_{12} = Pharmacokinetic rate constant for irreversible transfer between compartments 1 and 2 (see Fig. 2.1)
k_{el} = Pharmacokinetic rate constant for urinary/fecal elimination from compartment 1 (see Fig. 2.1)
k_{rc} = Transfer rate constant defined by Eq. (2.11)
$\underline{K}_{cs}$ = Equilibrium partition coefficient between stratum corneum and soil for absorbing chemical [(mass chemical)/(volume stratum corneum)]/[(mass chemical)/(mass soil)]
K_{cb} = Equilibrium partition coefficient SC and blood
K_{ow} = Equilibrium partition coefficient between octanol and water
L_c = Effective thickness of the SC
M_{el} = Cumulative mass of topical absorbed or IV dose eliminated in the excreta
M_{in} = Mass absorbed into the SC
M_{soil} = Mass of soil applied to the skin
M_{sys} = Cumulative mass of absorbed chemical entering compartment 1, equals the mass penetrating the skin
$\underline{P}_{cs}$ = Permeability coefficient across SC from soil, ($\underline{P}_{cs} = K_{cs} D_c / L_c$)
q_b = Cutaneous blood flow per unit area of skin
SC = Stratum corneum
t = Time period of exposure event
t_{exp} = Time of removal of the vehicle
V_1 = Volume of first compartment
V_2 = Volume of second compartment

Greek

α_c = Skin-blood transfer parameter, defined by Eq. (2.10)
β = Fraction of pharmacokinetic elimination pathway, defined by Eq. (2.2)
γ = Exposure parameter, defined by Eq. (2.7)
λ = Ratio of diffusion rate to elimination rate, defined by Eq. (2.6)
δ_d = Mass available for dermal absorption, $= M_{soil} \underline{C}_{soil}$

δ_{IV} = Mass of chemical administered intravenously
τ = Kinetically normalized time, ($\tau = b\,t$)
τ_{exp} = Kinetically normalized time at which the dermal dose is removed, ($\tau = b\,t_{exp}$)

Superscripts
c = Compartment model result
m = Membrane model result

2.7. References

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2.8. *Appendix: Development of Working Equations*

2.8.1. Bolus Equation

The bolus elimination rate was developed by applying mass balance equations around the animal's systemic compartment. Because the bolus is delivered directly into the blood, the skin compartments are not involved. For this systemic model the differential equation describing the mass balance is given as

$$V_1 \frac{dC_1}{dt} = -k_{el} C_1 V_1 - k_{12} C_1 V_1$$
$$\text{where } C_1 = \frac{\delta_{IV}}{V_1} \text{ at } t = 0 \tag{2A.1}$$

Eq. (2A.1) can be solved to describe the concentration in this systemic compartment. This concentration can then be substituted into the time rate of urinary-fecal elimination from compartment 1:

$$\frac{dM_{el}}{dt} = k_{el} C_1 V_1$$
$$\text{where } M_{el} = 0 \text{ at } t = 0 \tag{2A.2}$$

so that the rate of elimination can be evaluated. The cumulative elimination of an intravenous bolus IV injection is then

$$\frac{M_{el}}{\delta_{IV}} = \frac{k_{el}}{k_{el} + k_{12}}\left(1 - e^{\tau}\right) \tag{2A.3}$$

2.8.2. Eliminated Dose without Vehicle Removal

Mass balance equations written for the compartments shown in Fig. 2.1 are given below.

$$V_1 \frac{dC_1}{dt} = -k_{el} C_1 V_1 - k_{12} C_1 V_1 + \frac{dM_{sys}}{dt} \tag{2A.4}$$

where $C_1 = 0$ and $M_{sys} = 0$ at $t = 0$

The concentration in compartment 1 (C_1) can be symbolically evaluated with Laplace transforms,

$$C_1 = L^{-1}\left\{\frac{s\overline{M}_{sys}}{V_1(s+1)}\right\} \tag{2A.5}$$

and substituted into the expression for the rate of chemical elimination from the rapidly perfused body compartment:

$$\frac{dM_{el}}{dt} = k_{el} C_1 V_1 \tag{2A.6}$$

so that the cumulative mass of the dermally absorbed dose eliminated (M_{el}) can be evaluated. The completely general result, for the adopted systemic model, is:

$$M_{el} = \int_0^{\tau} \frac{k_{el}}{k_{el} + k_{12}} L^{-1}\left\{\frac{s\overline{M}_{sys}}{s+1}\right\} d\tau \tag{2A.7}$$

Once the expression for the mass into compartment 1 is chosen, (Eq. (2.4) for the compartment model or Eq. (2.8) for the membrane model), then the expression for the urinary excretion of the dermally absorbed dose (M_{el}) can be evaluated (i.e., Eqs. (2.12) or (2.13) respectively).

2.8.3. Eliminated Dose with Vehicle Removal

Eq. 2A.7 still applies to model elimination in experiments in which the vehicle is removed before all urine/feces has been collected. However, the amount of chemical

absorbed through the skin depends upon the time of removal of the vehicle τ_{exp}. For times before vehicle removal the amount systemically absorbed is given by Eqs. (2.8) or (2.4), while for times after removal the amount systemically absorbed is given by Eq. (2.14).

It is necessary to partition the Laplace transform over the time before and after exposure.

$$\overline{M}_{el} = \int_0^{\tau} M_{sys}\, e^{-s\tau}\, d\tau + \int_{\tau}^{\infty} M_{sys}\, e^{-s\tau}\, d\tau \tag{2A.8}$$

Where M_{sys} in the first integral represents mass leaving the skin for all times before removal and M_{sys} in the second integral represents the mass leaving the skin for all times after removal of the vehicle.

3. UNSTEADY-STATE ANALYSIS OF *IN VIVO* DERMAL ABSORPTION DATA FOR CHLOROFORM, TRICHLOROETHYLENE, AND TETRACHLOROETHYLENE INTO HAIRLESS GUINEA PIGS

3.1. Abstract

Steady-state permeability coefficients are the single most useful parameter for characterizing the barrier properties of skin to many toxic chemicals. This chapter compares unsteady-state and steady-state methods for analyzing *in vivo* dermal absorption data to determine permeability coefficients. Specifically, we examine the datasets from Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994) for chloroform, trichloroethylene, and tetrachloroethylene into hairless guinea pigs from aqueous solutions. In their analysis Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994) calculated permeability coefficients assuming all data were at steady state. Here we examined the steady-state assumption more closely and found that it was appropriate for CF and TCE but was not appropriate for PCE.

3.2. Introduction

Permeability is an important parameter for characterizing the transport resistance of a membrane. Strictly, the permeability of a chemical through a membrane is only meaningful when measured at steady state. For skin including both the stratum corneum (SC) and the viable epidermis (VE), the steady-state permeability of a chemical from a

given vehicle v, P_v, depends on the steady-state permeability coefficients for the SC (P_{cv}) and VE (P_{ev}):

$$\frac{1}{P_v} = \frac{1}{P_{cv}} + \frac{1}{P_{ev}} \tag{3.1}$$

which are defined as:

$$P_{cv} = \frac{K_{cv} D_c}{L_c} \tag{3.2}$$

$$P_{ev} = \frac{K_{ev} D_e}{L_e} \tag{3.3}$$

where K_{cv} is the equilibrium partition coefficient between the SC and a vehicle, and D_c is the diffusion coefficient of the chemical the SC, L_c is the thickness of the SC, K_{ev} is the VE-vehicle partition coefficient, and D_e and L_e are the diffusivity of chemical in and thickness of the VE respectively. If the vehicle does not alter the thermodynamic character of the SC or VE,

$$K_{ev} = K_{cv} / K_{ce} \tag{3.4}$$

where K_{ce} is the SC-VE partition coefficient, and the resistance of the SC-VE composite barrier is:

$$\frac{1}{P_v} = \frac{1}{P_{cv}}\left[1 + \frac{K_{ce} D_c L_e}{D_e L_c}\right] = \frac{1}{P_{cv}}(1 + B) \tag{3.5}$$

where the parameter B, defined as

$$B = \frac{D_c L_e K_{ce}}{D_e L_c} = \frac{P_{cv}}{P_{ev}} \tag{3.6}$$

measures the relative permeability of the SC to the VE. Many *in vitro* and *in vivo* experiments on human and animal skins have been conducted to determine skin permeability coefficients of various chemicals. In some experiments, the amount of chemical absorbed was measured. In others, the amount of chemical or radioactivity

which has crossed the skin barrier to appear in a receiving chamber, blood, or excreted materials (i.e., urine or feces) was determined. Since only the steady-state permeability is meaningful for comparing to other chemicals and exposure situations, it is important to know if *in vivo* or *in vitro* data were collected and analyzed to insure that a steady-state permeability value was obtained.

To derive permeability coefficients from *in vivo* experiments which follow blood, urine, or feces concentrations as a function of exposure time, t_{exp}, one must include systemic pharmacokinetics in the analysis. This requirement introduces additional experimentation and also uncertainties in the resulting percutaneous absorption parameters. Consequently, *in vivo* experiments which measure absorption directly have many advantages.

As already mentioned, permeability coefficients measured at steady state are preferred. Experimentally, this generally requires that changes in the vehicle concentration be small. (Steady-state P_v can be deduced from experiments with varying vehicle concentration, provided that rate of concentration change is known and is small relative to the rate of dermal absorption.) The common *in vivo* experiment which deposits chemical dissolved in a volatile vehicle on the skin surface is therefore inappropriate for determining P_v, since the vehicle concentration (C_v) changes rapidly and dramatically while the vehicle evaporates. Furthermore, the form of the deposited chemical after the vehicle evaporates (e.g., crystalline or amorphous solid or liquid) could profoundly but unaccountably effect absorption. Only a few *in vivo* experiments have directly measured absorption while keeping C_v essentially constant. A set of such experiments was recently reported for absorption of ^{14}C-labeled chloroform (CF), trichloroethylene (TCE), and tetrachloroethylene (i.e., perchloroethylene or PCE) into hairless guinea pigs (Bogen *et al.*, 1992; Bogen *et al.*, 1994) and these are considered here.

3.3. Previous Experiments and Analysis

A detailed experimental description is provided elsewhere (Bogen *et al.*, 1992; Bogen *et al.*, 1994). Sedated guinea pigs were immersed up to the neck in beakers of aqueous solution for 70 min and the amount of chemical remaining in the exposure solution was determined by liquid scintillation. Chemical loss from the exposure solution, modified for evaporation (which, as measured in separate control experiments, proved to be minor), was attributed to dermal absorption. Excretion efficiencies, measured by monitoring appearance of radioactivity in urine and fecal samples for 2 to 4 weeks following exposure, proved to be similar for dermal and subcutaneous delivery, supporting this assumption within the accuracy of the data. Inhalation was not eliminated as a potential route of exposure, so the measured excretion levels may be partly due to inhalation.

Penetration was observed in five separate guinea pigs, for each of the three chemicals. In each experiment, the first measurement was made at an exposure time of 10 min, and all reported values were normalized with respect to this first measurement. The initial concentrations, if known, were not reported. The 10 min concentrations were calculated from the net disintegrations per minute (measured disintegrations less disintegrations from appropriate control blanks). Dermal exposure was investigated for all three chemicals at concentration levels of 20-110 ppb. Additional experiments were performed on TCE at a concentration of 100 ppm.

The mass of radiolabeled PCE remaining in the exposure chamber when a guinea pig was present is reported in Table 3.1, relative to that measured at 10 min after exposure began. The same quantity, measured when no guinea pig was present (i.e., the chamber control experiment) is listed in Table 3.2. These tables can be used together to account for the depletion of the dermal exposure chamber that is attributed to percutaneous absorption. When exposed areas and vehicle volumes are correctly

Table 3.1 Mass of PCE Remaining Relative to that Measured at 10 min when a Guinea Pig was Present (Bogen *et al.*, 1992).

Relative Time (t_{exp} - 10 min)	Experiment Number*				
	1[a]	2[b]	3[c]	4[d]	5[e]
0	100	100	100	100	100
5	93.14	99.62	93.24	94.45	96.32
10	93.42	96.76	90.81	92.76	90.69
20	85.59	91.76	86.38	88.54	87.11
30	80.64	91.37	85.69	84.46	85.05
40	79.59	86.03	82.45	79.63	83.83
50	79.05	84.01	79.44	81.34	90.29
60	74.20	82.66	78.77	77.81	79.07

* Designated in Figure 5 of Bogen et al. (1994) as:

[a] Open circle [b] Solid circle [c] Open square [d] Solid square [e] Open triangle

Table 3.2 Mass of PCE Remaining Relative to that Measured at 10 min when no Guinea Pig was Present (Bogen *et al*., 1992).

Relative Time (t_{exp} - 10min)	Experiment Number*				
	1[a]	2[b]	3[c]	4[d]	5[e]
0	100	100	100	100	100
5	100.68	100.30	101.19	98.96	102.09
10	98.76	97.93	99.13	98.71	99.54
20	97.64	95.92	97.85	97.31	100.23
30	96.52	96.63	97.77	98.02	98.65
40	96.34	96.93	99.17	98.35	99.79
50	96.85	96.19	99.03	98.09	100.37
60	97.38	96.14	99.30	98.75	101.32
90	93.21	94.53	98.87	97.28	98.10
120	95.41	94.08	97.58	97.06	96.03
150	92.77	95.43	98.93	95.64	97.15
180	94.94	91.73	98.63	96.45	99.23

* Designated in Figure 5 of Bogen et al. (1994) as:

[a] Open circle [b] Solid circle [c] Open square [d] Solid square [e] Open triangle

accounted for, the data can be expressed relative to the amount absorbed at 10 min, and normalized by the mass that remains in the dermal exposure chamber at 10 min; these data are summarized in Table 3.3 for PCE. Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994) did not provide data tables for CF and TCE and, consequently, the data summarized in Tables 3.4 and 3.5 were obtained by digitizing figures and normalizing with respect to exposure area, vehicle volume, and the amount remaining in the solution at 10 min. The measurement at 40 min for CF (open triangle in Figure 4 of the Bogen *et al.* paper (Bogen *et al.*, 1994)) was missing or concealed, so it was excluded from all analysis.

Bogen *et al.*, (Bogen *et al.*, 1992; Bogen *et al.*, 1994) calculated skin permeabilities, from analysis of the chamber depletion data, with the equation:

$$P_w = \frac{(V_o - 2.0\ \text{ml})(S_{de} - S_{cc})}{100\%\,A} \tag{3.7}$$

where V_o is the initial chamber volume (about 500 mL), A is the area of exposed skin, and S_{de} and S_{cc} are the slopes of linear regressions to chamber depletion data normalized relative to the concentration measured at 10 min of exposure for the dermal-exposure and chamber-control experiments, respectively. The chamber volume was adjusted to account for sampling without replacement (i.e., 2.0 mL on average). The exposed area in cm^2 (A) of each guinea pig was estimated from the animals weight (w) in grams with the correlation $A = 24.5\,w^{0.394}$. The area was generally 250-300 cm^2. Chamber control experiments account for non-dermal mechanisms of depletion such as evaporation and adsorption to the chamber wall. In the analysis by Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994), P_w was calculated for each of the five animals and then averaged. The uncertainty reported is the standard deviation of the five P_w values.

Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994) reported the following calculated values for P_w : 0.13 cm/hr ± 0.08 for CF (uncertainty expressed as the 95%

Table 3.3 Normalized Mass of PCE Absorbed into Hairless Guinea Pigs (Relative to the Mass Absorbed at t_{exp} = 10 min) and Normalized by the Mass Remaining in the Vehicle at t_{exp}=10 min

	$\frac{\left[M_{in}(\text{at } t_{exp}) - M_{in}(\text{at } t_{exp} = 10 \text{ min})\right]}{VC_w^o - M_{in}(\text{at } t_{exp} = 10 \text{ min})}$						
Relative Time (t_{exp} - 10 min)	Experiment Number*						
	1[a]	2[b]	3[c]	4[d]	5[e]	Mean	Standard Deviation
0	0	0	0	0	0	0.0000	0
5	0.067	0.003	0.069	0.053	0.035	0.0453	0.0245
10	0.063	0.030	0.093	0.069	0.090	0.0688	0.0227
20	0.138	0.078	0.136	0.107	0.123	0.1165	0.0222
30	0.184	0.079	0.141	0.145	0.141	0.1380	0.0337
40	0.192	0.130	0.172	0.190	0.151	0.1669	0.0236
50	0.195	0.148	0.201	0.171	0.184	0.1795	0.0189
60	0.240	0.159	0.205	0.203	0.193	0.2000	0.0261
Exposed Area (cm^2)	254	266	282	282	275	271.8	-----
Solution Volume (cm^3)	577.7	543.8	495.3	525.1	530.4	534.5	-----

* Designated in Figure 5 of Bogen et al. (1994) as:

[a] Open circle [b] Solid circle [c] Open square [d] Solid square [e] Open triangle

Table 3.4 Normalized Mass of CF Absorbed into Hairless Guinea Pigs (Relative to the Mass Absorbed at t_{exp} = 10 min) and Normalized by the Mass Remaining in the Vehicle at t_{exp} = 10 min

	$\frac{\left[M_{in}(\text{at } t_{exp}) - M_{in}(\text{at } t_{exp} = 10 \text{ min})\right]}{VC_w^o - M_{in}(\text{at } t_{exp} = 10 \text{ min})}$							
Relative Time (t_{exp} - 10 min)	Experiment Number*							
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	Mean	Standard Deviation
0	0	0	0	0	0	0	0	0
5	-0.005	-0.011	-0.006	0.014	-0.014	-0.018	-0.0065	0.0103
10	0.033	0.031	-0.014	0.004	0.022	0.000	0.0125	0.0174
20	0.096	-0.019	0.054	0.043	0.032	0.026	0.0386	0.0343
30	0.036	0.038	0.091	0.049	N/A	0.009	0.0444	0.0267
40	0.039	0.068	0.060	0.075	0.032	0.026	0.0502	0.0188
50	0.084	0.086	0.115	0.066	0.053	0.050	0.0759	0.0224
60	0.115	0.126	0.171	0.041	0.094	0.020	0.0945	0.0510
Exposed Area (cm^2)	306	310	316	301	288	280	300.2	-----
Solution Volume (cm^3)	428.1	426.5	387.5	470.7	482.4	500.3	449.3	-----

* Designated in Figure 5 of Bogen et al. (1994) as:

[a] Open circle [b] Solid circle [c] Open square [d] Solid square [e] Open triangle [f] Solid triangle

Table 3.5 Normalized Mass of TCE Absorbed into Hairless Guinea Pigs (Relative to the Mass Absorbed at t_{exp} = 10 min) and Normalized by the Mass Remaining in the Vehicle at t_{exp}=10 min

	$\frac{\left[M_{in}(\text{at } t_{exp}) - M_{in}(\text{at } t_{exp} = 10 \text{ min})\right]}{VC_w^o - M_{in}(\text{at } t_{exp} = 10 \text{ min})}$						
Relative Time (t_{exp} - 10 min)	Experiment Number*						
	1[a]	2[b]	3[c]	4[d]	5[e]	Mean	Standard Deviation
0	0	0	0	0	0	0.0000	0
5	0.033	-0.023	0.007	0.043	0.021	0.0161	0.0231
10	0.041	-0.010	0.012	0.030	0.008	0.0162	0.0176
20	0.060	-0.009	0.043	0.037	0.042	0.0345	0.0233
30	0.078	-0.021	0.044	0.062	0.073	0.0471	0.0360
40	0.086	0.009	0.052	0.066	0.103	0.0630	0.0321
50	0.107	0.033	0.059	0.105	0.148	0.0903	0.0405
60	0.120	0.052	0.058	0.096	0.193	0.1036	0.0511
Exposed Area (cm^2)	257	273	252	263	257	260.4	----
Solution Volume (cm^3)	449.9	478.6	509.4	488.0	520.7	489.3	----

* Designated in Figure 5 of Bogen et al. (1994) as:

[a] Open diamond [b] Solid diamond [c] Solid square [d] Open circle [e] Open square

confidence interval based on the reported coefficient of variation), 0.23 cm/hr ± 0.08 for the low concentration TCE experiments, 0.21 cm/hr ± 0.24 for the high concentration TCE experiments, 0.37 cm/hr ± 0.13 for PCE.

3.4. Theory

A detailed description of the two membrane model of skin exposed to a constant concentration vehicle has been presented elsewhere (Cleek and Bunge, 1993). The results of that model of interest here are the equations describing the mass flux in and out of the skin during the unsteady and steady-state periods. In the steady-state period the amount of absorption becomes linear (in exposure time), and the cumulative mass into the skin (M_{in}^{∞}) becomes:

$$\frac{M_{in}^{\infty}}{A\,C_v^o} = \frac{P_{cv}\,t_{exp}}{1+B} + K_{cv}\,L_c\,\frac{1+3B+3B^2}{3(1+B)^2} \tag{3.8}$$

For short exposure times the chemical will penetrate only a short distance into the SC, and the penetration rate is faster than at steady state. During this unsteady-state period, cumulative mass entering the skin (M_{in}^{uss}) depends upon the square-root of time as:

$$\frac{M_{in}^{uss}}{A\,C_v^o} = 2K_{cv}\sqrt{\frac{D_c\,t_{exp}}{\pi}} = 2\sqrt{\frac{P_{cv}\,K_{cv}\,L_c\,t_{exp}}{\pi}} \tag{3.9}$$

The unsteady-state period should last while t_{exp} < about 2.4 $t_{lag,c}$ where $t_{lag,c}$ is the lag time for transfer across the SC alone.

3.5. Results and Discussion

Figure 3.1 shows the average cumulative mass absorbed (adjusted for differences in vehicle volumes and exposure areas) and one standard deviation for each time point plotted relative to the first measurement at 10 min (t_{exp} - 10 min = 0). The cumulative mass absorbed is plotted relative to the mass absorbed at 10 min (i.e., [M_{in}(at t_{exp})-M_{in}(at

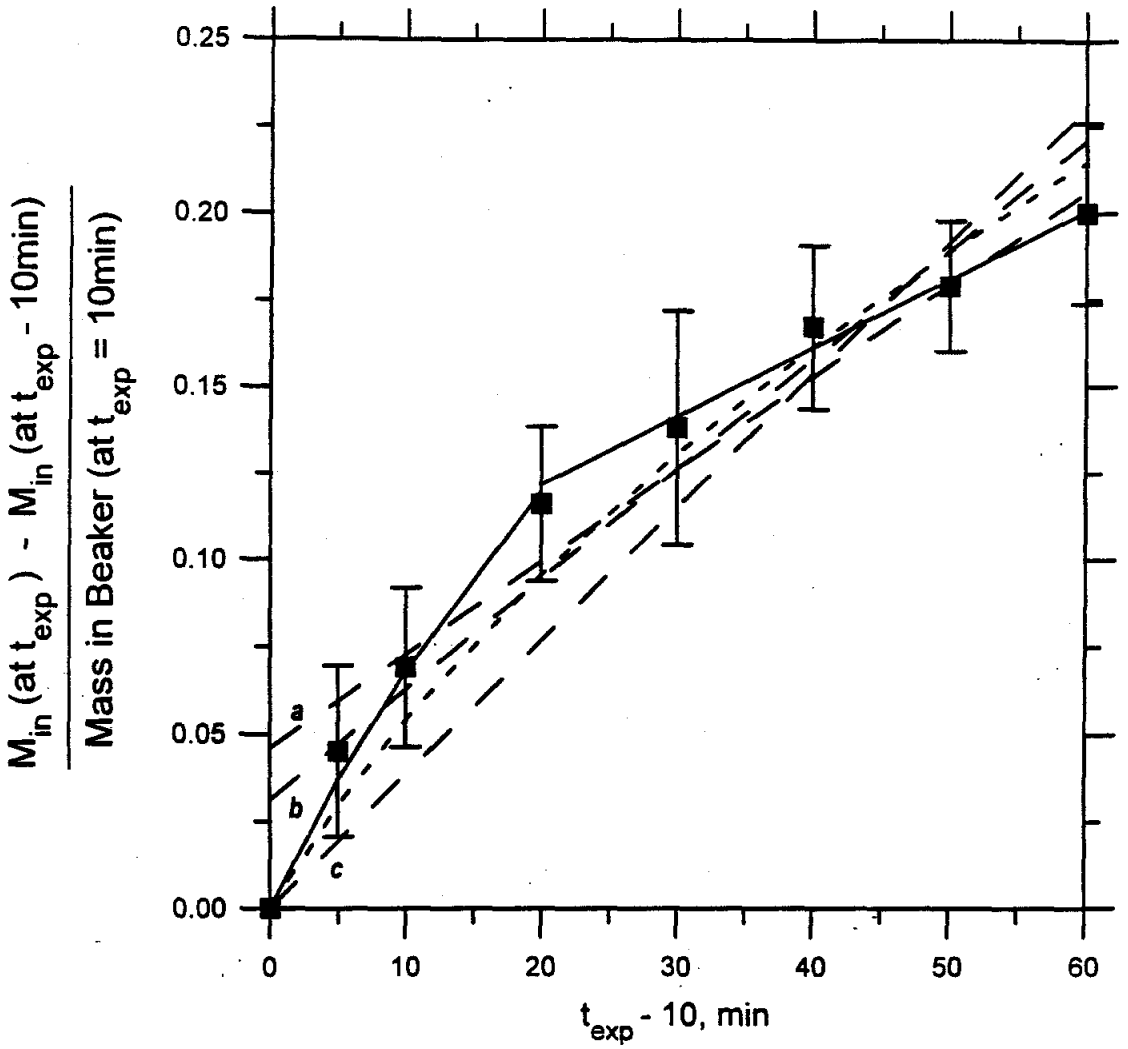

Figure 3.1 Cumulative mass of PCE dermally absorbed in hairless guinea pigs normalized with respect to the mass available for absorption 10 min after the exposure began. Linear regressions in t (lines labeled a, b and c), linear regressions in $\sqrt{t}$ (short dashes), and combined regression in $\sqrt{t}$ and t (solid curve).

t_{exp} = 10 min)]/[VC_w^o-M_{in}(at t_{exp} = 10 min)]), where V is the average chamber volume and C_w^o is the constant chamber concentration. Consequently, the general equation for a line in Figure 3.1 is of the form:

$$\frac{\left[M_{in}(\text{at } t_{exp}) - M_{in}(\text{at } t_{exp} = 10 \text{ min})\right]}{V C_w^o \left[1 - \frac{M_{in}(\text{at } t_{exp} = 10 \text{ min})}{V C_w^o}\right]} = S\left(t_{exp} - 10 \text{ min}\right) + I \tag{3.10}$$

If a line in Fig 3.1 represents the steady-state data, then the definition of the slope (S) and intercept (I) are deduced by comparing Eqs. (3.8) and (3.10)

$$S = \frac{A P_{cw}}{V(1+B)\left[1 - \frac{M_{in}(\text{at } t_{exp} = 10 \text{ min})}{V C_w^o}\right]} \tag{3.11}$$

$$I = \frac{A C_w^o \left[P_{cw}(10 \text{ min}) + L_c K_{cw}\left(\frac{1+3B+3B^2}{3(1+B)^2}\right)\right] - M_{in}(\text{at } t_{exp} = 10 \text{ min})}{V C_w^o \left[1 - \frac{M_{in}(\text{at } t_{exp} = 10 \text{ min})}{V C_w^o}\right]} \tag{3.12}$$

where A is the average area of exposure, and V is the average volume of solution in the beaker. Because Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994) report absorption relative to that which occurred in the first 10 min, the mass absorbed in the first 10 min must be known or estimated to calculate the permeability from the slope. If steady state is achieved within the first 10 min of exposure, then M_{in}(at t_{exp} = 10 min) is represented by Eq. (3.10) and the intercept, I, should be approximately zero.

Figure 3.1 shows three different linear regressions (the dashed lines labeled as *a*, *b*, *c*) each implying different assumptions. The data point at t_{exp} - 10 min = 0 was not included in the line *a* regression of the average cumulative mass absorbed values (S = 0.16 ± 0.06 hr^{-1} and I = 0.046 +0.043/-0.059), thereby making the assumption that all data, except the (t_{exp} - 10 min = 0) data point are from exposure times longer than the

time to reach steady state (i.e., 10 min < t*≤ 15 min, where t* represents the time to reach steady state). The regressed S and I values are reported as the mean ± the 95% confidence interval. Unless noted otherwise, we report 95% confidence limits (lower 2.5% and upper 97.5%) determined by superimposing the normal distribution functions of S and I from regressions of each of the five animals (5000 trials for each animal were generated by Crystal Ball (Decisioneering, 1993)).

The line *c* regression ($S = 0.23\ hr^{-1} \pm 0.07$) included all average cumulative mass absorbed data points while forcing I = 0. In this approach, the absorption rate is assumed to reach steady state prior to the first data point at t_{exp} - 10 min = 0 (i.e., t* ≤ 10 min) with only a small error in the first measurement of the chemical mass remaining in the beaker (i.e., at t_{exp} - 10 min = 0).

The line *b* regression included the first time point (i.e., t_{exp} - 10 min = 0) while allowing I ≠ 0 ($S = 0.19 \pm 0.07\ hr^{-1}$ and $I = 0.031 \pm 0.042$). When using this regression procedure, one has assumed that all data points (including the t_{exp} - 10 min = 0 data point) were at steady state (i.e., t* ≤ 10 min), with a non-zero error in the measurement of the chemical mass remaining in the beaker at t_{exp} - 10 min = 0. To the extent that measurement errors were randomly distributed, the magnitude of I should represent the error in the first measurement of chemical mass in the beaker. If all of the data points were at steady state, and the measurement error is random, we expect I for type *b* regression lines from replicate experiments to vary randomly around zero. The fact that I from all five replicates are positive supports our suspicion that steady state was not reached within the first 10 min.

To calculate P_{cw} from S and I we must know M_{in}(at t_{exp} = 10 min). For PCE, Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994) reported P_{cw} = 0.37 (±0.13) cm/hr. calculated by averaging slopes from linear regressions of each of the five experiments (including all data points but not forcing I = 0) and assuming that M_{in} (at t_{exp} = 10 min)

and B were both small (i.e., ≅ zero). If, as Bogen *et al.* assumed, $t^* \leq 10$ min, then M_{in}(at $t_{exp} = 10$ min) is calculated from Eq. (3.8), leading to the conclusions that I = 0 in Eq. (3.12) and that S in Eq. (3.11) depends on the unknown value of ($K_{cw} L_c$) in addition to P_{cw}. Unfortunately, insufficient information is provided by Bogen *et al.* to estimate ($K_{cw} L_c$) separately from P_{cw}. If we arbitrarily assume that the chemical capacity of the stratum corneum is small (i.e., $K_{cw} L_c = 0$), then the mass absorbed relative to the initial mass in the beaker, M_{in}(at $t_{exp} = 10$ min)/(V C_w^o) ≅ A P_{cw}(10 min/60 min/hr)/V (the average V/A was 1.97 cm), and $P_{cw} = 0.36$ cm/hr. Assuming that M_{in}(at $t_{exp} = 10$ min) ≈ 0, we calculated that $P_{cw} = 0.37$ (±0.13) cm/hr, exactly as estimated by Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994). Since $\log K_{ow} = 3.40$ for PCE, assuming B ≈ 0 is reasonable, but it is unlikely that the capacity of the stratum corneum is insignificant (i.e., $K_{cw}L_c \neq 0$). Consequently, M_{in}(at $t_{exp} = 10$ min)/(V C_w^o) will be larger than estimated above, leading to smaller values for P_{cw}.

We assumed that $t^* > 10$ min and used Eq. (3.9) to estimate M_{in}(at $t_{exp} = 10$ min)/(V C_w^o) $=2(A / V)\sqrt{P_{cw} K_{cw} L_c / \pi}\sqrt{(10 \text{ min} / 60 \text{ min} / \text{hr}}$. Substituting this relationship into Eqs. (3.11) and (3.12), P_{cw} and $K_{cw} L_c$ can be deduced from S and I which are known from linear regression of the steady-state data.

According to Eq. (3.9), any data points in Figure 3.1 at exposure times less than t* should be a function of $\sqrt{t_{exp}}$ rather than t_{exp}. Indeed, the early exposure data are much better predicted by the best fit (short-dashed curve) in Figure 3.1 which was forced to be zero at time zero, providing evidence that Eq. (3.9) correctly represents unsteady-state absorption.

The data at longer exposure times appear to be nearly linear with t_{exp} suggesting that steady state was eventually established. Accordingly, we assumed various values for t*, and fit the data at which $t_{exp} \leq t^*$ to a square-root of time function, and the data at which $t_{exp} > t^*$ to a linear function of t_{exp}. The slope, intercept, P_{cw} and $K_{cw}L_c$ determined

for all possible transition times (t*) by assuming various number of points (2 through all 8) are at steady state. The results are summarized in Table 3.6a,b. The transition time (t*) with the minimum residual is between 20 and 30 min (i.e., 10 min < t* - 10 min ≤ 20 min). The result is shown as the solid curve and line in Figure 3.1. If correct, this represents a $t_{lag,c}$ of between 8.3 and 12.5 min (i.e., $t_{lag,c} \sim t^*/2.4$).

The calculations summarized in Table 3.6a,b are based on the mean value of the absorption for all 5 animals at each time point. Regression was also applied to each of the individual animals assuming 5 steady-state data points. The results are summarized in Table 3.7a,b. Regressions following the strategies referred to earlier as *a*, *b*, and *c* were also applied to the individual animals assuming 7, 8, and 8* (i.e., also forcing a zero intercept) steady-state data points, respectively . These results are summarized in Tables 3.8a,b - 3.10.

From the linear regressions for each animal when t_{exp} - 10 min ≥ 20 min (S = 0.12 ± 0.10 cm/hr and I = 0.077 +0.070/-0.085), we determined P_{cw} = 0.22 (± 0.15) cm/hr and $K_{cw} L_c$ = 0.90 (+0.44/-0.57) cm using Eqs. (3.11) and (3.12). To determine the indicated confidence intervals, we stochastically generated the mean and distribution functions for P_{cw} and ($K_{cw} L_c$) separately for each animal using Crystal Ball (Decisioneering, 1993) and assuming normal distribution functions for S and I (5000 trials for each animal). We then superimposed these distribution functions for P_{cw} and ($K_{cw} L_c$) to determine the mean values and 95% confidence limits.

Using P_{cw} = 0.22 and $K_{cw} L_c$ = 0.90 cm, we estimate that M_{in}(at t_{exp} = 10 min)/(V C_w^o) ≈ 0.10, which is more than 1/3 of the total amount absorbed in the entire 70 min exposure (i.e., M_{in}(at t_{exp} = 10 min)/(V C_w^o) ≈ 0.30). The 0.37 (± 0.13) cm/hr value calculated by Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994) is almost two times larger and significantly different than the P_{cw} value of 0.22 (± 0.15) cm/hr, calculated here

Table 3.6a Slope (S), Intercept (I), P_{cw} and L_cK_{cw} for Different Regressions to the Mean Percutaneous Absorption (for 5 Animals) of PCE in the Hairless Guinea Pig

Steady-State Points	Slope (S) (hr^{-1}) Mean	± 95% Confidence	Intercept (I) Mean	± 95% Confidence
8*	0.2301	0.0395	0	0
8	0.1869	0.0489	0.0305	0.0275
7	0.1646	0.0392	0.0463	0.0236
6	0.1495	0.0423	0.0576	0.0275
5	0.1248	0.0264	0.0768	0.0187
4	0.1187	0.0561	0.0818	0.0437
3	0.0988	0.1756	0.0995	0.1483
2	0.1228	0	0.0769	0

Table 3.6b

Steady-State Points	$M_{in}/(VC_w)$ (at t_{exp}=10)	Method A[a] Permeability (cm/hr) Mean	± 95%	Method B[b] Permeability (cm/hr) Mean	± 95%	Method B[b] L_cK_{cw} (cm) Mean	± 95%
8*	N/A	0.4524	0.0777	N/A[c]	N/A[c]	N/A[c]	N/A[c]
8	N/A	0.3676	0.0962	N/A[c]	N/A[c]	N/A[c]	N/A[c]
7	0.108	0.3237	0.0771	0.2886	0.067	0.7364	0.123
6	0.108	0.2940	0.0833	0.2622	0.072	0.8081	0.143
5	0.105	0.2455	0.0520	0.2195	0.045	0.9151	0.098
4	0.104	0.2335	0.1103	0.2091	0.097	0.9407	0.225
3	0.099	0.1944	0.3454	0.1750	0.304	1.0268	0.775
2	0.104	0.2415	N/A	0.2163	N/A	0.9120	N/A

* Zero intercept forced

[a] $P_{cw} = (V/A)*S$ (note that average V/A = 1.97 cm). M_{in}(at t_{exp}=10 min)/(VC_w) and B are assumed zero

[b] In this method B is assumed to be zero and M_{in}(at t_{exp}=10 min)/(VC_w) is calculated with Eq. (3.9) and then permeability and L_cK_{cw} are calculated from Eqs. (3.11) and (3.12).

[c] No unsteady-state data so L_cK_{cw} which is calculated from unsteady-state data can not be calculated

Table 3.7a Slope (S), Intercept (I), P_{cw} and L_cK_{cw} for PCE Absorption into each Guinea Pig Assuming Five Steady-State Data Points (i.e., 10 min < t* - 10 min ≤ 20 min)

Guinea Pig Number**	Slope (S) (hr^{-1})		Intercept (I)	
	Mean	± 95%	Mean	± 95%
1[a]	0.1290	0.0900	0.1033	0.0636
2[b]	0.1384	0.0783	0.0262	0.0553
3[c]	0.1178	0.0562	0.0922	0.0397
4[d]	0.1294	0.1154	0.0766	0.0816
5[e]	0.1091	0.0367	0.0856	0.0259

Table 3.7b

Guinea Pig Number**	$M_{in}/(VC_w)$ (at t_{exp} = 10 min)	Permeability* (cm/hr)		L_cK_{cw}* (cm)	
		Mean	± 95%	Mean	± 95%
1[a]	0.1170	0.2239	0.154	1.1160	0.328
2[b]	0.0835	0.2494	0.138	0.5094	0.294
3[c]	0.1074	0.2068	0.097	1.0152	0.207
4[d]	0.1073	0.2272	0.200	0.9227	0.426
5[e]	0.1003	0.1929	0.064	0.9491	0.135

* In this method B is assumed to be zero and M_{in}(at t_{exp}=10 min)/(VC_w) is calculated with Eq. (3.9) and then permeability and L_cK_{cw} are calculated from Eqs. (3.11) and (3.12).
** Designated in Figure 5 of Bogen *et al.* (Bogen *et al.*, 1994) as:
[a] Open circle [b] Solid circle [c] Open square [d] Solid square [e] Open triangle

Table 3.8a,b Slope (S), Intercept (I), P_{cw} and L_cK_{cw} for PCE Absorption into each Guinea Pig Assuming Seven Steady-State Data Points (i.e., 0 min < t* - 10 min ≤ 5 min)

Experiment Number	Slope (S) (hr^{-1}) Mean	± 95%	Intercept (I) Mean	± 95%
1[a]	0.1892	0.0698	0.0570	0.0420
2[b]	0.1697	0.0438	0.0025	0.0264
3[c]	0.1474	0.0367	0.0696	0.0221
4[d]	0.1651	0.0563	0.0493	0.0339
5[e]	0.1521	0.0576	0.0532	0.0347

Table 3.8b

Experiment Number	$M_{in}/(VC_w)$ (at t_{exp} = 10 min)	Permeability* (cm/hr) Mean	± 95%	L_cK_{cw}* (cm) Mean	± 95%
1[a]	0.1244	0.3256	0.1185	0.8654	0.2160
2[b]	0.0614	0.3128	0.0800	0.2198	0.1460
3[c]	0.1128	0.2571	0.0630	0.9010	0.1155
4[d]	0.1100	0.2888	0.0974	0.7629	0.1775
5[e]	0.1066	0.2671	0.1000	0.7755	0.1826

* In this method B is assumed to be zero and M_{in}(at t_{exp}=10 min)/(VC_w) is calculated with Eq. (3.9) and then permeability and L_cK_{cw} are calculated from Eqs. (3.11) and (3.12).
** Designated in Figure 5 of Bogen *et al.* (Bogen *et al.*, 1994) as:
[a] Open circle [b] Solid circle [c] Open square [d] Solid square [e] Open triangle.

Table 3.9 Slope (S), Intercept (I) and Permeability Coefficients for PCE Absorption into each Guinea Pig Assuming Eight Steady-State Data Points (i.e., t* < 10 min)

Experiment Number**	Slope (S) (hr^{-1})		Intercept* (I)		Permeability* (cm/hr)	
	Mean	± 95%	Mean	± 95%	Mean	± 95%
1[a]	0.2166	0.0712	0.0376	0.0401	0.4259	0.1399
2[b]	0.1707	0.0331	0.0016	0.0186	0.3356	0.0651
3[c]	0.1810	0.0648	0.0459	0.0365	0.3557	0.1274
4[d]	0.1888	0.0593	0.0325	0.0334	0.3712	0.1166
5[e]	0.1777	0.0623	0.0350	0.0351	0.3493	0.1225

* In this method B and M_{in}(at t_{exp} = 10 min)/(VC_w) are assumed to be zero.
** Designated in Figure 5 of Bogen *et al.* (Bogen *et al.*, 1994) as:
[a] Open circle [b] Solid circle [c] Open square [d] Solid square [e] Open triangle

Table 3.10 Slopes (S) and P_{cw} Calculated for PCE Absorption into each Guinea Pig Assuming Eight Steady-State Data Points (i.e., t* < 10 min) and Forcing I = 0

Experiment Number**	Slope (S) (hr^{-1})		Permeability* (cm/hr)	
	Mean	± 95%	Mean	± 95%
1[a]	0.2698	0.0529	0.5304	0.1040
2[b]	0.1730	0.0180	0.3401	0.0354
3[c]	0.2458	0.0564	0.4832	0.1109
4[d]	0.2347	0.0448	0.4614	0.0881
5[e]	0.2272	0.0477	0.4467	0.0938

* In this method B and M_{in}(at t_{exp} = 10 min)/(VC_w) are assumed to be zero.
** Designated in Figure 5 of Bogen *et al.* (Bogen *et al.*, 1994) as:
[a] Open circle [b] Solid circle [c] Open square [d] Solid square [e] Open triangle

using data from $t_{exp} \geq 30$ min and correcting for absorption during the first 10 min of exposure. Calculating permeability coefficients from direct measurements of absorption which include unsteady-state data always overestimates the true permeability.

We have also examined the CF and TCE data and determined that, for these compounds (MW = 119.4 and 131.4, respectively for CF and TCE, compared to 165.8 for PCE), t* for the guinea pig is approximately equal to or less than the time of their first data point at 10 min. Tables 3.11 and 3.12 contain slopes and intercepts from regression analysis of CF dermal absorption. The results in Table 3.11 are based on linear regression on the mean values of CF absorption into all 6 hairless guinea pigs at each time point. The slopes calculated when 2 through 8 data are considered steady state do not differ significantly. Regression following the strategy referred to earlier as *b* was also applied to the individual animals assuming 8 steady-state data points. These results are summarized in Table 3.12. Tables 3.13 and 3.14 contain slopes and intercepts from the analysis of TCE dermal absorption. The results in Table 3.13 are based on linear regression on the mean values of TCE absorption into all 5 hairless guinea pigs at each time point. The slopes from regressions where 2-8 data points are considered steady state do not differ significantly. Regression following the strategy referred to earlier as *b* was also applied to the individual animals assuming 8 steady-state data points. These results are summarized in Table 3.14.

Some negative intercepts were found when the *b* regression strategy was applied to absorption data for the separate animals, supporting our belief that steady state was nearly attained within the first 10 min of exposure. When the PCE data was analyzed with the *b* regression strategy, intercepts for all five animals were positive, indicating that steady state probably was not attained within the first 10 min.

The observed transition times (t*) for CF and TCE are reasonably consistent with transition times calculated using the observed transition time for PCE and an intuitive

Table 3.11 Slopes and Intercepts for Linear Regressions to the Mean Value (Average over 5 Animals) of Absorption of CF

Datapoints[a]	Slope		Intercept	
	Mean	95%	Mean	95%
8[b]	0.0898	0.0112	0	N/A
8	0.0959	0.0191	-0.0042	0.0107
7	0.0989	0.0243	-0.0064	0.0146
6	0.0905	0.0280	-0.0001	0.0182
5	0.0860	0.0473	0.0034	0.0335
4	0.1056	0.0719	-0.0130	0.0555
3	0.1330	0.1541	-0.0373	0.1300
2	0.1120	N/A	-0.0175	N/A

[a] Number of points included in the regression

[b] Intercept zeroed

Table 3.12 Slopes and Intercepts from Linear Regression Analysis (all 8 points included) of CF Absorption into Six Guinea Pigs

	Slope		Intercept	
Animal	Mean	95%	Mean	95%
1	0.0944	0.0757	0.0074	0.0426
2	0.1259	0.0597	-0.0165	0.0336
3	0.1684	0.0605	-0.0165	0.0340
4	0.0601	0.0476	0.0096	0.0268
5	0.0822	0.0423	-0.0049	0.0241
6	0.0432	0.0375	-0.0052	0.0211

Table 3.13 Slopes and Intercepts for Linear Regressions to the Mean Value (Average over 5 Animals) of Absorption of TCE

Datapoints[a]	Slope		Intercept	
	Mean	95%	Mean	95%
8[b]	0.1025	0.0064	0	N/A
8	0.1010	0.0116	0.0011	0.0065
7	0.1001	0.0153	0.0017	0.0092
6	0.1063	0.0157	-0.0029	0.0101
5	0.1088	0.0265	-0.0049	0.0187
4	0.1181	0.0475	-0.0126	0.0368
3	0.1218	0.3081	-0.0159	0.2602
2	0.0798	N/A	0.0238	N/A

[a] Number of points included in the regression

[b] Intercept zeroed

Table 3.14 Slopes and Intercepts from Linear Regression Analysis (all 8 points included) of TCE Absorption into Five Guinea Pigs

	Slope		Intercept	
Animal	Mean	95%	Mean	95%
1	0.1081	0.0230	0.0173	0.0130
2	0.0584	0.0426	-0.0224	0.0239
3	0.0613	0.0234	0.0068	0.0131
4	0.0889	0.0343	0.0149	0.0192
5	0.1880	0.0349	-0.0107	0.0196

method for extrapolating transition times for chemicals with different MW. Assuming t* for PCE is 20 to 30 min and adjusting the SC diffusion coefficient for MW using a proposed separation of the Potts and Guy permeability correlation (Cleek and Bunge, 1993) we estimate that t* is between 10 and 16 min for CF and between 12 and 18 min for TCE. Although the Potts and Guy permeability correlation is for human rather than guinea pig stratum corneum, significant differences in the MW dependency are not expected. However, the Potts and Guy permeability correlation was developed from a database of hydrocarbons (with only a few halogenated chemicals included) and so the transition time (t*) estimated for halogenated chemicals might be smaller than predicted. That is, halogenated chemicals have a higher ratio of MW/molecular volume than unhalogenated compounds, so, correlations involving MW, that were developed from primarily unhalogenated hydrocarbon data, calculate properties (e.g., transition times) for halogenated chemicals assuming molecular volumes that are larger than actual.

Figure 3.2 shows the residuals calculated when the mean mass of CF (Fig. 3.2A), TCE (Fig. 3.2B), and PCE (Fig. 3.2C) (normalized with respect to the mass available for absorption 10 min after exposure began) was linearly regressed, assuming that steady state was reached prior to the first time point (i.e., $t^* - 10 \text{ min} \leq 0$) and allowing for a variable intercept. Residuals for CF and TCE show the randomness that is expected for residuals steady-state dermal absorption measurements plotted as a function of time. For PCE relatively rapid short time absorption rates and relatively slower long-time absorption rates apparently indicate that the early-time PCE data is unsteady-state.

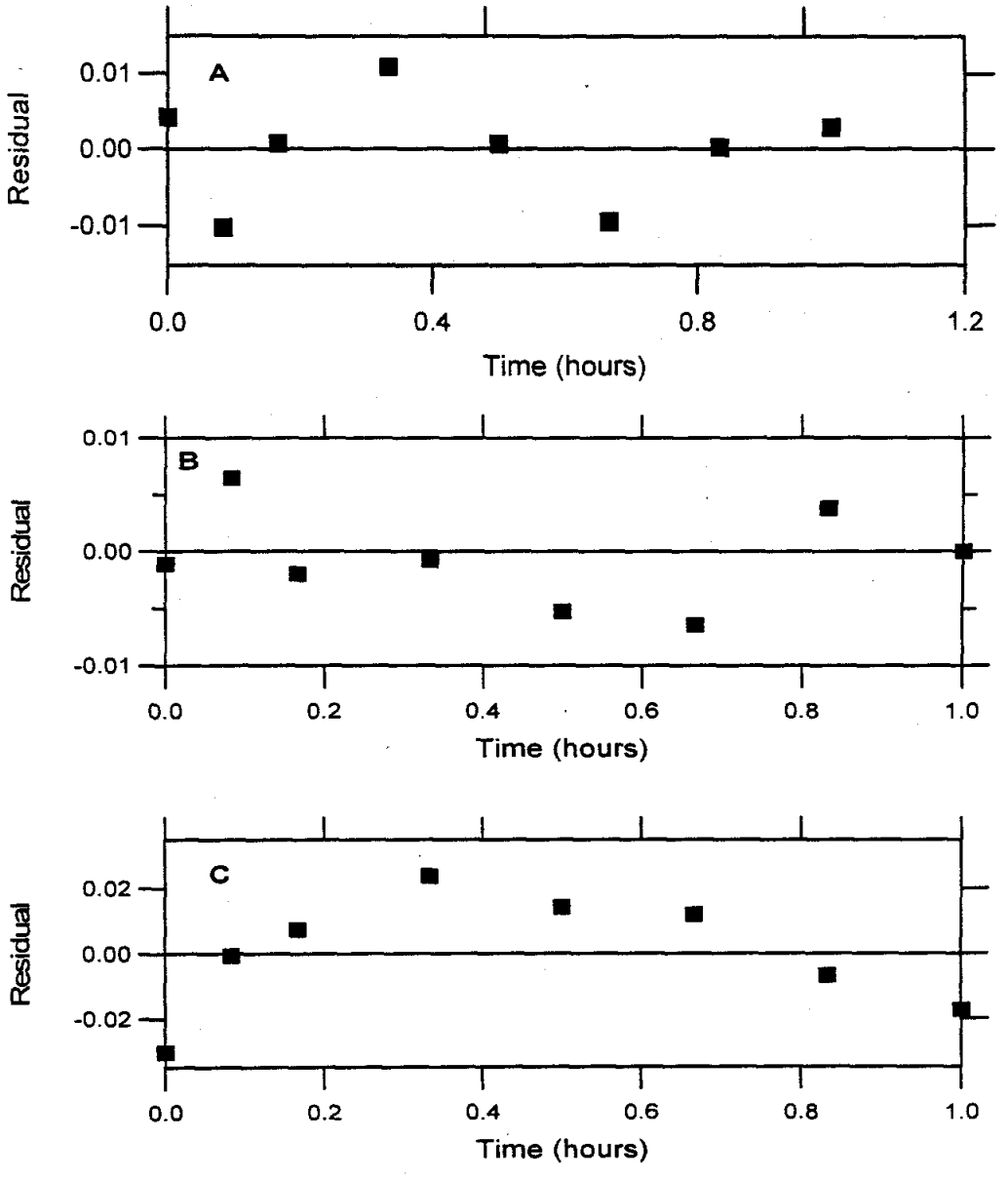

Figure 3.2 Residuals between the mean mass (normalized with respect to the mass available for absorption 10 min after the exposure began) of (A) CF, (B) TCE, and (C) PCE absorbed into five or six (for CF) hairless guinea pigs and a linear regression to all eight datapoints allowing for a nonzero intercept.

3.6. Conclusions

For comparing absorption of different chemicals or the same chemical under different conditions, permeability coefficients are most meaningful when derived from steady-state data. In analyzing their data for *in vivo* dermal absorption of CF, TCE and PCE into hairless guinea pigs, Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994) assumed that steady state had been reached in less than 10 min. In a more detailed analysis, we have shown that this assumption may be reasonable for CF and TCE but probably was not for PCE. We estimate that 20-30 minutes were required for the absorption of PCE to become steady state. The permeability coefficient calculated for PCE when only the data from $t_{exp} \geq 30$ min are used and considering the amount of absorption which occurred during the first 10 min of exposure is 0.22 (±0.15) cm/hr. This is almost two times larger and significantly different from the permeability coefficient of 0.37 (±0.13) cm/hr reported by Bogen *et al.* (Bogen *et al.*, 1992; Bogen *et al.*, 1994). These results are consistent with the theoretical expectation that analysis of dermal uptake data which include unsteady-state values should produce estimates of the permeability coefficient that are larger than the steady-state value.

3.7. Notation

A = Surface area of chemical exposure
B = A parameter measuring the SC/VE permeability ratio
C_v^0 = Concentration of the absorbing chemical in the vehicle; assumed to remain constant during the exposure period, t_{exp}
C_w^0 = Aqueous concentration of the absorbing chemical; assumed to remain constant during the exposure period, t_{exp}
D_c = Effective diffusivity of the absorbing chemical in the SC
VE = Viable epidermis
I = Intercept defined by Eq. (3.6)
K_{cv} = Equilibrium partition coefficient between the SC and vehicle for the absorbing chemical
K_{cw} = Equilibrium partition coefficient between the SC and water for the absorbing chemical
L_c = Effective thickness of the SC
M_{in} = Cumulative mass absorbed into the SC during an exposure period, t_{exp}
MW = Molecular weight of the absorbing chemical
P_{cv} = Steady-state permeability of the SC from a specified vehicle
P_{cw} = Steady-state permeability of the SC from water
P_{ev} = Steady-state permeability of the VE from a specified vehicle
P_{ew} = Steady-state permeability of the VE from water
P_v = Steady-state permeability of the SC-VE composite membrane from a specified vehicle
P_w = Steady-state permeability of the SC-VE composite membrane from water
S = Slope defined by Eq. (3.5)
S_{cc} = Slope in regression of chemical depletion data in chamber control experiment
S_{de} = Slope in regression of chemical depletion data in dermal exposure experiment
SC = Stratum corneum
t^* = Time to approximately reach steady state
t_{exp} = Time of exposure to absorbing compound
$t_{lag,c}$ = Lag time across the SC, equals $D_c/(6L_c^2)$
V_o = Initial volume of dermal exposure chamber

Superscripts
uss = Unsteady-state period of absorption
∞ = Steady-state period of absorption

3.8. References

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4. A COMPARISON OF SIXTEEN SKIN PERMEABILITY CORRELATIONS WITH THE FLYNN PERMEABILITY DATABASE

4.1. Abstract

Risk assessment requires an estimate of the stratum corneum permeability coefficient for compounds that have not been measured or have been measured unsatisfactorily. Usually these estimates are based on correlations developed from known permeability coefficients for compounds that have been previously measured. Sixteen correlations, which predict skin permeability coefficients based on the molecular weight (MW) and octanol-water partition coefficient (K_{ow}) of the penetrating molecule, were compared against the well known Flynn database. Correlations developed from a diverse group of compounds are superior at prediction than those developed from compounds of very similar structure and properties. This analysis provides a better understanding of the limitations of predictive stratum corneum permeability coefficient correlations and should guide risk assessors and others in deciding among the many correlations available for estimating permeability coefficients. Additionally, this study indicates that permeability coefficients can be more accurately estimated if correlations are based on validated measurements. Data validation criteria identified as important include fraction unionized, temperature, and type of skin.

4.2. Introduction

Contaminated terrestrial waters and chemically disinfected waters frequently contact human skin with the potential for waterborne contaminants (environmental contaminants and disinfection byproducts, respectively) to be dermally absorbed.

Reasonably estimating the amount of chemical absorbed by the skin is important for determining safe levels of contamination.

The stratum corneum (SC) permeability coefficient is one of the key parameters for estimating dermal absorption. Fortunately, permeability coefficients for many compounds have been measured from aqueous solution, and informed decisions about dermal uptake can now be made. When decisive experimental measurements are not available, predictive correlations provide a reasonable substitute. Many correlations have been developed, but not all provide adequate estimates for all chemicals of concern. In this chapter, 16 different correlations are compared to the large collection of permeability coefficients from Flynn (Flynn, 1990).

4.3. Background

Most attempts to develop predictive correlations for permeability coefficients have recognized contributions from molecular size and from the solubility within the SC lipids. The availability, appealing physical interpretation, and relative success in describing a wide range of biological processes, make molecular weight (MW) and logarithmically-transformed octanol-water partition coefficients ($\log K_{ow}$) by far the most widely used parameters for correlating skin penetration. Correlations based on $\log K_{ow}$ alone have assumed either that the effect of MW is minor (which may be correct if the MW range of the regressed data set was narrow), or that $\log K_{ow}$ and MW are so strongly correlated that the effect of MW is represented by $\log K_{ow}$ alone. All correlations studied in this analysis predict permeability coefficients with one or more of these parameters.

One of the dominant questions facing the modeling of dermal absorption is whether hydrophilic compounds and ionized species penetrate the SC by a different pathway than uncharged lipophilic chemicals. It is widely held that lipophilic compounds partition into and penetrate through the lipid domains of the SC. A number of

investigators have examined permeability coefficient and $\log K_{ow}$ data with a more complex model in which the transport mechanism changes for penetrants of differing polarity. In particular, the apparent independence of permeability coefficients upon lipophilicity (i.e., $\log K_{ow}$) for polar compounds has led to the hypothesis that these compounds penetrate the SC by a polar pathway (Flynn, 1990; Kasting *et al.*, 1992). Some of the models examined here consider separate pathways for hydrophilic and lipophilic compounds.

4.4. Results and Discussion

4.4.1. The Correlations

Table 4.1 contains some of the published correlations for estimating permeability coefficients for chemicals penetrating human SC from aqueous vehicles, P_{cw} [cm/hr]. In some cases, the correlations have been rearranged, or are condensed (but equivalent) forms of the original. Also listed are the chemical classes and range of MW and $\log K_{ow}$ (noted as suggested range) upon which each correlation was developed.

Since most of the permeability coefficients used in developing these correlations are determined with intact epidermis (SC and viable epidermis (VE)), the correlations actually predict the permeability coefficient of the entire epidermis, P_w, which is related to the permeability coefficients through the SC (P_{cw}) and the VE (P_{ew}) as:

$$\frac{1}{P_w} = \frac{1}{P_{cw}} + \frac{1}{P_{ew}} \tag{4.1}$$

Except for very lipophilic penetrants (approximately $\log K_{ow} > 4$), the permeability coefficient through the entire epidermis (i.e., P_w) is not different from that through the SC alone (i.e., P_{cw}), so that these correlations are often considered to predict SC permeability coefficients. This issue is addressed more quantitatively in Chapter 9, where we present

Table 4.1 Predictive Permeability Coefficient Correlations for Human Skin

Model	Equation Reference	Chemical Class	Permeability Correlation [cm/hr]	Data Reference	Suggested Range
1	Abraham *et al.* (1995)	Misc (n=43)	$\log P_w = -2.184 + 0.85 \log K_{ow} - 0.012\ MW$	Not Specified[a]	Not Specified
2	Vecchia & Bunge (1996)[b]	Misc (n=170)	$\log P_w = -2.44 + 0.514 \log K_{ow} - 0.0050\ MW$	Detailed in Chapter 5	$18<MW<500$ $-1.3<\log K_{ow}<4.3$
3[c]	Bronaugh & Barton (1991)	Flynn Database (n≈90)	$\log P_w = -2.61 + 0.67 \log K_{ow} - 0.0061\ MW$	Flynn (1990)	$18<MW<765$ $-3<\log K_{ow}<6$
4	Brown & Rossi (1989)	Alkanols, phenols, drugs (n=39)[d]	$\log P_w = -1.0 + 0.75 \log K_{ow} - \log(120 + K_{ow}^{0.75})$	Scheuplein (1973), Roberts (1977), Michaels (1975)	$32<MW<765$ $-0.8<\log K_{ow}<4.6$
5	Cleek & Bunge (1993)[e]	Flynn Database (n≈90)	$\log P_{cw} = -2.8 + 0.74 \log K_{ow} - 0.006\ MW$ $P_w = \frac{P_{cw}}{1 + \frac{P_{cw}\sqrt{MW}}{2.6}}$	Flynn (1990)	$18<MW<765$ $-3<\log K_{ow}<6$
6	El Tayar *et al.* (1991)	Steroids (n=11)	$\log P_w = -5.324 + 0.80 \log K_{ow}$	Anderson *et al.* (1988)	$403<MW<503$ $1.4<\log K_{ow}<5.5$

Model	Equation Reference	Chemical Class	Permeability Correlation [cm/hr]	Data Reference	Suggested Range
7	El Tayar *et al.* (1991)	Phenolics (n=18)	$\log P_w = -5.515 + 2.39 \log K_{ow} - 0.37(\log K_{ow})^2$	Roberts *et al.* (1977)	94<MW<197 0.8<$\log K_{ow}$<3.7
8	Flynn & Amidon (in EPA, 1992)	Flynn Database (n≈90)	$\ln P_w = -3.31 + 0.79 \ln K_{ow} - 1.45 \ln MW$	Flynn (1990)	18<MW<765 -3<$\log K_{ow}$<6
9	Flynn (1990)	Flynn Database (n≈90)	For MW ≤ 150 $\log P_w = -3.0 \quad (\log K_{ow} < 0.5)$ $\log P_w = -3.5 + \log K_{ow} \quad (0.5 \le \log K_{ow} < 3)$ $\log P_w = -0.5 \quad (\log K_{ow} > 3)$ For MW > 150 $\log P_w = -5.0 \quad (\log K_{ow} < 0.5)$ $\log P_w = -5.5 + \log K_{ow} \quad (0.5 \le \log K_{ow} < 3.5)$ $\log P_w = -1.5 \quad (\log K_{ow} > 3.5)$	Flynn (1990)	18<MW<765 -3<$\log K_{ow}$<6
10	Kasting *et al.* (1992)	Misc.[f]	$P_w = \left[\frac{1}{P_{lip} + P_{pol}} + \frac{1}{P_{aq}}\right]^{-1}$ $\log P_{lip} = -2.87 + \log K_{ow} - 0.0078\ MW$ $P_{pol} = 1.0 \times 10^{-5}\sqrt{300/MW}$ $P_{aq} = 0.15\sqrt{300/MW}$	Detailed in Ref.	18<MW<518 -1.4<$\log K_{ow}$<6.3

Model	Equation Reference	Chemical Class	Permeability Correlation [cm/hr]	Data Reference	Suggested Range
11	McKone & Howd (1992)	Misc.[g] (n=51)	$\log P_w = -2.40 + \log(0.24 + 3K_{ow}^{0.8}) - 0.6 \log MW$	Detailed in Ref.	18<MW<227 $-1.4<\log K_{ow}<4.6$
12	Michaels *et al.* (1975)[h]	Drugs (n=10)	$\log P_w = -3.6 + \log K_{mw} + \log(1.16 + 3x10^{-6} K_{mw}) - \log(0.16 + 0.002 K_{mw})$ K_{mw} = mineral oil / water partition coefficient Plotted results assumed $K_{mw} \approx K_{ow}$	Michaels *et al.* (1975)	165<MW<765 $1<\log K_{ow}<4$
13	Morimoto *et al.* (1992)	Drugs (n=16)	$P_w = 4.21x10^{-4} K_{ow}^{0.75} + 9.83x10^{-5}$	Morimoto *et al.* (1992)	130<MW<358 $-4.7<\log K_{ow}<4.0$
14	Potts & Guy (1992)	alkanols, acids, diols (n=23)	$\log P_w = -2.24 + 0.81 \log K_{ow} - 0.013 MW$	Scheuplein & Blank (1967)	18<MW<160 $-1.4<\log K_{ow}<3.0$
15	Potts & Guy (1992)	Flynn Database (n≈90)	$\log P_w = -2.74 + 0.71 \log K_{ow} - 0.0061 MW$	Flynn (1990)	18<MW<765 $-3<\log K_{ow}<6$
16	Siddiqui *et al.* (1989)	Steroids (n=7)	$\log P_w = -6.66 + 1.05 \log K_{ow}$	Siddiqui *et al.* (1989)	288<MW<476 $1.0<\log K_{ow}<3.5$

[a] Although not directly specified, there is some indication that this data is largely composed of the alkanols, alkanoic acids and alkanediols of Scheuplein and Blank (1967) and the phenols reported by Roberts *et al.* (1977).

[b] See also Chapter 5, Eq. 5.28.

[c] The Bronaugh and Barton model (Model 3) is essentially identical to the Potts and Guy model (Model 15).

[d] Data adjusted to 31°C by assuming the permeability coefficient doubles for every 10 °C increase in temperature.

[e] Modification of Model 15.

[f] This database of more than 130 permeability measurements shares some permeability measurements with the Flynn database. Most of the additional values were measured either with shed snake skin as the membrane (13 values), or with human skin from the vehicle propylene glycol (78 values).

[g] Includes many of the permeability measurements for chemicals of MW < 227 tabulated in the Flynn database. *In vivo* guinea pig permeability measurements for 3 chemicals were also included. This equation ignores blood flow resistances. An SC thickness of 25μm is assumed, per the recommendation of the authors.

[h] Lipophilicity is represented with the mineral oil-water partition coefficient (K_{mw}) rather than the more conventional $\log K_{ow}$. For unknown reasons, these two measures of lipophilicity are poorly correlated for these ten chemicals. However, $\log K_{ow}$ is higher on average and the permeability is overestimated when K_{ow} is substituted for K_{mw}.

measured ratios of P_{cw}/P_{ew} and develop predictive correlations based on the lipophilicity of the penetrating chemical. One of the models in Table 4.1 (Model 5), explicitly incorporates the resistance due to the VE tissues (Cleek and Bunge, 1993) by incorporating the ratio of SC to VE permeability coefficients, called B (i.e., $B = P_{cw}/P_{ew}$). Occasionally permeability coefficients are measured with the dermis present, which presents an additional barrier to the penetration of lipophilic compounds. This barrier may effect *in vitro* penetration of the more lipophilic chemicals but probably does not contribute during *in vivo* absorption where the dermis layer is well perfused with capillaries.

All of the correlations examined in this work consist of parameters developed from experimental measurements. That is, the correlations are empirical in the sense that the coefficients were determined through regression of experimental data. Nevertheless, some permeability coefficient models are appropriately termed semi-theoretical, because the chosen functional form of their parameters conforms with a theoretical model. For example, the Potts and Guy correlation (Model 15) was developed assuming a theoretically expected exponential dependency upon molecular size as represented by MW. By contrast, Model 8 (US EPA, 1992) assumed that permeability varies linearly with molecular size (i.e., MW). Consequently, calculations of permeability using Models 8 and 15 over a wide range of molecular size give very different results. Unlike the other models in Table 4.1, Model 7 assumes that permeability varies with $(\log K_{ow})^2$.

Figures 4.1 - 4.3 compare the correlations of Table 4.1 (evaluated at MW ≈ 100, 300, and 500, respectively) with experimental data (Flynn, 1990) at comparable MW ranges: 50 > MW > 150, 250 > MW > 350, and 450 > MW > 550, respectively. The $\log K_{ow}$ values presented by Flynn (Flynn, 1990) were used to correlate the experimental data. Figure 4.1 clearly illustrates, with some notable exceptions, that most of the 16 correlations are adequate at predicting the available skin permeability coefficient data for

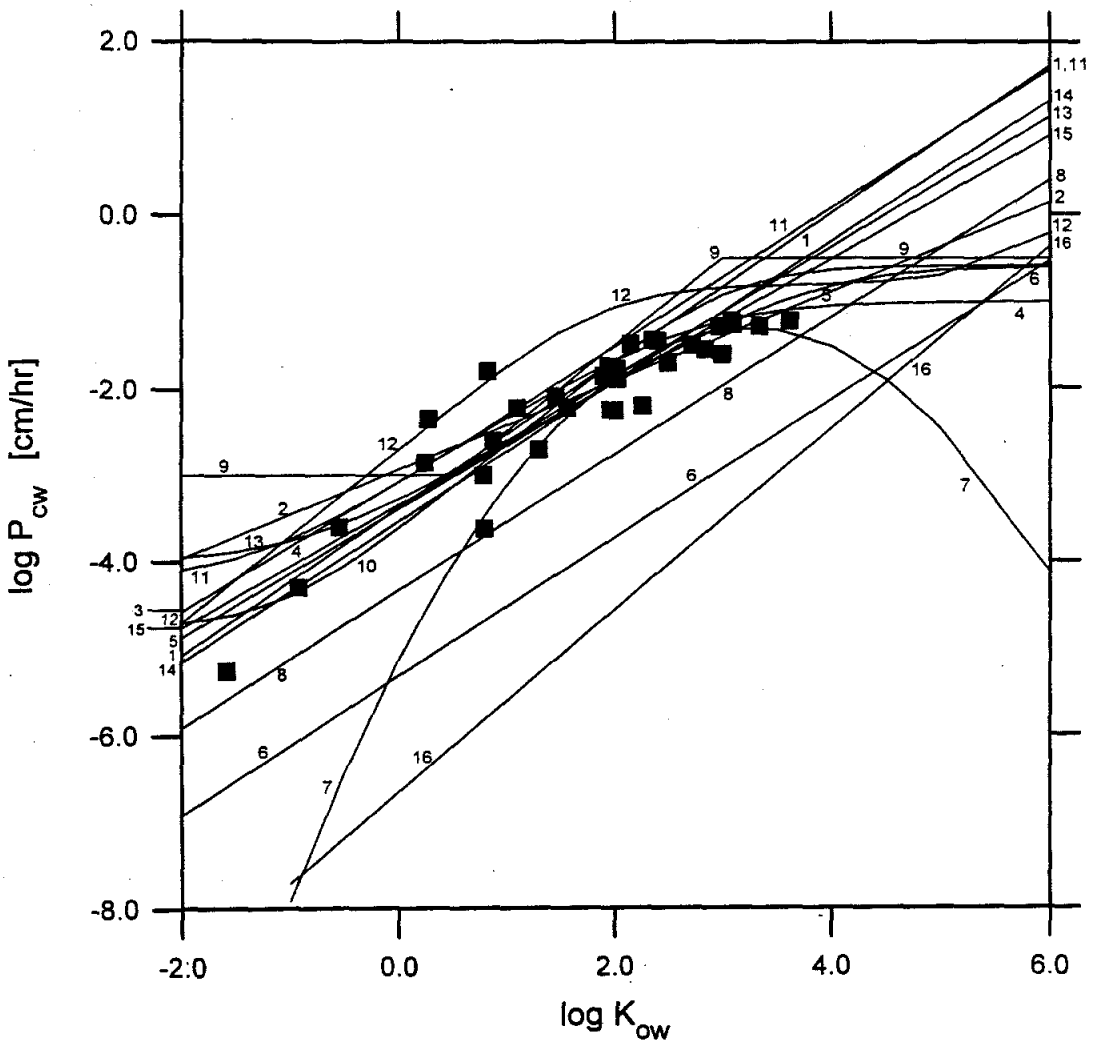

Figure 4.1 Permeability coefficient correlations assuming MW = 100 compared to experimental data from the Flynn permeability coefficient database for chemicals of 50 < MW < 150.

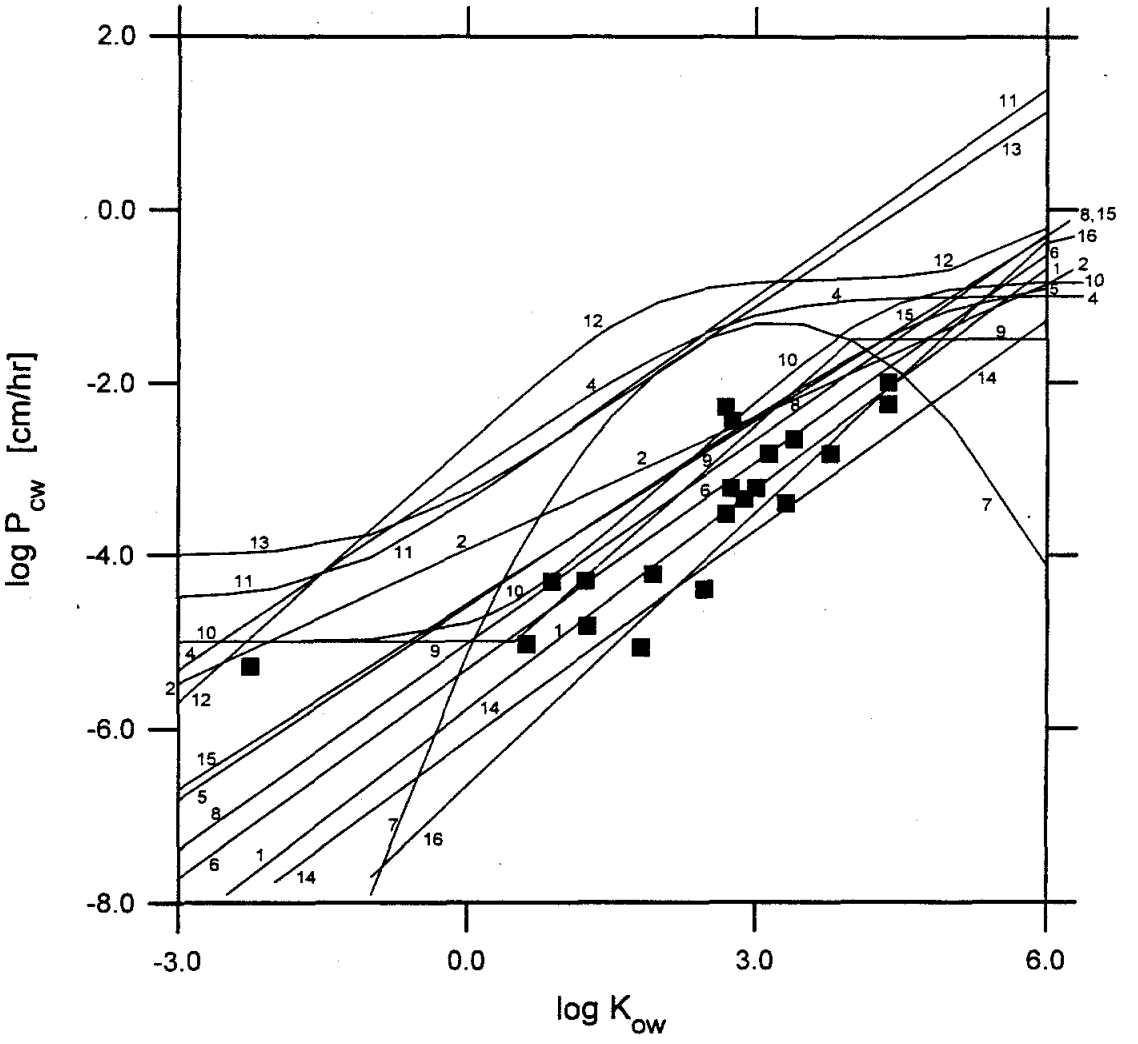

Figure 4.2 Permeability coefficient correlations assuming MW = 300 compared to experimental data from the Flynn permeability coefficient database for chemicals of 250 < MW < 350.

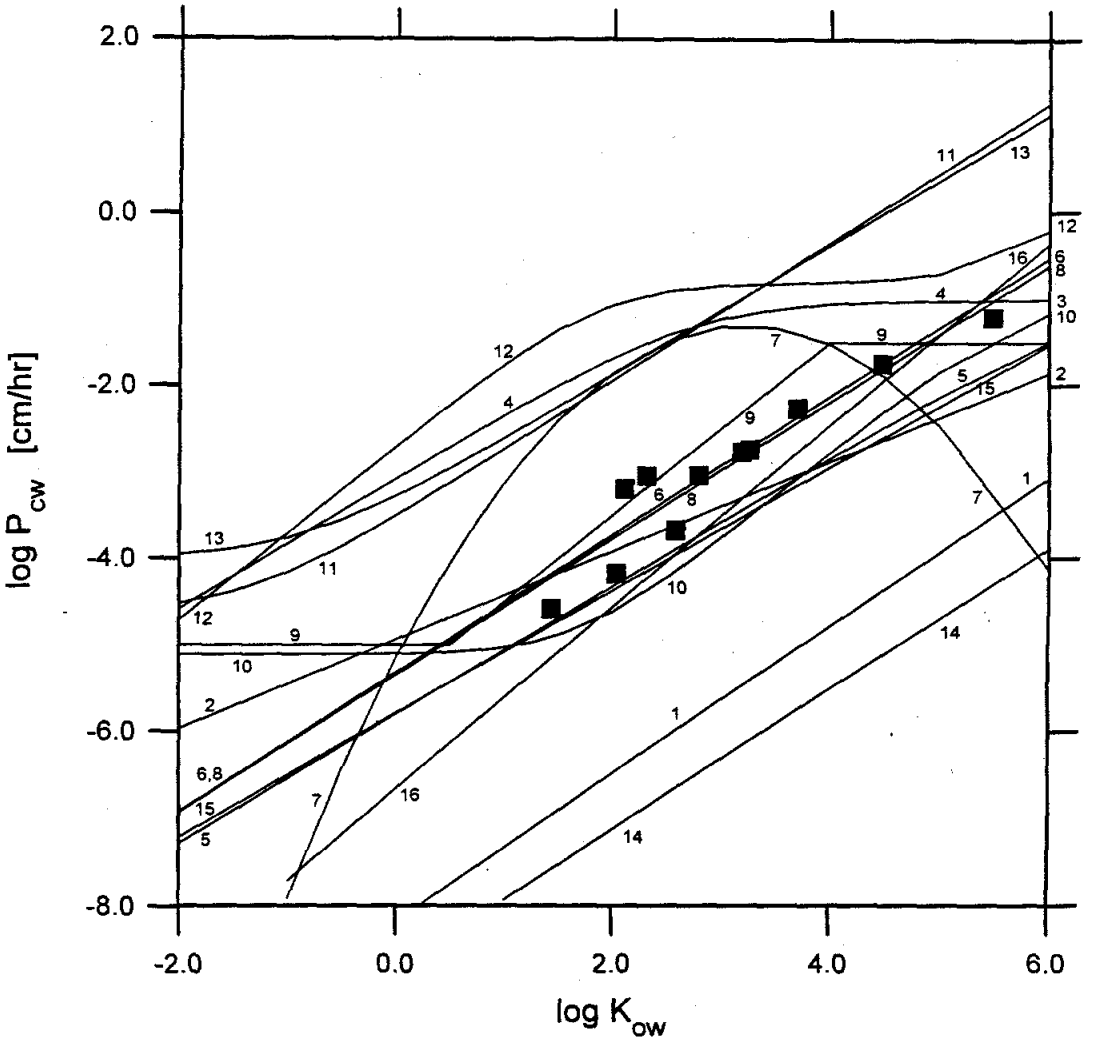

Figure 4.3 Permeability coefficient correlations assuming MW = 500 compared to experimental data from the Flynn permeability coefficient database for chemicals of 450 < MW < 550.

chemicals of low MW. Though the permeability coefficient correlations displayed in Fig. 4.1 are in relative agreement, the data uncertainty is nearly three orders of magnitude. It will be shown later that a portion of this variability can be explained in terms of operational variables (temperature, state of ionization, etc.) which effect the measured permeability coefficient. The remaining residual either indicates that lipophilicity ($\log K_{ow}$) and MW are not able to model the important aspects of skin permeability coefficients, or that experimental uncertainties and individual skin variability limit the prediction of skin permeability coefficients. Figure 4.2 shows analogous results, although the uncertainty in the data and the discrepancy between correlations have increased.

At the highest MW, Figure 4.3 shows that most of the correlations more poorly describe the data, primarily because the datasets on which many of these correlations were developed included only a few or no chemicals with a MW this high. Because it was derived from similar chemicals, the El Tayar model (Model 6) based on the hydrocortisone-21-yl-esters reasonably describes these data. This figure dramatically shows the adverse effects of extrapolating permeability coefficient correlations outside the range of the training database.

Based on the results in Figures 4.1-4.3, we are able to make the following observations: (1) many of the correlations predict a similar dependence on K_{ow}; (2) the distinctive differences between correlations are in their prediction of the effect of MW; (3) nearly all of the correlations reasonably predict the experimental data for chemicals of MW $\approx$ 100 (exceptions are Models 6, 7 and 16); (4) differences between correlations and between predicted and experimental values increase with the MW of the penetrating chemical; (5) some of the correlations in Table 4.1 were developed from data with a limited range of properties, and, not surprisingly, those correlations do not perform well when used outside of that range; examples of this are Models 11, 14, and 16. Models 11

and 14 were developed for chemicals with low MW (and therefore, perform well at low MW and more poorly at higher MW). Model 16 was developed for high MW chemicals (and therefore, performs well at high MW but more poorly at lower MW).

4.4.2. The Flynn Database

The Flynn database (Flynn, 1990) has been used so extensively in the development of permeability coefficient correlations that many of its features are common knowledge. Flynn assembled 97 human skin permeability coefficients for 94 compounds with a relatively broad range of properties ($18 < MW < 765$) and ($-3 < \log K_{ow} < 6$). It is important to recognize that only a few data are available for chemicals at the extremes of $\log K_{ow}$ or high MW. The database consisted of measured permeation, primarily *in vitro*, from (mostly) aqueous solutions. Because of its size and diversity, this database has been widely used in developing permeability coefficient correlations. Five of the correlations listed in Table 4.1 were developed from the Flynn database (i.e., Model 3, 5, 8, 9, and 15) with subtle changes in the form of equations or slightly different $\log K_{ow}$ values. Three of the correlations in Table 4.1 (i.e., Models 6, 7, and 12 among others) are based on subsets of the Flynn database.

Though consisting of a relatively diverse set of toxic and pharmacological compounds, the Flynn database illustrates that hydrophilic chemicals have been much less studied than lipophilic chemicals, perhaps because hydrophilic chemicals penetrate more slowly than lipophilic chemicals of similar size. Of the 97 assembled permeability coefficients for 94 different chemicals, only nine compounds (2,3-butanediol, ethanol, 2-ethoxy ethanol, methanol, N-nitrosodiethanolamine, ouabain, sucrose, scopolamine, water) have $\log K_{ow} \leq 0.0$ and only four (N-nitrosodiethanolamine, ouabain, sucrose, water) of those are at $\log K_{ow} \leq -1$. More data needs to be collected so that penetration of ionic and hydrophilic chemicals can be better quantified.

Appendix 4A contains the Flynn database, along with MW, $\log K_{ow}$, the temperature the permeability coefficient was determined at, the fraction unionized, and the permeability coefficient predicted by Model 15. Two sets of $\log K_{ow}$ values were given, Set A and Set B. The Set A values of $\log K_{ow}$, most of which were tabulated by Flynn (Flynn, 1990) and reprinted exactly by US EPA (US EPA, 1992), were used in developing several of the permeability coefficient correlations based on the Flynn database. Flynn (Flynn, 1990) did not list $\log K_{ow}$ values for four chemicals (chlorpheniramine, diethylcarbamazine, N-nitrosodiethanolamine, and ouabain) and so Set B values were used for these chemicals in analysis, unless specified otherwise. To indicate that these values were not provided in the original Flynn database we enclose the $\log K_{ow}$ values for these four chemicals in parenthesis (e.g., for chlorpheniramine (3.39)). The Set B $\log K_{ow}$ values are from the StarList of Hansch *et al.* (Hansch *et al.*, 1995) unless contained in brackets (e.g., for chloroxylenol [3.48]), in which case they were calculated using Daylight (PCModels, 1995). A StarList or calculated value for $\log K_{ow}$ was not obtained for 17-hydroxypregnenolone.

The fraction of the penetrating compound that was unionized was computed by SPARC (SPARC, 1995). The program SPARC [SPARC Performs Automated Reasoning in Chemistry] is an expert system for the estimation of chemical and physical reactivity; its computational algorithms are based on considerations of molecular structure that are arrived at using the reasoning process that an expert chemist might apply in reactivity analysis. The computational approaches in SPARC are a blending of conventional linear free-energy theory (LFET) and perturbed molecular orbital (PMO) methods. In general, SPARC utilizes LFET to compute thermodynamic properties and PMO theory to describe quantum effects such as delocalization energies or polarizabilities of π-electrons. SPARC-calculated and IUPAC pK_a values for more than 3500 different compounds have been calculated and compared. For this statistical comparison, the regression coefficient,

r^2 was 0.994 and the root mean square (RMSE) was 0.37 (Hilal *et al.*, 1995). These statistics indicate a high level of predictive power.

The Flynn database permeability coefficients are compared with the Potts & Guy correlation (Model 15) in Fig. 4.4. The dashed, upper and lower horizontal lines distinguish those permeability coefficients which are under-estimated or over-estimated by more than an order of magnitude (i.e., factor of ten). This plot does not indicate great correlation (approximately 3 orders of magnitude discrepancy between data and prediction), but is an improvement over the discrepancy between data and correlations (approximately six orders of magnitude) shown in Fig. 4.3.

The Flynn database includes three *in vivo* measurements (ethylbenzene, styrene, and toluene from Dutkiewicz and Tyras (Dutkiewicz and Tyras, 1967; Dutkiewicz and Tyras, 1969)) which are designated in Fig. 4.4 by stars. These measurements consistently exceed the predictions. The fact that the measurements are high is expected from the experimental protocol and the procedure for calculating permeability from the absorption data. The measurements were made on hands and palmar skin is more permeable than abdominal, forearm, back or forehead skin (Scheuplein and Blank, 1971). Permeability coefficients were calculated from loss of chemical in the vehicle before steady state was established. This also should overestimate the steady-state permeability coefficient, which correlations such as Model 15 are meant to approximate. Finally, their permeability coefficients were calculated using the vehicle concentration at the end of the experiment.

Permeability coefficients of several compounds are distinguished in Figure 4.4. The measured permeability coefficient of hydrocortisone (Hadgraft and Ridout, 1987) determined from a 5% ethanol vehicle (ethanol can enhance penetration (Roberts and Anderson, 1975)), and the permeability coefficient of naproxen (Chowhan and Pritchard, 1978) determined from an aqueous gel vehicle, may not be representative of the

exclusively aqueous vehicles. The permeability coefficient of etorphine (Jolicoeur *et al.*, 1992) is questionable since it is much larger than permeability coefficients measured for compounds with very similar structure and size (see (Jolicoeur *et al.*, 1992)). Also, the permeability coefficient measured for etorphine using hairless mouse skin was much more consistent with the data from these related chemicals, suggesting the human skin measurement may not be reliable. The permeability coefficient of digitoxin (Michaels *et al.*, 1975) is distinguished because digitoxin is the largest molecule in the database (MW = 765) and is expected to require the longest time to reach steady state. Unfortunately, since an exposure time was not reported in the investigation, we can not assess whether steady state was attained.

Finally, the permeability coefficients of fluocinonide and sucrose (Anderson *et al.*, 1988) were excluded based on the recommendation of Anderson (Anderson, 1995). Anderson noted a discrepancy between the permeability coefficient of fluocinonide in the experimental notebook (1.7×10^{-2} cm/hr) and that reported in their paper (1.7×10^{-3} cm/hr) (Anderson *et al.*, 1988). The correct value has not been decisively resolved. Anderson also suggested excluding their reported permeability coefficient for sucrose. He suggested that subsequent studies by Peck (Peck, 1996) explored the permeability of polar penetrants in much greater depth and solved some of the problems leading to variability in permeability coefficients for these compounds.

Figure 4.4 also compares the permeability coefficients of ionized and unionized compounds. It has been repeatedly verified (e.g., (Michaels *et al.*, 1975; Roy and Flynn, slower rate than the corresponding unionized form. Frequently, unionized species penetrate between one and two orders of magnitude faster than ionized species of the same compounds. Consequently, the fraction of unionized chemical available for penetration should greatly influence the magnitude of the observed permeability coefficient. When the ionized species penetrates approximately 100 times more slowly

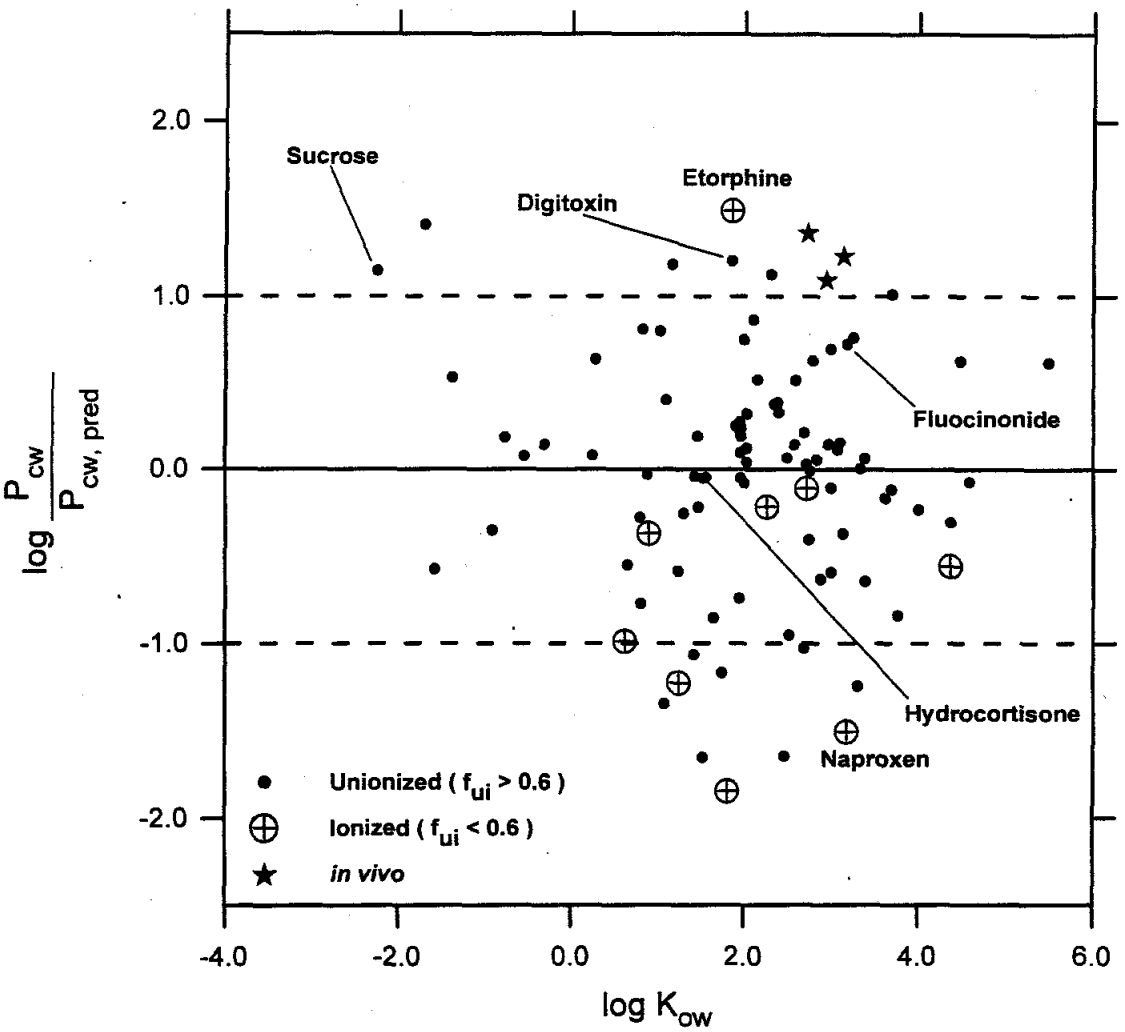

Figure 4.4 Comparison of experimental permeability coefficients with those predicted by the Potts and Guy correlation (Model 15).

1989) among others) that ionized forms of chemicals penetrate through skin at a much through skin than the unionized species and a significant fraction of the compound exists in an unionized form (greater than 10%), it is approximately correct to disregard the ionized species concentration and calculate permeability coefficients based on the concentration of only the unionized species. However, permeability coefficients reported for nine compounds in the Flynn database (atropine, codeine, etorphine, fentanyl, hydromorphone, meperidine, morphine, naproxen, salicylic acid) were measured at a pH where at least 40% of the chemical existed in an ionized state, but appear to have been calculated using the total concentration (i.e., including the concentration of the ionized and unionized species).

We believe that the permeability coefficient that is reported for etorphine and permeability coefficients that are summarized for six other chemicals (sufentanil, fentanyl, meperidine, codeine, hydromorphone, and morphine) by Jolicoeur *et al.* (Jolicoeur *et al.*, 1992), all of which appear in the Flynn database (Flynn, 1990), are based on the total concentration rather than the concentration of the unionized chemical, but, we can not be certain until we confirm this with the authors. Six compounds (codeine, etorphine, hydromorphone, meperidine, morphine, and naproxen), were more than 90% ionized in the vehicle. As indicated in Fig. 4.4, these permeability coefficients are lower on average than predictions developed with the entire Flynn database (e.g., Model 15). Clearly, chemical ionization is one source of uncertainty in the Flynn database.

Figure 4.5 examines the effect of temperature on permeability coefficients in the Flynn database. Permeability coefficients measured at lower temperatures (i.e., 25-26°C) are smaller on average than other permeability coefficients (and are overestimated on average by Model 15), while permeability coefficients measured at higher temperatures (i.e., 37°C) are larger on average than other permeability coefficients (and are underestimated on average by Model 15). To quantify this observation, the log residuals

(defined as $\log P_{cw}$ - $\log P_{cw, pred}$) were calculated: at 25-26°C the mean log residual is -0.195 (low), at 30-32°C the mean log residual is 0.005 (almost zero), and at 37°C the mean log residual is +0.330 (high). These residuals indicate that the permeability coefficient approximately doubles when the temperature increases by 5-7°C. These are in approximate agreement with observations by Scheuplein and Blank that the permeability coefficient can more than double over the 10-12°C temperature range common to dermal absorption experiments (Scheuplein and Blank, 1971). Consequently, differences in temperature used to measure the permeability coefficients causes some of the uncertainty in predicting the Flynn database.

4.4.3. Other Databases

Other databases exhibit similar problems. The database compiled by Wilschut and others (Wilschut *et al.*, 1995) is a more extensive compilation than that of Flynn, but, many of the added measurements are not of the same quality. Duplicate permeability coefficients for the alkanols studied by Scheuplein and Blank were included, effectively weighting these measurements. Some measurements were included (Barry *et al.*, 1985) despite the fact that the vehicle was 50% ethanol, which would likely have damaged the skin (Roberts and Anderson, 1975). Additionally, measurements for ionized compounds were included. For example, Wilschut *et al.* (Wilschut *et al.*, 1995) chose to include the permeability coefficient values for the 100% ionized rather than the unionized form for six drugs (diclofenac, diethylamine salicylate, indomethacin, naproxen, piroxicam, salicylic acid), although Singh and Roberts (Singh and Roberts, 1994) reported both. The relevance of correlating permeability coefficients determined for the ionized compound with $\log K_{ow}$ determined for the unionized compound is questionable.

Kasting and others (Kasting *et al.*, 1992; Kasting *et al.*, 1987) compiled a database of more than 130 permeability coefficient measurements. Many of the measurements overlap with the Flynn database. However, others were measured with shed snake skin as

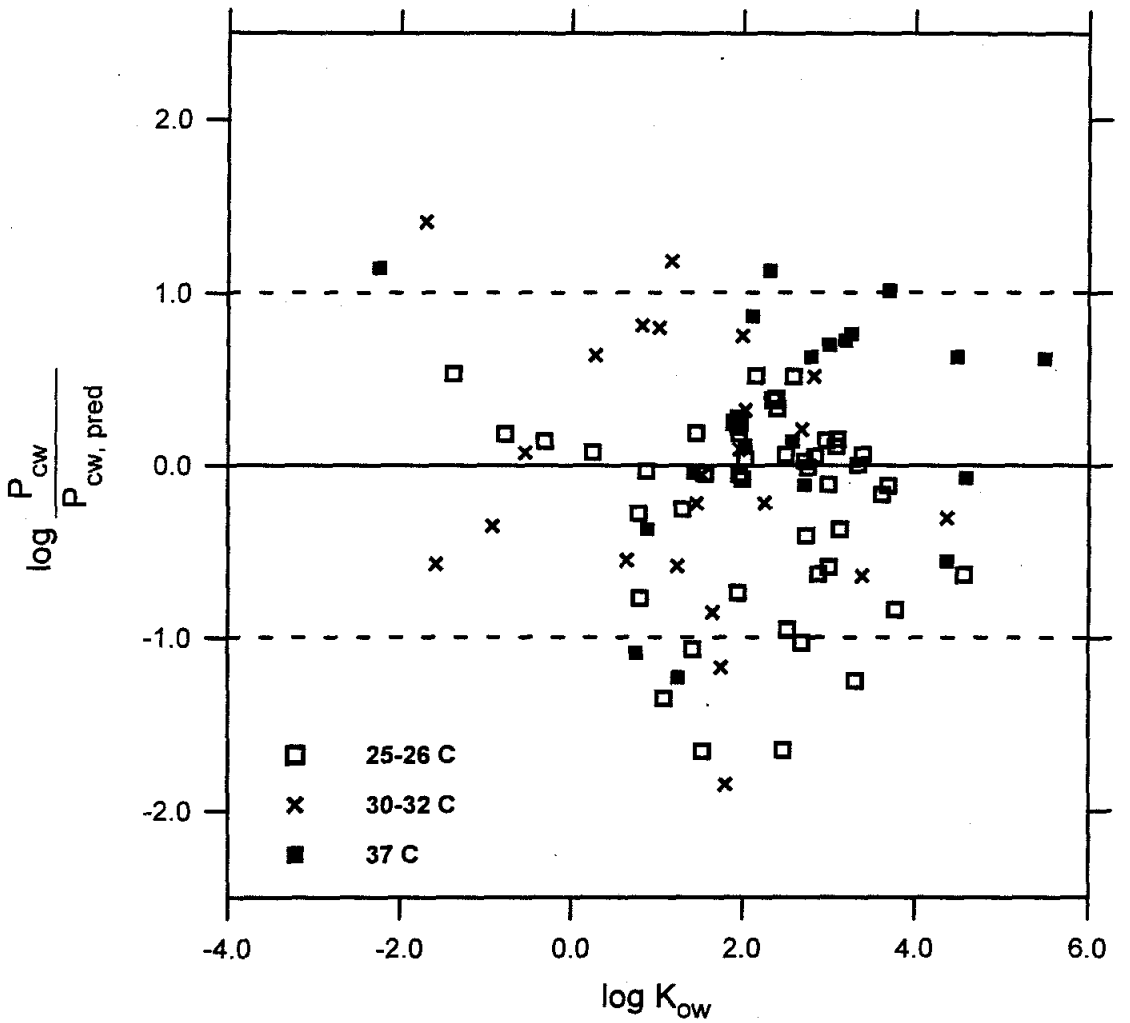

Figure 4.5 The effect of temperature on experimental permeability coefficients compared to those predicted by the Potts and Guy correlation (Model 15). *In vivo* data (ethylbenzene, styrene, and toluene) and measurements made at unknown temperature (naproxen and benzyl alcohol) are not shown.

the membrane (13 values), or with human skin but from propylene glycol vehicles (78 values). Permeability coefficients in snake skin have been reported to mimic human skin (Itoh *et al.*, 1990) but the relationship has not been precisely elucidated (see Chapter 7 of this thesis for a larger discussion of snake skin). Permeability coefficients measured from propylene glycol solution were adjusted to account for partition coefficient differences (using the propylene glycol-water partition coefficient), but other effects (e.g., propylene glycol induced damage or enhancement) were not considered.

McKone and Howd (McKone and Howd, 1992) formed an additional database consisting mostly of permeability coefficient measurements for chemicals from the Flynn database (Flynn, 1990) with MW < 227. Permeability coefficient measurements for three chemicals measured *in vivo* in hairless guinea pigs were also included.

As one final example, the database assembled by Morimoto *et al.* (Morimoto *et al.*, 1992) to develop Model 13 was independent of the Flynn database. Several compounds were ionized.

4.4.4. The Predictors $\log K_{ow}$ and MW

Permeability coefficients and $\log K_{ow}$ are both effected by chemical ionization. Permeability coefficients measured for ionized compounds are lower than permeability coefficients measured for unionized compounds (Michaels *et al.*, 1975; Roy and Flynn, 1989). $\text{Log}K_{ow}$ measured with both ionized and unionized species present, and without correction for ionization, are lower than $\log K_{ow}$ measured when only unionized chemical is present, because the ionized form has a higher water solubility. Permeability coefficients measured for unionized chemicals are generally correlated with $\log K_{ow}$ also measured for the unionized chemical. Different approaches are used to correlate permeability coefficients determined for partially ionized chemicals (i.e., the permeability coefficients observed when unionized and ionized species are simultaneously penetrating

skin) with $\log K_{ow}$. Among other models, Models 9 and 15 correlate several permeability coefficients for chemicals which were > 40% ionized (e.g., atropine, naproxen, salicylic acid) with $\log K_{ow}$ measured for the unionized penetrant. Another approach being used, is to correlate permeability coefficients for partially ionized chemicals with $\log K_{ow}$ values measured at the same pH (i.e., the same proportions of ionized and unionized species). This method assumes that the partitioning of ionized and unionized species into octanol is representative of their partitioning into stratum corneum. Model 13 (Morimoto *et al.*, 1992) was developed using this method. In Chapter 5 we further discuss ways to correlate permeability coefficients for partially ionized chemicals with $\log K_{ow}$ and make recommendations on how this should be done.

All correlations of permeability coefficients which use MW and $\log K_{ow}$ as predictors include correlation among these two parameters. Figure 4.6 examines the relationship between $\log K_{ow}$ (Set A from Appendix 4A) and MW for all 94 chemicals in the Flynn permeability coefficient database. There is a slight positive correlation between $\log K_{ow}$ and MW (i.e., $\log K_{ow}$ increases with increasing MW), although the correlation coefficient is quite low ($r^2 = 0.07$). In addition, at least two chemicals (sucrose and ouabain) are clear exceptions to this trend. The poor correlation coefficient of the $\log K_{ow}$ with MW regression does not prove that $\log K_{ow}$ and MW are independent. Indeed, closer examination reveals that some homologous series of chemicals within the database (for example the normal alkanols) exhibit strong correlation between $\log K_{ow}$ and MW as shown in Fig. 4.4. Alternatively, the steroids exhibit almost no correlation between these two parameters because the MW is nearly constant over a wide range of $\log K_{ow}$. We are led to conclude that the poor correlation coefficient for the entire database arises from combining several groups of chemicals with different correlation between $\log K_{ow}$ and MW. Correlations developed from a chemical series (e.g., the normal alkanols), with a strong $\log K_{ow}$-MW correlation, are most effected by the

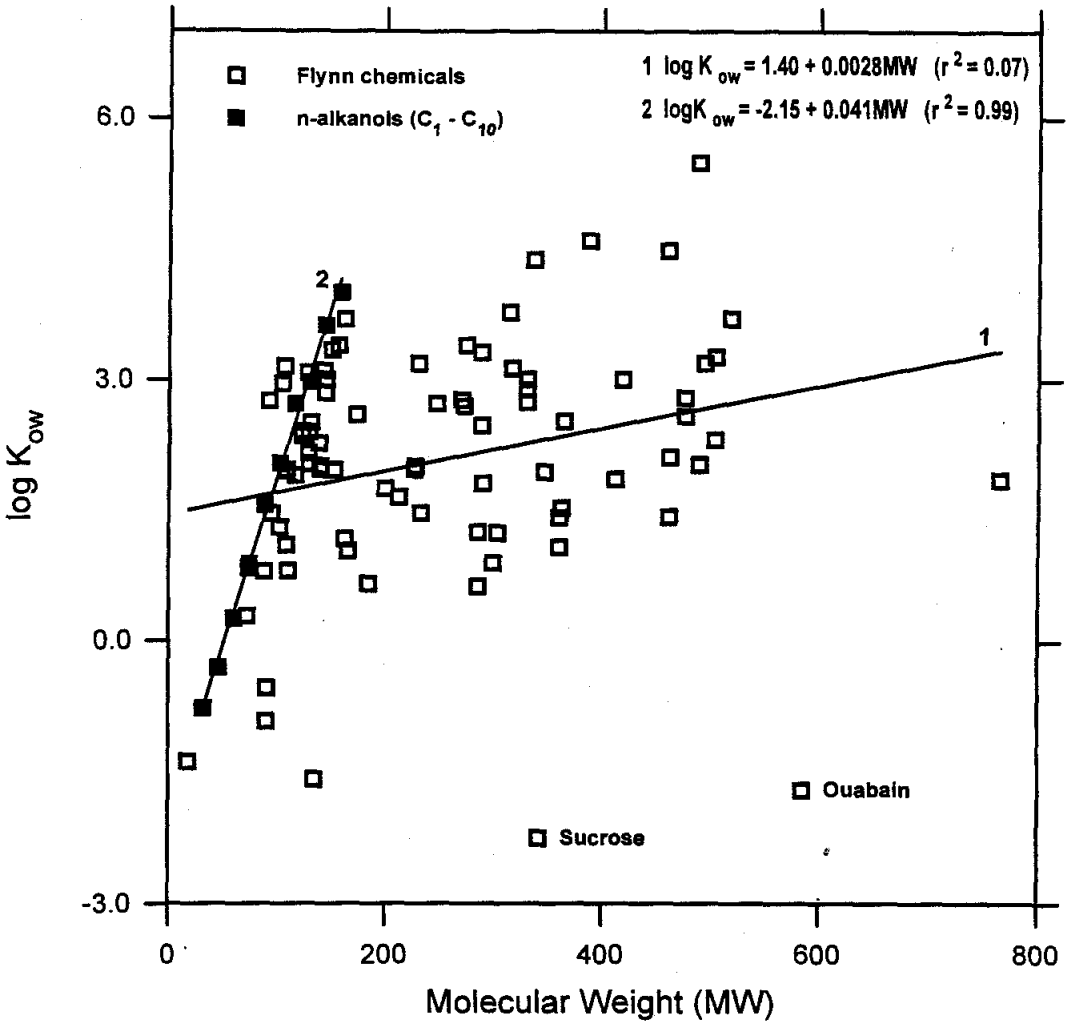

Figure 4.6 Correlation between lipophilicity ($\log K_{ow}$) and molecular weight (MW) for all Flynn database compounds.

correlation and least able to make predictions for chemicals with different correlation between $\log K_{ow}$ and MW. Correlations developed from a database containing many different compounds and chemical series (each with a different type of correlation) are best able to make predictions for chemicals outside of the database.

4.4.5. Reanalysis of the Flynn Database

Several excellent correlations for estimating skin permeability coefficients from aqueous solution are based on the Flynn database, but permeability coefficients in this database were measured at different temperatures, and different levels of ionization (from unionized to completely ionized). The correlations do not account for these differences, and therefore do not make optimal use of the permeability coefficient measurements in the database. In this section, we explore the Flynn database in several ways: (1) using $\log K_{ow}$ that are tabulated by Flynn (Flynn, 1990) (i.e., the $\log K_{ow}$ values from Set A) and comparing results to analysis when $\log K_{ow}$ are recommended by Hansch *et al.* (Hansch *et al.*, 1995) (i.e., the $\log K_{ow}$ values from Set B), (2) making an adjustment for ionization and comparing results to analysis with no adjustment for ionization, (3) incorporating the temperature of the permeability coefficient measurement into analysis and comparing results with the analysis results when no temperature incorporation was made, and (4) analyzing a critically validated portion of the Flynn database and comparing the results to an analysis of the entire Flynn database.

First, we compare results when the Flynn database is analyzed using $\log K_{ow}$ from the Flynn tabulation (Flynn, 1990) and using the recommended values of Hansch *et al.* (Hansch *et al.*, 1995). Using a standard multiple linear regression program, JMP (SAS Institute, 1995), and the Set A list of $\log K_{ow}$ values (from Appendix 4A), we obtained the following fit to the entire Flynn database without adjustment for ionization,

$$\log P_{cw}\ [\mathrm{cm/hr}] = -2.77(0.16) + 0.677(0.06)\log K_{ow} - 0.0057(0.0005)\,\mathrm{MW}$$
$$(n = 97,\ r^2 = 0.698\ \ r^2_{adj} = 0.691,\ \mathrm{RMSE} = 0.730,\ \mathrm{F-Ratio} = 108.4) \qquad (4.2)$$

Uncertainties in parentheses are standard errors of the regression coefficients. Equation (4.2) shows that approximately 69.8% of the variability in $\log P_{cw}$ is explained by variation in $\log K_{ow}$ and MW. The r^2(adj.) statistic is analogous to r^2 but allows for more relevant comparisons between models with different numbers of fitted parameters (JMP User's Guide (SAS Institute, 1995)). Specifically, $(1 - r^2)$ = error sum of squares/total sum of squares and $(1 - r^2(\mathrm{adj.}) = (1 - r^2)(n - 1)/(n - p)$ where n = # of data points and p = # of parameters. RMSE is the root mean square error of the model, which is zero when the model perfectly correlates the data. When presented in an equation, F-Ratio is the model F-Ratio (sum of squares for the model divided by the degrees of freedom for the model) / (sum of squares for the error divided by the degrees of freedom for the error), and when presented for a term such as the MW or $\log K_{ow}$, the F-Ratio is the effect F-Ratio (sum of squares for the effect divided by the degrees of freedom for the effect) / (sum of squares for the error divided by the degrees of freedom for the error). The model F-ratio = 1 when there is zero correlation with the parameters and is large for correlations with good predictive power. Because the number of fitted parameters is in the denominator of the F-Ratio, changes in the model F-Ratio with an increase in the number of parameters should reflect the effect on predictive power relative to the number of fitted parameters. Thus, a correlation with a larger number of parameters might give a higher r^2 (or r^2(adj.)) but a lower F-Ratio than a correlation with fewer parameters. This would indicate that the improvement in predictive power (as indicated by a larger r^2) was not as large per parameter as for the equation with fewer parameters.

Alternatively, using the Set B list of $\log K_{ow}$ values (from Appendix 4A), we obtained the following fit to the entire Flynn database without adjustment for ionization,

$$\log P_{cw}\ [\text{cm / hr}] = -2.69(0.20) + 0.53(0.06)\log K_{ow} - 0.0045(0.0006)\ \text{MW}$$
$$(n = 97,\ r^2 = 0.568\ \ r^2_{adj} = 0.559,\ \text{RMSE} = 0.873,\ \text{F} - \text{Ratio} = 61.7) \tag{4.3}$$

Equation (4.3) shows that approximately 56.8% of the variability in $\log P_{cw}$ is explained by variation in $\log K_{ow}$ and MW.

The fit obtained with $\log K_{ow}$ values tabulated by Flynn ($r^2 = 0.698$) is superior to that obtained with $\log K_{ow}$ values reported by Hansch *et al.* ($r^2 = 0.568$). Similarly, the Set A values of $\log K_{ow}$ will also provide better fits than the Set B values of $\log K_{ow}$ in regressions discussed later. This may occur because for some compounds the $\log K_{ow}$ values tabulated by Flynn were measured at the same conditions by the same investigators as P_{cw}. Specifically, the $\log K_{ow}$ values from Set A and Set B are quite different for the set of hydrocortisone esters (Anderson *et al.*, 1988). The Set B values were calculated using Daylight and are not the Hansch StarList values. Generally, we favor the use of Hansch *et al.* recommended values, despite these statistics, because they have been validated and are fully documented.

Next, we compare results when the Flynn database is adjusted for ionization to results when there is no adjustment for ionization. Specifically, permeability coefficients measured for partially ionized penetrants were divided by the fraction unionized when no more than 90% of the compound was ionized, and permeability coefficients for six chemicals (i.e., codeine, etorphine, hydromorphone, meperidine, morphine, and naproxen) were excluded because more than 90% of the compound was ionized. Using multiple linear regression with the Set A values of $\log K_{ow}$, we obtained the following fit to the ionization-adjusted Flynn database:

$$\log P_{cw}\ [\text{cm / hr}] = -2.72(0.15) + 0.68(0.05)\log K_{ow} - 0.0057(0.0005)\ \text{MW}$$
$$(n = 91,\ r^2 = 0.724,\ r^2_{adj} = 0.717,\ \text{RMSE} = 0.691,\ \text{F} - \text{Ratio} = 115.2) \tag{4.4}$$

Equation (4.4) shows that approximately 72.4% of the variability in $\log P_{cw}$ is explained by variation in $\log K_{ow}$ and MW.

Alternatively, using multiple linear regression and the Set B values of $\log K_{ow}$, we obtained the following fit to the ionization-adjusted Flynn database:

$$\log P_{cw}\ [\mathrm{cm/hr}] = -2.63(0.19) + 0.53(0.06)\log K_{ow} - 0.0045(0.0006)\,\mathrm{MW}$$
$$(n = 91,\ r^2 = 0.586,\ r^2_{adj} = 0.577,\ \mathrm{RMSE} = 0.845,\ \mathrm{F-Ratio} = 62.4) \qquad (4.5)$$

Equation (4.5) shows that approximately 58.6% of the variability in $\log P_{cw}$ is explained by variation in $\log K_{ow}$ and MW.

Comparison of Eqs. (4.4) and (4.2), and Eqs. (4.5) and (4.3) show that adjustment for ionization reduces the variability of permeability coefficients in the Flynn database and improves predictability. The statistics change only slightly because there are many other sources of variability and only a few compounds in the Flynn database are partially ionized.

Thirdly, we compare results when temperature is incorporated into the analysis to results when temperature is not incorporated. Our examination of the Flynn database showed that temperature was an observable source of uncertainty which can potentially be reduced by properly incorporating temperature into the model. Assuming that temperature alters the diffusion coefficient through an Arrhenius process, temperature can be used to modify the MW term in Eq. (4.5). Using the Set B values of $\log K_{ow}$, we obtain the following fit to the ionization-adjusted Flynn database:

$$\log P_{cw}\ [\mathrm{cm/hr}] = -2.60(0.19) + 0.532(0.06)\log K_{ow} - 1.42(0.18)\left(\frac{\mathrm{MW}}{\mathrm{T}}\right) \qquad (4.6)$$
$$(n = 91,\ r^2 = 0.597,\ r^2_{adj} = 0.588,\ \mathrm{RMSE} = 0.833,\ \mathrm{F-Ratio} = 65.3)$$

Equation (4.6) shows that approximately 59.7% of the variability in $\log P_{cw}$ is explained by variation in $\log K_{ow}$ and (MW/T). Equation (4.6) incorporates temperature in a physically realistic way without creating an additional adjustable parameter.

Equations (4.6) and (4.5) indicate that more of the variability in skin permeability coefficient data can be explained by temperature inclusive correlations than can be

explained by a correlation which does not incorporate temperature. Temperature differences are a small contribution to uncertainty but effect many measurements.

Finally, we compare results for the entire Flynn database to results for a validated fraction of the Flynn database. Several measurements from the Flynn database were withheld from analysis for various reasons. Permeability coefficients for ionizable penetrants were adjusted (by dividing the observed permeability coefficient by the fraction ionized) or excluded (when more than 90% is ionized) as described previously. The three *in vivo* measurements for ethylbenzene, styrene and toluene (Dutkiewicz and Tyras, 1967; Dutkiewicz and Tyras, 1969) were excluded from the analysis for reasons described previously. The hydrocortisone measurement (Hadgraft and Ridout, 1987) and the naproxen measurement (Chowhan and Pritchard, 1978) were excluded because they were measured from non-aqueous vehicles. The permeability coefficient of digitoxin was excluded because it is likely that it was not measured at steady state. The permeability coefficients of fluocinonide and sucrose (Anderson *et al.*, 1988) were excluded as recommended by the author (Anderson, 1995).

Using the Set B values of $\log K_{ow}$ we obtained the following fit to this validated database:

$$\begin{aligned} &\log P_{cw}\ [\mathrm{cm/hr}] = -2.76(0.20) + 0.52(0.06)\log K_{ow} - 0.0041(0.0006)\,\mathrm{MW} \\ &(n = 84,\ r^2 = 0.537,\ r^2_{adj} = 0.526,\ \mathrm{RMSE} = 0.820,\ \mathrm{F-Ratio} = 47.0) \end{aligned} \tag{4.7}$$

Equation (4.7) shows that approximately 53.7% of the variability in $\log P_{cw}$ is explained by variation in $\log K_{ow}$ and MW.

Temperature effects were incorporated into the analysis of this validated database by assuming that temperature alters the diffusion coefficient through an Arrhenius process. Using the Set B values of $\log K_{ow}$ we obtain the following fit to this validated database:

$$\log P_{cw}\ [\text{cm / hr}] = -2.72(0.20) + 0.53(0.06)\log K_{ow} - 1.32(0.20)\left(\frac{MW}{T}\right) \tag{4.8}$$

$$(n = 84,\ r^2 = 0.549,\ r^2_{adj} = 0.538,\ \text{RMSE} = 0.809,\ F - \text{Ratio} = 49.4)$$

Equation (4.8) shows that approximately 54.9% of the variability in $\log P_{cw}$ is explained by variation in $\log K_{ow}$ and (MW/T).

Interestingly, removal of questionable data did not reduce the variability in the P_{cw} correlation. The fit of Eq. (4.7) is poorer than the fit obtained when the entire unadjusted Flynn database is analyzed (i.e., Eq. (4.3)) using the same values of $\log K_{ow}$. Goodness-of-fit statistics usually, but not always, increase when measurements with questionable validity are removed. Reduction of variability is not the only criteria for model discrimination; models should be based on data which are physically relevant and have been consistently measured and analyzed. Permeability coefficients that have been determined or calculated inconsistently (e.g., ionized and unionized chemicals) and permeability coefficients from different physical situations (e.g., non-aqueous vehicles, animal skin, etc.) should be excluded from analysis whether or not the goodness-of-fit statistic improves, remains the same, or worsens.

Like other correlations based on the Flynn database, Eqs. (4.7) and (4.8) are trained with permeability coefficients for compounds of diverse structure and properties (as measured by MW and $\log K_{ow}$), and so should be useful for predicting permeability coefficients for a wide range of organic compounds. We recommend that Equations (4.7) and (4.8) are more valid than correlations based on the entire Flynn database because effects of ionization (and temperature also for Eq. (4.8)) have been accounted for and the data have been more thoroughly reviewed.

The analysis described here begins validation of permeability coefficient data by considering some operational variables which are most easily assessed. Some of the variability which can not be explained by the criteria proposed here may be explained by

differences in experimental protocols, and these effects are addressed more fully in Chapter 5. Through data validation, more certain methods of estimating human skin permeability coefficients should be possible.

4.5. Conclusions

Skin permeability coefficients can be predicted with semi-theoretical correlations developed from a large and diverse set of valid permeability coefficient measurements. We have compared 16 correlations from the literature with a large and well known permeability coefficient database (the Flynn database with 97 measurements for 94 different chemicals). Correlations developed from large and diverse databases are best able to predict this database; correlations developed from small or non-diverse datasets may have excellent fit statistics but have poor predictive utility beyond the dataset from which they were developed.

Permeability coefficient data should be critical evaluated before being included in correlation development. A portion of the variance in permeability coefficients in the Flynn and other databases can be explained by different experimental factors (e.g., temperature, fraction of chemical that is ionized, vehicle type, etc.). Based on reasonable quality criteria, some permeability coefficients from the Flynn database should not be used to develop correlations. Application of quality criteria to permeability coefficient data are discussed more extensively in Chapter 5.

4.6. Notation

f_{ui}	=	Fraction of the total chemical dose that is unionized in the vehicle
K_{ow}	=	Octanol-water partition coefficient of the penetrating chemical
K_{mw}	=	Mineral-water partition coefficient of the penetrating chemical
MW	=	Molecular weight of the absorbing chemical
P_{cw}	=	Steady-state permeability of the SC from water
$P_{cw,pred}$	=	Predicted steady-state permeability of the SC from water
P_{ew}	=	Steady-state permeability of the VE from water
P_w	=	Steady-state permeability of the SC-VE composite membrane from water
SC	=	Stratum corneum
T	=	Absolute temperature (Kelvin)
VE	=	Viable epidermis

4.7. References

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4.8. Appendix 4A The Flynn Database

Table 4A.1 Information Relevant to Interpretation of the Permeability Coefficients in the Flynn Database

Compound	MW	Set A $\log K_{ow}$[a]	Set B $\log K_{ow}$[b]	T (°C)	f_{ui}	P_{exp} (cm/hr)	P_{pred}[c] (cm/hr)	Reference
Aldosterone	360.4	1.08	1.08	26	1	3.0E-6	6.7E-5	Scheuplein et al., 1969
Amobarbital	226.3	1.96	2.07	30	1	2.3E-3	1.8E-3	Hadgraft & Ridout, 1987
Atropine	289.4	1.81	1.83	30	0.55	8.6E-6	6.0E-4	Michaels et al., 1975
Barbital	184.2	0.65	0.65	30	1	1.1E-4	3.9E-4	Hadgraft & Ridout, 1987
Benzyl alcohol	108.1	1.1	1.10	25	1	6.0E-3	2.4E-3	Roberts, 1976
4-Bromophenol	173.0	2.59	2.59	25	1	3.6E-2	1.1E-2	Roberts et al., 1977
2,3-Butanediol	90.1	-0.92	-0.92	30	1	<5.0E-5	1.1E-4	Scheuplein & Blank, 1971
Butanoic acid	88.1	0.79	0.79	25	1	1.0E-3	1.9E-3	Scheuplein & Blank, 1971
n-Butanol	74.1	0.88	0.88	25	1	2.5E-3	2.7E-3	Scheuplein & Blank, 1971
2-Butanone	72.1	0.28	0.29	30	1	4.5E-3	1.0E-3	Scheuplein & Blank, 1971
Butobarbital	212.2	1.65	1.73	30	1	1.9E-4	1.4E-3	Hadgraft & Ridout, 1987
4-Chlorocresol	142.6	3.10	3.10	25	1	5.5E-2	3.9E-2	Roberts et al., 1977
2-Chlorophenol	128.6	2.15	2.15	25	1	3.3E-2	1.0E-2	Roberts et al., 1977
4-Chlorophenol	128.6	2.39	2.39	25	1	3.6E-2	1.5E-2	Roberts et al., 1977
Chloroxylenol	156.6	3.39	[3.48]	25	1	5.9E-2	5.1E-2	Roberts et al., 1977
Chlorpheniramine	274.8	(3.39)	3.39	30	0.96	2.2E-3	9.7E-3	Michaels et al., 1975
Codeine	299.3	0.89	1.14	37	<0.1	4.9E-5	1.2E-4	Roy and Flynn, 1989
Cortexolone	364.5	2.52	2.52	26	1	7.5E-5	6.6E-4	Scheuplein et al., 1969
Cortexone	330.5	2.88	2.88	26	1	4.5E-4	1.9E-3	Scheuplein et al., 1969
Corticosterone	346.5	1.94	1.94	26	1	6.0E-5	3.3E-4	Scheuplein et al., 1969
Cortisone	360.5	1.42	1.47	26	1	1.0E-5	1.2E-4	Scheuplein et al., 1969
o-Cresol	108.1	1.95	1.95	25	1	1.6E-2	9.6E-3	Roberts et al., 1977
m-Cresol	108.1	1.96	1.96	25	1	1.5E-2	9.7E-3	Roberts et al., 1977

Compound	MW	Set A $\log K_{ow}$ [a]	Set B $\log K_{ow}$ [b]	T (°C)	f_{ui}	P_{exp} (cm/hr)	P_{pred} [c] (cm/hr)	Reference
p-Cresol	108.1	1.95	1.94	25	1	1.8E-2	9.6E-3	Roberts et al., 1977
n-Decanol	158.3	4.0[d]	4.57	25	1	8.0E-2	1.4E-1	Scheuplein & Blank, 1973
2,4-Dichlorophenol	127.6	3.08	3.06	25	1	6.0E-2	4.6E-2	Roberts et al., 1977
Diethylcarbamazine	199.3	(1.75)	[1.75]	30	1	1.3E-4	1.9E-3	Michaels et al., 1975
Digitoxin	764.9	1.86[e]	2.83	30	1	1.3E-5	8.1E-7	Michaels et al., 1975
Ephedrine	165.2	1.03	0.93	30	0.92	6.0E-3	9.5E-4	Michaels et al., 1975
β-Estradiol	272.4	2.69	4.01	26	1	3.0E-4	3.2E-3	Scheuplein et al., 1969
β-Estradiol (2)	272.4	2.69	4.01	30	1	5.2E-3	3.2E-3	Michaels et al., 1975
Estriol	288.4	2.47	2.45	26	1	4.0E-5	1.8E-3	Scheuplein et al., 1969
Estrone	270.4	2.76	3.13	26	1	3.6E-3	3.7E-3	Scheuplein et al., 1969
Ethanol	46.1	-0.31	-0.31	25	1	8.0E-4	5.7E-4	Scheuplein & Blank, 1971
2-Ethoxy ethanol	90.1	-0.54	-0.32	30	1	2.5E-4	2.1E-4	Scheuplein & Blank, 1971
Ethyl benzene	106.2	3.15	3.15	24	1	1.2	7.0E-2	Dutkiewicz & Tyras, 1967
Ethyl ether	74.1	0.83	0.89	30	1	1.6E-2	2.5E-3	Scheuplein & Blank, 1971
4-Ethylphenol	122.2	2.4	2.58	25	1	3.5E-2	1.6E-2	Roberts et al., 1977
Etorphine	411.5	1.86	[1.41]	37	<0.1	3.6E-3	1.2E-4	Jolicoeur et al., 1992
Fentanyl	336.5	4.37	4.05	37	0.59	5.6E-3	2.0E-2	Roy and Flynn, 1989
Fentanyl (2)	336.5	4.37	4.05	30	0.85	1.0E-2	2.0E-2	Michaels et al., 1975
Fluocinonide	494.6	3.19	3.19	37	1	1.7E-3	3.2E-4	Anderson et al., 1988
Heptanoic acid	130.2	2.5	[2.41]	25	1	2.0E-2	1.7E-2	Scheuplein, 1967
n-Heptanol	116.2	2.72	2.72	25	1	3.2E-2	3.0E-2	Scheuplein & Blank, 1971
Hexanoic acid	116.2	1.9	1.92	25	1	1.4E-2	7.9E-3	Scheuplein, 1967
n-Hexanol	102.2	2.03	2.03	25	1	1.3E-2	1.2E-2	Scheuplein & Blank, 1971
Hydrocortisone (HC)	362.5	1.53	1.61	26	1	3.0E-6	1.4E-4	Scheuplein et al., 1969
Hydrocortisone (HC) (2)	362.5	1.53	1.61	30	1	1.2E-4	1.4E-4	Hadgraft & Ridout, 1987
[HC-21-yl]-N,N dimethyl	489.6	2.03	[0.88]	37	1	6.7E-5	5.1E-5	Anderson et al., 1988

Compound	MW	Set A $\log K_{ow}$ [a]	Set B $\log K_{ow}$ [b]	T (°C)	f_{ui}	P_{exp} (cm/hr)	P_{pred} [c] (cm/hr)	Reference
succinamate								
[HC-21-yl]-hemipimelate	504.6	3.26	[1.82]	37	0.80	1.8E-3	3.1E-4	Anderson et al., 1988
[HC-21-yl]-hemisuccinate	462.5	2.11	[0.91]	37	0.78	6.3E-4	8.6E-5	Anderson et al., 1988
[HC-21-yl]-hexanoate	460.6	4.48	[3.28]	37	1	1.8E-2	4.2E-3	Anderson et al., 1988
[HC-21-yl]-hydroxy hexanoate	476.6	2.79	[1.29]	37	1	9.1E-4	2.1E-4	Anderson et al., 1988
[HC-21-yl]-octanoate	488.7	5.49	[4.34]	37	1	6.2E-2	1.5E-2	Anderson et al., 1988
[HC-21-yl]-pimelamate	503.6	2.31	[0.82]	37	1	8.9E-4	6.7E-5	Anderson et al., 1988
[HC-21-yl]-proprionate	418.5	3.0	[1.69]	37	1	3.4E-3	6.8E-4	Anderson et al., 1988
[HC-21-yl]-succinamate	461.6	1.43	[0.17]	37	1	2.6E-5	2.9E-5	Anderson et al., 1988
Hydromorphone	285.3	1.25	[0.55]	37	<0.1	1.5E-5	2.5E-4	Roy and Flynn, 1989
17-Hydroxypregnenolone	330.5	3.0	(3.0)	26	1	6.0E-4	2.3E-3	Scheuplein et al., 1969
17-Hydroxyprogesterone	330.5	2.74	3.17	26	1	6.0E-4	1.5E-3	Scheuplein et al., 1969
Isoquinoline	129.2	2.03	2.08	30	0.99	1.7E-2	8.1E-3	Hadgraft & Ridout, 1987
Meperidine	247.0	2.72	2.45	37	<0.1	3.7E-3	4.8E-3	Roy and Flynn, 1989
Methanol	32.0	-0.77	-0.77	25	1	5.0E-4	3.3E-4	Scheuplein & Blank, 1971
Methyl-[HC-21-yl]-succinate	476.6	2.58	[1.38]	37	1	2.1E-4	1.5E-4	Anderson et al., 1988
Methyl-[HC-21-yl]-pimelate	518.6	3.7	[2.20]	37	1	5.4E-3	5.2E-4	Anderson et al., 1988
Methyl-4-hydroxy benzoate	152.1	1.96	1.96	25	1	9.1E-3	5.2E-3	Roberts et al., 1977
Morphine	285.3	0.62	0.76	37	<0.1	9.3E-6	9.0E-5	Roy and Flynn, 1989
2-Napthol	144.2	2.84	2.70	25	1	2.8E-2	2.5E-2	Roberts et al., 1977
Naproxen	230.3	3.18	3.34	NR	<0.1	4.0E-4[f]	1.3E-2	Chowhan & Pritchard, 1978
Nicotine	162.2	1.17	1.17	30	0.95	1.9E-2	1.3E-3	Hadgraft & Ridout, 1987
Nitroglycerine	227.1	2.0[g]	[0.98]	30	1	1.1E-2	2.0E-3	Michaels et al., 1975

Compound	MW	Set A $\log K_{ow}$ [a]	Set B $\log K_{ow}$ [b]	T (°C)	f_{ui}	P_{exp} (cm/hr)	P_{pred} [c] (cm/hr)	Reference
3-Nitrophenol	139.1	2.0	2.00	25	1	5.6E-3	6.7E-3	Roberts et al., 1977
4-Nitrophenol	139.1	1.96	1.91	25	1	5.6E-3	6.3E-3	Roberts et al., 1977
N-nitrosodiethanolamine	134.1	(-1.58)	[-1.58]	32	1	5.5E-6	2.1E-5	Bronaugh et al., 1981
n-Nonanol	144.3	3.62	4.26	25	1	6.0E-2	8.8E-2	Scheuplein & Blank, 1971
Octanoic acid	144.2	3.0	3.05	25	1	2.5E-2	3.2E-2	Scheuplein, 1967
n-Octanol	130.2	2.97	3.00	25	1	5.2E-2	3.7E-2	Scheuplein & Blank, 1971
Ouabain	584.6	(-1.70)	-1.70	30	1	7.8E-7	3.0E-8	Michaels et al., 1975
Pentanoic acid	102.1	1.3	1.39	25	1	2.0E-3	3.6E-3	Scheuplein, 1967
n-Pentanol	88.2	1.56	1.56	25	1	6.0E-3	6.7E-3	Scheuplein & Blank, 1971
Phenobarbital	232.2	1.47	1.47	30	1	4.5E-4	7.6E-4	Hadgraft & Ridout, 1987
Phenol	94.1	1.46	1.46	25	1	8.2E-3	5.2E-3	Roberts et al., 1977
Pregnenolone	316.5	3.13	4.22	26	1	1.5E-3	3.5E-3	Scheuplein et al., 1969
Progesterone	314.5	3.77	3.87	26	1	1.5E-3	1.0E-2	Scheuplein et al., 1969
n-Propanol	60.1	0.25	0.25	25	1	1.4E-3	1.2E-3	Scheuplein & Blank, 1971
Resorcinol	110.1	0.80	0.80	25	1	2.4E-4	1.4E-3	Roberts et al., 1977
Salicylic acid	138.1	2.26	2.26	30	0.49	6.3E-3	1.0E-2	Hadgraft & Ridout, 1987
Scopolamine	303.4	1.24	[-0.20]	30	0.76	5.0E-5	1.9E-4	Michaels et al., 1975
Styrene	104.1	2.95	2.95	24	1	6.4E-1	5.2E-2	Dutkiewicz & Tyras, 1969
Sucrose	342.3	-2.25	-3.70	37	1	5.2E-6	3.7E-7	Anderson et al., 1988
Sufentanyl	387.5	4.59	3.95	37	0.90	1.2E-2	1.4E-2	Roy and Flynn, 1989
Testosterone	288.4	3.31	3.32	26	1	4.0E-4	7.0E-3	Scheuplein et al., 1969
Thymol	150.2	3.34	3.30	25	1	5.3E-2	5.1E-2	Roberts et al., 1977
Toluene	92.1	2.75	2.73	24	1	1.0	4.4E-2	Dutkiewicz & Tyras, 1969
2,4,6-Trichlorophenol	162.0	3.69	3.69	25	1	5.9E-2	7.7E-2	Roberts et al., 1977
Water	18.01	-1.38	-1.38	25	1	5.0E-4	1.5E-4	Scheuplein & Blank, 1971
3,4-Xylenol	122.2	2.35	2.23	25	1	3.6E-2	1.5E-2	Roberts et al., 1977

[a] $LogK_{ow}$ tabulated by Flynn (Flynn, 1990) and reprinted exactly by US EPA (US EPA, 1992). Values for four chemicals (chlorpheniramine, diethylcarbamazine, N-nitrosodiethanolamine, and ouabain) contained within parenthesis are from the Set B list. According to Flynn (Flynn, 1990): (1) $logK_{ow}$ were taken from the permeability coefficient references where these data were provided in addition to permeability coefficients, and (2) the actual source of many of these data and the source of values not co-listed with permeability coefficients is the invaluable, extensive compilation of C. Hansch and L. Leo.

[b] $LogK_{ow}$ taken from the StarList of Hansch (Hansch *et al.*, 1995), unless contained within Brackets (e.g., for chloroxylenol [3.48]), in which case it was calculated using Daylight (PCModels, 1995). The $logK_{ow}$ for 17-hydroxypregnenolone is contained in parenthesis to designate that Set A values were used for this chemical.

[c] Predicted by the Potts and Guy permeability coefficient correlation (Model 15).

[d] Interpreted from other values in homologous series.

[e] Value reported for $logK_{butanol/water}$

[f] Interpreted from data obtained with gels containing naproxen in solution.

[g] Value reported for partitioning into "oils".

5. ESTIMATING HUMAN SKIN PERMEABILITY OF ORGANIC CHEMICALS FROM AQUEOUS SOLUTIONS

5.1. Introduction

Obtaining a viable estimate of the amount of compound absorbed into the skin is important for reasons related to either medicinal therapy or to protect human health during exposure to environmental pollutants and toxic compounds. Experimentally determined permeability coefficients are useful for quantifying the dermal absorption of compounds through the skin, and have been determined for many compounds in many different exposure scenarios. However, measurements do not exist for many compounds with the potential for dermal absorption. Hence, the motivation for correlating the available permeability data in terms of more readily available parameters (e.g., molecular weight and octanol-water partition coefficients).

Sixteen correlations that estimate the permeability of skin to aqueous organic compounds, in terms of the penetrating chemical's molecular weight (MW) and octanol-water partition coefficient (K_{ow}) were reviewed in Chapter 4 along with several databases on which these correlations were developed. We identified numerous measurements in the most commonly utilized databases which are inconsistent with the database in various ways. For example, some measurements were made at unsteady state. Others were measured from vehicles other than water alone. Still other measurements were animal and not human skin. Also the different penetration rates of ionized and unionized penetrants were overlooked. Permeability coefficients for ionogenic compounds were frequently calculated using the total vehicle concentration, rather than concentration of

the unionized species, despite the fact that ionized species penetrate more slowly through stratum corneum (SC) than unionized species. Using the Flynn database, we showed that such permeability coefficients were consistently lower than expected. Finally, temperature was usually not incorporated into the analysis of permeability coefficients, although permeability coefficients in the Flynn database measured at 37°C were larger (on average) than permeability coefficients measured at 25-26°C.

Permeability coefficients can be more accurately predicted if more of the many skin penetration studies are critically evaluated and utilized. We have already found that chemical ionization, temperature, and certain model-conformity criteria (e.g., steady state) influence permeability. Additional criteria pertaining to the predictor parameters used in the permeability correlation (e.g., MW and K_{ow}) will also be discussed. In this work we develop and apply quality criteria to a large database of experimentally determined permeability coefficients. These data provide the means to develop mechanistically relevant predictive models of percutaneous absorption. Finally, correlations describing these data are presented and discussed.

5.2. Data Validation Criteria

All permeability coefficients analyzed in this chapter were measured *in vitro* through human skin from aqueous vehicles. Prior to developing correlations, each data point was critically reviewed with respect to several criteria deemed important to permeability coefficient values. Permeability coefficients reported without pertinent details were reserved for future analysis, allowing time for contact with the authors. Three collectively exhaustive (taken together they contain all permeability measurements we have considered) and mutually exclusive (measurements appearing in one database do not appear in others) databases are presented: (1) a fully-validated database which meet all of the quality criteria (containing the measurements used to develop correlations), (2)

a provisional database reserved for subsequent analysis (containing measurements which are missing pertinent information with respect to the quality criteria), and (3) an excluded database (containing measurements which do not meet one or more of the validation criteria). Details about how the permeability coefficients were extracted from the original references and their validation are contained in Appendix 5B.

Data in the fully-validated database were required to meet five criteria: (1) the temperature must be known and be between 20-40°C, (2) more than 10% of the penetrating compound must be in an unionized form, (3) a valid $\log K_{ow}$ (either a recommended value of Hansch *et al.* (Hansch *et al.*, 1995) or else a value calculated using Daylight software which was developed from these recommended K_{ow} (PCModels, 1995)) must represent the penetrating molecule (usually the unionized compound), (4) the measurement must have been determined at steady state, and (5) the donor and receptor fluids do not compromise (more than water does) the barrier of the skin. Steady-state permeability coefficients require a constant vehicle concentration (or adjustment to account for changing vehicle concentration).

The excluded database includes a few measurements of ionic chemicals which are valid in all respects except that a suitable $\log K_{ow}$ is not available. Although these measurements can not be used to develop predictive correlations, information can be obtained about the penetration of these ions (cations, anions, zwitterions) without attempting to correlate them with $\log K_{ow}$.

5.2.1. Adjustment for Ionization

Permeability coefficients have been measured for chemicals which exhibit very diverse ionization behavior. Many compounds are essentially unionized at the pH of the experiment (i.e., typically $2 < pH < 10$). However, many others exist in equilibrium with a charged (frequently protonated amines or dissociated carboxylic acid) species (e.g.,

aniline, caffeine, codeine, isoquinoline, ibuprofen, and nicotine among others). Some compounds are essentially always ions (e.g., paraquat or tetraethylammonium bromide). Others are either charged or zwitterionic (i.e., net neutral) but never unionized (e.g., 5-fluorouracil). Still others coexist as a complex mixture of zwitterionic, charged, and unionized species (e.g., 2-amino-4-nitrophenol, dopamine, hydromorphone, isoprenaline, levodopa, morphine, nicotinic acid). The relative rates of penetration of anionic, cationic, and zwitterionic species are not precisely known. Sznitowska and colleagues have measured the penetration of net anionic, cationic, and zwitterionic forms of several amino acids and claim that permeability coefficients are essentially the same (within experimental error) when measured for the different ionic forms (Sznitowska *et al.*, 1993). Intuition suggests that zwitterionic (net neutral) species may penetrate through the skin more rapidly than species carrying a charge and we will look for this trend in our database.

Ionized organic compounds do not penetrate through the SC as rapidly as unionized compounds. For example, Figure 5.1a shows that the absorption of fentanyl and sufentanil (Roy and Flynn, 1990) at different extents of ionization is accurately correlated with the fraction unionized (f_{ui}). Fentanyl and sufentanil are weak bases which ionize more as the pH decreases. The reference unionized permeability coefficients (P_{cw} = 0.0339 cm/hr for fentanyl and P_{cw} = 0.0327 cm/hr for sufentanil) were determined by averaging the observed permeability coefficients that were measured when the fraction unionized (determined from calculated pK_a values for fentanyl (7.0 at 37°C) and sufentanil (6.2 at 37°C)), was more than 0.99 (see also Appendix 5B). The appendix Table 5A.1 permeability coefficients for fentanyl and sufentanil are not used because they are reported for skins from different individuals, while in this figure it is convenient to plot multiple measurements from the skin of a single individual. Typical of the penetration of other organic molecules, this plot shows that the permeability coefficient

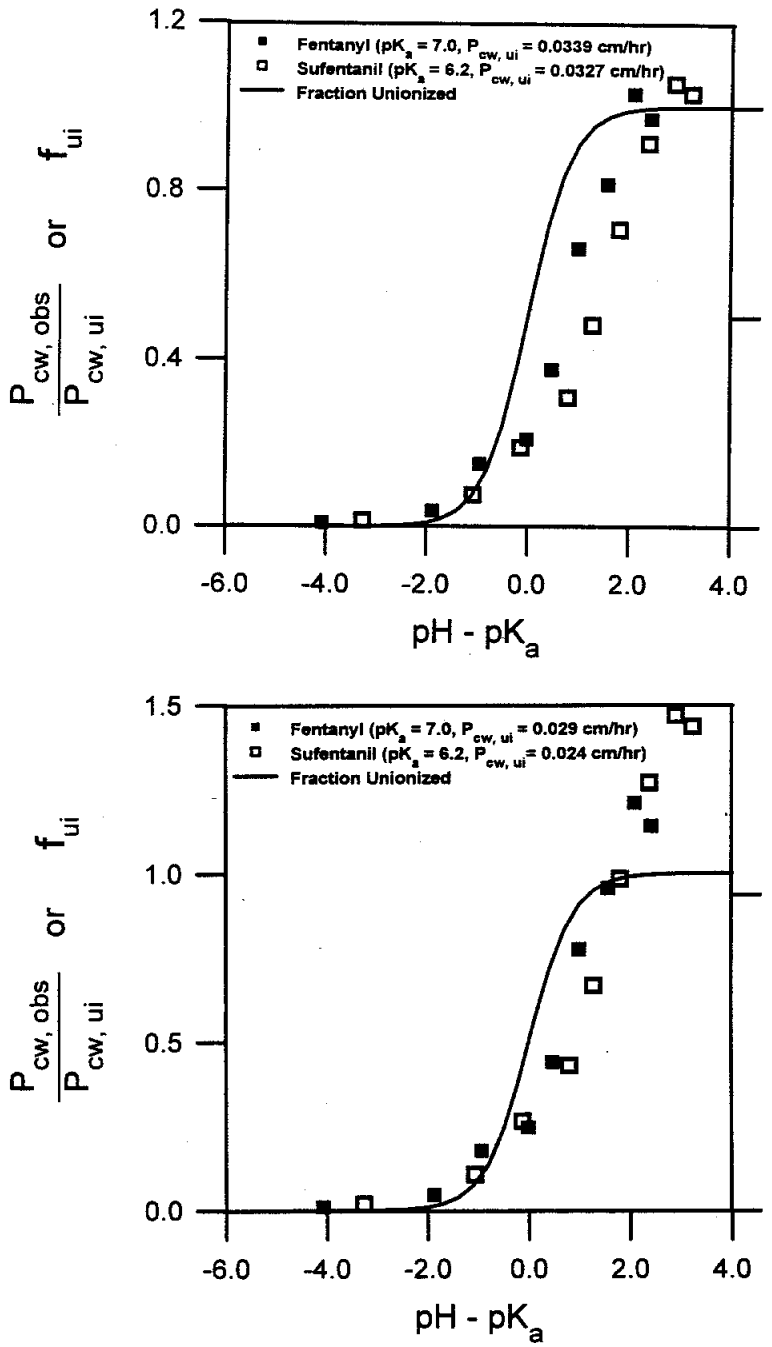

Figure 5.1 Observed permeability coefficients ($P_{cw,obs}$) normalized by $P_{cw,ui}$ for weak bases fentanyl and sufentanil at a range of pH (Roy and Flynn, 1990) compared to f_{ui}: (a) $P_{cw,ui}$ was calculated by averaging all $P_{cw,obs}$ values at pH where $f_{ui} > 0.99$, (b) $P_{cw,ui}$ was calculated by averaging all $P_{cw,obs}$ values at pH where $f_{ui} \geq 0.1$.

observed for an ionized molecule is substantially less than for an unionized molecule. We have found that SC permeability coefficients for unionized compounds are frequently one-two orders of magnitude larger than permeability coefficients for ionized forms of the same compound. The exact relationship should depend upon the chemical and in particular upon the lipophilicity of the unionized chemical. Specifically, the penetration rates for unionized and ionized forms of the same chemical should be more similar when the unionized chemical is hydrophilic and more different when it is lipophilic.

Figure 5.1b compares the observed permeability coefficients with unionized permeability coefficients calculated with permeability coefficients that were determined for compounds under conditions where they were partially ionized. The reference unionized permeability coefficients (P_{cw} = 0.029 cm/hr for fentanyl and P_{cw} = 0.024 cm/hr for sufentanil) were determined by dividing all of the observed permeability coefficients by the fraction unionized, calculated using the pK_a values of fentanyl (7.0 at 37°C) and sufentanil (6.2 at 37°C), and averaging permeability coefficients at all pH for which at least 10% of the compound is unionized (see also Appendix 5B). Unless otherwise specified, permeability coefficients for the unionized species were calculated using permeability coefficients determined for the partially but not significantly ($f_{ui} > 0.1$) ionized species. The permeability coefficients measured at high pH (i.e., basic solution) exceed the unionized permeability coefficients (i.e., $P_{cw,\ observed}/P_{cw,\ unionized} > 1$ at high pH). This probably occurs because the pK_a values for these compounds are not precisely known. Roy and Flynn (Roy and Flynn, 1989; Roy and Flynn, 1990) reported pK_a values (8.9 for fentanyl and 8.5 for sufentanil at 37C) without reference, which are quite different from the calculated pK_a values we used (using SPARC, which will be discussed later). However, the pK_a values which we have calculated appear to be more consistent with the data than the pK_a values reported by Roy and Flynn (Roy and Flynn, 1989; Roy and Flynn, 1990). Estimation of unionized permeability coefficients using permeability

coefficients measured on partially ionized compounds, although theoretically and experimentally supported, may be strongly dependent upon the knowledge of relatively accurate and validated pK_a values.

To a good approximation, penetration can be attributed to the unionized species alone, particularly when the fraction unionized is not too low (i.e., $f_{ui} > 0.1$). Consequently, we calculated unionized-species permeability coefficients by dividing the observed permeability coefficient (calculated using the total concentration) by the fraction unionized. When more than 90% of the compound is ionized (i.e., $f_{ui} < 0.1$), the rate of penetration of the ionized species can not be neglected. Thus, we require that the data in the fully-validated database meet the criteria that $f_{ui} \geq 0.1$. Assuming that unionized compounds penetrate two orders of magnitude faster than ionized forms of the same compound, this limit will ensure that errors in estimating the SC permeability coefficient should not exceed 10%

The fraction of unionized compound in the vehicle, f_{ui}, for compounds with one dominant acid-base reaction can be calculated from the acid-base dissociation constant (pK_a) and the vehicle pH (Schwarzenbach *et al.*, 1993):

$$f_{ui} = \frac{1}{\left(1+10^{g}\right)} \tag{5.1}$$

where the exponent, $g = (pH - pK_a)$ for acids and $(pK_a - pH)$ for bases. We have used the vehicle pH rather than the skin pH (typically about pH = 4) because the skin has low buffer capacity to balance a vehicle with large volume.

SPARC [SPARC Performs Automated Reasoning in Chemistry] (SPARC, 1995) was used to calculate pK_a values at 25°C. The program SPARC is an expert system for the estimation of chemical and physical reactivity. Its computational algorithms are based on considerations of molecular structure that are arrived at using the reasoning process that an expert chemist might apply in reactivity analysis. The computational approaches

in SPARC blend conventional linear free energy theory (LFET) and perturbed molecular orbital (PMO) methods. In general, SPARC utilizes LFET to compute thermodynamic properties and PMO theory to describe quantum effects such as delocalization energies or polarizabilities of π-electrons. SPARC-calculated and IUPAC pK_a values for more than 4000 different compounds have been calculated and compared. For this statistical comparison, the regression coefficient, r^2 was 0.994 and the root mean square error was 0.37 (Hilal *et al.*, 1995).

Frequently the pK_a is not known precisely enough for small differences in temperature to effect it (Schwarzenbach *et al.*, 1993). Nevertheless, we have adjusted all pK_a values for temperature by using an integrated form of the van't Hoff (Smith and Van Ness, 1987):

$$\ln\left[\frac{(K_a)_2}{(K_a)_1}\right] = \frac{-\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{5.2}$$

Enthalpies of ionization, ΔH (given for the acid dissociation reaction with the equilibrium constant K_a), used in adjusting pK_a values for temperature, were estimated from enthalpies of protonation presented elsewhere (Sober, 1968): 0.0 for carboxylic acids, 5.0 for phenolic compounds, 10 for amines (primary, secondary, or tertiary), 7.5 for aniline (or amines attached directly to an aromatic ring system), and 5 for aromatic nitrogen (pyridine derivatives and isoquinoline). We assume that the enthalpy of ionization is constant over a small temperature range (25-37°C) and disregard the temperature dependence of heat capacities. Multiple pK_a values for the same molecule were adjusted independently for effects of temperature.

When buffer solutions were not used and the pH was not reported, we calculated the expected natural pH using the solution concentration, all pK_a values, and assuming neutral water prior to chemical addition. A general treatment of simultaneous equilibrium is required, involving equations for all linearly-independent reactions, the

water dissociation reaction ($K_a = 1.0x10^{-14}$), a molecular balance on the active species, and an equation requiring solution electroneutrality (Brescia *et al.*, 1975).

5.2.2. Selection of K_{ow}

There are three different approaches for correlating permeability coefficients for ionogenic compounds with $\log K_{ow}$. The best approach, assuming that only unionized species penetrate, is to jointly use the unionized-species permeability coefficient and the unionized-species K_{ow}. The next best approach is to disregard ionization effects and use the observed (for all ionized and unionized species) permeability coefficient and the observed (for all ionized and unionized species) K_{ow} measured at the same conditions (i.e., the same pH, concentration, f_{ui}, and temperature). This approach assumes that the ability of unionized, ionized, and zwitterionic species to partition into octanol from water is representative of their ability to partition into and permeate through SC from water. The least appropriate method and a flaw of several existing correlations (e.g., those based on the Flynn database as discussed in Chapter 4) is to use the observed permeability coefficient (based on the total concentration of ionized and unionized species) and the octanol-water partition coefficient for the unionized chemical. The three approaches are equivalent for compounds that are unionized or exist in only one state of ionization (e.g., paraquat, tetraethylammonium ion, and 5-fluorouracil at low pH).

In the following analysis, we have assumed that only the unionized species penetrate the SC. Therefore, we calculate the permeability of the unionized species and correlate it with the preferred $\log K_{ow}$ values (★) reported by Hansch and colleagues (Hansch *et al.*, 1995), which were 'measured as or converted to the neutral form', were preferentially selected. When these recommended values were not available, Daylight software (PCModels, 1995) was used to calculate surrogates. These calculated surrogates are generated using algorithms trained to the database of preferred values. These neutral

form values of K_{ow} are larger than those that would be measured if the chemical is partly ionized, because the ionized species are more water soluble.

5.3. Development of Correlations for Permeability Coefficients

The steady-state permeability across the SC from an aqueous vehicle (P_{cw}) into an infinite sink depends on the diffusivity of the chemical in the SC (D_c), the SC thickness (L_c), and the equilibrium partition coefficient between the SC and the water vehicle (K_{cw}) as given below (Crank, 1975):

$$P_{cw} = \frac{K_{cw} D_c}{L_c} \tag{5.3}$$

where K_{cw} is defined as the concentration of chemical in the SC (mass/volume of SC at absorbing conditions) divided by the equilibrium concentration in the vehicle (mass/volume) (Parry *et al.*, 1990). As defined here, D_c is an effective diffusivity based on the SC thickness rather than the true diffusivity based on the actual molecular diffusion path length, which is not known.

The barrier resistance provided by the SC is $1/P_{cw}$. However, for intact skin the barrier includes the viable epidermis (VE) in addition to the SC, and the steady-state resistance ($1/P_w$) is the sum of these two resistances:

$$\frac{1}{P_w} = \frac{1}{P_{cw}} + \frac{1}{P_{ew}} \tag{5.4}$$

where the steady-state permeability through the VE from an aqueous vehicle is defined as

$$P_{ew} = \frac{K_{ew} D_e}{L_e} \tag{5.5}$$

K_{ew} is the VE-water partition coefficient, D_e and L_e are the diffusivity of chemical in and thickness of the VE, respectively. If the vehicle itself does not alter the thermodynamic character of the SC or VE,

$$K_{ew} = \frac{K_{cw}}{K_{ce}} \tag{5.6}$$

and the resistance of the SC-VE composite barrier (i.e., the intact epidermis) is then

$$\frac{1}{P_w} = \frac{1}{P_{cw}}\left[1 + \frac{K_{ce} D_c L_e}{D_e L_c}\right] = \frac{1}{P_{cw}}(1 + B) \tag{5.7}$$

where the parameter B, defined as

$$B = \frac{D_c L_e K_{ce}}{D_e L_c} = \frac{P_{cw}}{P_{ew}} \tag{5.8}$$

measures the relative permeability of the SC to the VE and is independent of the vehicle, provided that the vehicle has not altered physicochemically the SC or VE. Hence, B-values from one vehicle, such as water, can be used in calculations for other vehicles.

Because diffusivity in the VE is much larger than in the SC, B is often small and the permeability across the SC-VE barrier nearly equals the permeability of the SC alone. However, the VE is much more hydrophilic than the SC and K_{ce} will be similar to partitioning between lipophilic and hydrophilic solvents, such as octanol and water. Consequently, chemicals with large $\log K_{ow}$ will have large values for B, indicating that the resistance across the VE is larger than the resistance across the SC. When B is large, the total permeability of the combined SC-VE barrier no longer depends solely on the SC permeability. These highly lipophilic compounds enter the relatively hydrophilic VE with difficulty, thereby causing the total permeability of the combined SC-VE barrier to be less than the permeability across only the SC. Since many chemicals of environmental interest are highly lipophilic, dermal absorption estimates should include effects of the SC-VE combined barrier.

Most permeability coefficients have been measured with SC-VE composite membranes (i.e., P_w) rather than with isolated SC membranes (i.e., P_{cw}), however, the database still predicts the SC permeability coefficient because for most compounds the

VE is unimportant. Only a few permeability coefficients have been measured for compounds lipophilic enough that the VE may provide a significant resistance to penetration. Most correlations, including the correlations developed in this Chapter, do not adequately incorporate the VE resistance and are effectively correlations for the SC rather than correlations for the SC-VE composite. In Chapter 9 we develop a predictive correlation of the B-parameter (defined by Eq. (5.8)) and then use Eq. (5.7) to represent the resistance of the SC-VE composite.

We begin by developing a model which assumes, based on Eq. (5.3) that SC permeability coefficients can be modeled in terms of another two-phase partition coefficient ($\log K_{ow}$) and molecular weight (which is anticipated to influence the rate of diffusion). Two subsequently developed models account for the anticipated effect of temperature on the diffusion coefficient, and make a density correction so that molecular weight is more representative of molecular volume for heavy-element molecules. Next, a model is developed that allows permeability coefficients for lipophilic and hydrophilic compounds (this distinction to be defined by the model) to depend differently upon size and lipophilicity. Finally, a linear solvation-energy relationship (LSER) is developed using a subset of the database to gain mechanistic information and evaluate the performance of MW and $\log K_{ow}$.

5.3.1. Derivation of the Conventional Correlation

Equation (5.3) is the basis for several correlations of SC permeability coefficients (e.g., (Potts and Guy, 1992)). The SC-water partition coefficient is assumed to be related to the octanol-water partition coefficient through a power function of the general form:

$$\log K_{cw} = \log(a) + f \cdot \log K_{ow} \tag{5.9}$$

where the parameter f accounts for differences in lipophilic character of the SC lipids (Potts and Guy, 1992). The SC-water partition coefficient data in Chapter 6 indicate that the intercept (i.e., log(a)) predicted by Eq. (5.9) is small and negative. The diffusion of

small molecules in rubbery polymers is generally considered to be an activated process which varies exponentially with the size of the penetrant (Mulder, 1991):

$$D_c = D_o \exp(-\beta \cdot MV) \tag{5.10}$$

where D_o is the diffusion coefficient of a hypothetical molecule having zero molecular volume (MV), and β is a constant. Equations (5.9) and (5.10) can then be combined as indicated by Eq. (5.3). Potts and Guy (Potts and Guy, 1992) showed no significant degradation in correlation quality when MV was replaced by MW and recommended the general functional form:

$$\log P_{cw} = \left[\log(a) + \log(D_o / L_c)\right] + f \cdot \log K_{ow} + \beta^{\#} \cdot MW \tag{5.11}$$

where $\beta^{\#} = \beta \log(e)$. However, their dataset consisted primarily of hydrocarbons. One would expect that MV would be better than MW for describing a chemically more heterogeneous dataset (e.g., including halogenated hydrocarbons. Analysis of skin permeability measurements with Eq. (5.11) will provide values for [log (a) + log (D_o/L_c)], f, and $\beta^{\#}$ which have attributable physicochemical meaning.

5.3.2. Incorporation of the Effect of Temperature

The effect of temperature on P_{cw} has been explored experimentally by Blank and colleagues (Blank *et al.*, 1967) who showed that P_{cw}, measured for the normal alcohols, increased approximately 2.9 fold for a temperature increase of 10°C. Likewise, in Chapter 4 we observed that residuals (measurement - correlation prediction) between measurements and our conventional correlation were positive (on average) for measurements made at temperatures greater than 30°C and negative (on average) for measurements made at temperatures less than 30°C. We now analyze the data with an equation that explicitly incorporates the temperature effect in the hope of reducing unexplained variance.

According to free-volume theory the transport of small molecules in rubbery polymers is viewed as an activated process in which the diffusion coefficient obeys an Arrhenius-type relationship (Mulder, 1991):

$$D_c = D_o \exp(-E_a / RT) = D_o \exp(-\beta_2 \cdot MV / T) \tag{5.12}$$

where E_a is a size-dependent activation energy required for the penetrant to make a diffusional jump, β_2 is a constant, and T is the absolute temperature.

Assuming that MW is an appropriate substitute for MV, Eqs. (5.12) and (5.9) can be substituted into Eq. (5.3) to obtain an expression for the SC permeability coefficient of the form:

$$\log P_{cw} = [\log(a) + \log(D_o / L_c)] + f \cdot \log K_{ow} + \beta_2^{\#} \cdot \frac{MW}{T} \tag{5.13}$$

where $\beta_2^{\#} = \beta_2 \log(e)$. In Eq. (5.13) temperature is assumed to reduce the activation energy for transport of penetrants through the SC, primarily acting through the MW dependence. Equation (5.13) has the same number of adjustable parameters as the conventional correlation.

5.3.3. Liquid Density (LD) Corrections and LD-Temperature Corrections

Equation (5.10) shows that, according to free-volume theory, the SC diffusion coefficient is influenced by the MV of the penetrating compound. As already described, MV is often replaced by MW. Since the MW of most hydrocarbons are very similarly related to MV this is often not a bad assumption (i.e., MV ≈ b(MW) where b is approximately the same for all chemicals). However, this assumption can introduce systematic errors if the database includes chemicals composed of heavy elements such as halogens (e.g., b is probably different for chlorinated organics than for hydrocarbons). By correcting the MW with an experimental liquid density for each compound, it might be possible to reduce these errors without having to know or calculate the true molecular volume.

We will incorporate a pseudo liquid density correction to make MW more closely resemble MV using two approaches. In the first approach, we assume that the coefficient of thermal expansion is the same for all compounds. The coefficient of thermal expansion, K_T, is a thermodynamic property of materials (i.e., $K_T \equiv \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_P$) that to a first approximation can be considered constant for all organic liquids. Based on values reported by Welty and colleagues (Welty *et al.*, 1986) for several liquids (water, aniline, ammonia, dichlorodibromomethane (Freon-12), n-butyl alcohol, benzene, glycerin) in the temperature range of 80-100°F, K_T varies between $8.3\text{x}10^{-5}$/K for water to $9.6\text{x}10^{-4}$/K for dichlorodibromomethane. The K_T for these compounds differ by only a factor of 11.5. An experimental liquid density, ρ_{ref}, which was measured at a temperature T_{ref}, is adjusted to the temperature of the permeability coefficient measurement (T) using K_T:

$$\rho(\text{at T}) = \rho_{ref} \cdot \exp[K_T(T_{ref} - T)] \tag{5.14}$$

The density ρ is a pseudo liquid density calculated through a hypothetical adjustment of an experimental density (ρ_{ref}) without accounting for phase changes. Using the fact that MV is closely approximated by MW normalized by this pseudo liquid density (i.e., MW/ρ) and using Eq. (5.11) we obtain an expression for $\log P_{cw}$:

$$\begin{aligned}\log P_{cw} &= [\log(a) + \log(D_o / L_c)] + f \cdot \log K_{ow} \\ &\quad + \beta^{\#} \cdot \frac{MW}{\rho_{ref}} \cdot \exp[-K_T(T_{ref} - T)]\end{aligned} \tag{5.15}$$

If, in addition, the effect of temperature on the permeability coefficient is included, we obtain an expression of the form:

$$\begin{aligned}\log P_{cw} &= [\log(a) + \log(D_o / L_c)] + f \cdot \log K_{ow} \\ &\quad + \beta_2^{\#} \cdot \frac{MW}{T\rho_{ref}} \cdot \exp[-K_T(T_{ref} - T)]\end{aligned} \tag{5.16}$$

Equations (5.15) and (5.16) are equivalent except for the temperature in the denominator of the last term of Eq. (5.16).

In the second approach, we assume that the product of the thermal expansion coefficient and the critical temperature is constant. The critical temperature, T_c, is the highest temperature, at any pressure, that a pure material can exist in vapor/liquid equilibrium. Starting with the definition of K_T and the theorem of corresponding states (which suggests that properties of materials are similar when related to their critical point temperature, pressure and volume), it is easy to show that the product $K_T T_c$ is approximately the same for all liquids. Using tabulated K_T values (Welty *et al.*, 1986) for several liquids (water, aniline, ammonia, dichlorodibromomethane (Freon-12), n-butyl alcohol, benzene, glycerin) in the temperature range 80-100°F and T_c for these compounds (Reid *et al.*, 1987), we found that the product $K_T T_c$ varies from a low of 0.054 for water to a high of 0.368 for dichlorodibromomethane. The product differs by only a factor of 6.82 for these compounds. Equation (5.17) is analogous to Eq. (5.14) but uses the product $K_T T_c$ to adjust densities for temperature:

$$\rho(\text{at } T) = \rho_{ref} \cdot \exp\left[K_T T_c \left(\frac{T_{ref}}{T_c} - \frac{T}{T_c}\right)\right] \tag{5.17}$$

Using the fact that the MV is closely approximated by MW normalized by this pseudo liquid density (i.e., MW/ρ) and using Eq. (5.11) we obtain an improved expression for $\log P_{cw}$ which is analogous to Eq. (5.15):

$$\log P_{cw} = \left[\log(a) + \log(D_o / L_c)\right] + f \cdot \log K_{ow} + \beta^{\#} \cdot \frac{MW}{\rho_{ref}} \cdot \exp\left[-K_T T_c \left(\frac{T_{ref}}{T_c} - \frac{T}{T_c}\right)\right] \tag{5.18}$$

Like before, the separate effect of temperature on the permeability coefficient can also be included to produce an expression which is analogous in form to Eq. (5.16):

$$\log P_{cw} = \left[\log(a) + \log(D_o / L_c)\right] + f \cdot \log K_{ow} + \beta_2^{\#} \cdot \frac{MW}{T\rho_{ref}} \cdot \exp\left[-K_T T_c \left(\frac{T_{ref}}{T_c} - \frac{T}{T_c}\right)\right] \tag{5.19}$$

In summary, Eqs. (5.15) and (5.18) adjust for only liquid density while Eqs. (5.16) and (5.19) adjust for temperature in addition to liquid density.

5.3.4. Derivation of a Two-Mechanism Model

Until now we have essentially ignored the fact that the SC is a heterogeneous membrane composed of lipophilic domains (the intercellular lipids) and hydrophilic domains (the cellular protein). The protein and lipid domains in the SC are histologically revealed as a mosaic of cornified cells containing cross-linked keratin filaments and intercellular lipid-containing regions (Elias, 1981). The cellular protein is not itself a homogeneous domain but the differences within this domain are small when compared with differences between the lipids and this protein phase. Many researchers believe that lipophilic compounds penetrate the SC by a transcellular pathway through the lipid domains (Anderson *et al.*, 1988). The mechanism for penetration of hydrophilic compounds is subject to more controversy.

Many authors have suggested that dermal absorption of hydrophilic and lipophilic compounds is governed by separate mechanisms (Flynn, 1990; Kasting *et al.*, 1992; Kasting *et al.*, 1987; Michaels *et al.*, 1975; Morimoto *et al.*, 1992). Potts and Guy performed an analysis of the Flynn database where they concluded that one mechanism was sufficient to describe all data (Potts and Guy, 1992). In their investigation, partially ionized and unionized compounds were all correlated in terms of $\log K_{ow}$ for the unionized compound.

We will revisit the question of separate mechanisms for penetration of lipophilic and hydrophilic compounds with a larger set of data, including several more measurements for hydrophilic compounds. Furthermore, analysis of this database will be

more meaningful because (1) steps have been taken to avoid unequal comparisons between partially ionized and unionized species, and (2) partially ionized species are not correlated with $\log K_{ow}$ for the unionized compound.

To test the 2-mechanism hypothesis we have developed a mathematical model with a different linear dependence on $\log K_{ow}$ for hydrophilic (P_{cw}^{H}) and lipophilic compounds (P_{cw}^{L}):

$$\log P_{cw}^{H} = a + (b + c) \cdot \log K_{ow} + d \cdot MW \tag{5.20}$$

$$\log P_{cw}^{L} = (a - c \cdot \log K_{ow}^{\#}) + b \cdot \log K_{ow} + d \cdot MW \tag{5.21}$$

Equations (5.20) and (5.21) can be combined to represent hydrophilic and lipophilic chemicals:

$$\log P_{cw} = a + b \cdot \log K_{ow} + c \cdot \left(\log K_{ow} - \log K_{ow}^{\#}\right)\delta + d \cdot MW \tag{5.22}$$

where $K_{ow}^{\#}$, representing the transition between hydrophilic and lipophilic mechanisms, is determined in the regression. The indicator variable δ has the following values:

$$\begin{aligned} \delta = 1 \quad &\text{if} \quad K_{ow} \geq K_{ow}^{\#} \\ \delta = 0 \quad &\text{if} \quad K_{ow} < K_{ow}^{\#} \end{aligned} \tag{5.23}$$

In this development, we have assumed that the effect of MW is not a function of the pathway. If the permeability coefficients for hydrophilic compounds do not depend upon K_{ow} then $(b + c) = 0$ and Eq. (5.22) simplifies to yield:

$$\log P_{cw} = a + c \cdot \left(\log K_{ow} - \log K_{ow}^{\#}\right)\delta + d \cdot MW \tag{5.24}$$

In Eq. (5.24) the parameter a represents the maximum value for $\log P_{cw}^{H}$ and $(a - c \cdot \log K_{ow}^{\#})$ is the maximum value for $\log P_{cw}^{L}$.

5.3.5. Linear Solvation-Energy Analysis

The theoretical foundations of Linear Solvation-Energy Relationships (LSER) have been described elsewhere (Abraham *et al.*, 1994; Cramer *et al.*, 1993; Famini and Penski, 1992), and LSER have been developed to interpret and predict SC permeability coefficients (Abraham *et al.*, 1995; El Tayar *et al.*, 1991). Appendix 5C contains an introduction to LSER.

Briefly, LSER have been successful at correlating free energy-dependent properties exhibited by a class of solutes in the same solvent. In this application, the parameters of the model (i.e., the solvatochromic parameters α, β, π, and V_x) correspond to the solutes rather than the solvent. Thus, correlations of the general form,

$$\log(\lambda) = \log(\lambda_0) + a \cdot \alpha + b \cdot \beta + c \cdot \pi + d \cdot V_x \tag{5.25}$$

were found for various solvent-dependent, free energy-based properties, λ. The solute solvatochromic parameters have the following physicochemical interpretation: α is the effective hydrogen-bond acidity, β is the effective hydrogen-bond basicity, π is the solute dipolarity/polarizability, and V_x (units of $cm^3/mol/100$) is the characteristic volume of McGowans (Abraham and McGowan, 1987) which is calculated simply from molecular structure and is independent of intermolecular forces such as hydrogen bonding. For a given property and set of compounds, the coefficients $\log(\lambda_0)$ and the parameters a through e are determined using multilinear regression analysis of data.

SC permeability coefficients can be modeled with a LSER using an equation of the form:

$$\log(P_{cw}) = \log(P_{cw}^{o}) + a \cdot \alpha + b \cdot \beta + c \cdot \pi + d \cdot V_x \tag{5.26}$$

This equation assumes that the difference in the chemical potential (between solute in the vehicle and in the skin) driving the solute across the SC results from differences in van Der Waals forces, size effects, polarizability, and the preference of a solute to act as an electron donor and an electron acceptor in hydrogen bonds.

5.4. Results and Discussion

Table 5A.1 contains the fully-validated database of permeability coefficients. The provisional database is contained in Table 5A.2. Permeability coefficients that were excluded from further analysis are listed in Table 5A.3. The adjustment of pK_a values for temperature and the calculation of the fraction unionized and the natural pH for unbuffered solutions at known concentration are summarized in Table 5A.4.

5.4.1. Examination of the Data

Figure 5.2 shows the fully-validated database and several excluded and provisional measurements plotted as a function of $\log K_{ow}$. Several labeled measurements in Figure 5.2 require further discussion. Digitoxin, the highest MW compound studied (MW = 764.9), was excluded from the validated database because the exposure time was not specified and very long times are expected before steady state is attained. Likewise, ouabain (MW = 584.6) was made provisional because an exposure time was not specified. Other excluded data includes the measurements of fluocinonide and sucrose reported by Anderson *et al.* (Anderson *et al.*, 1988). According to Anderson (Anderson, 1995) an unresolvable discrepancy exists between the original notebook and the published value for fluocinonide. Anderson recommended that the permeability coefficient for sucrose be excluded because it was not measured with a technique that is necessary to measure permeability coefficients of hydrophilic compounds (see further documentation in Appendix 5B). Etorphine was excluded because the permeability coefficient reported was unusually large relative to permeability coefficients for similarly structured chemicals. We found in Chapter 7 that the permeability coefficient for etorphine in hairless mouse skin, measured in the same investigation, is of a much more realistic magnitude. Perhaps, the human skin used to study etorphine was damaged or otherwise compromised.

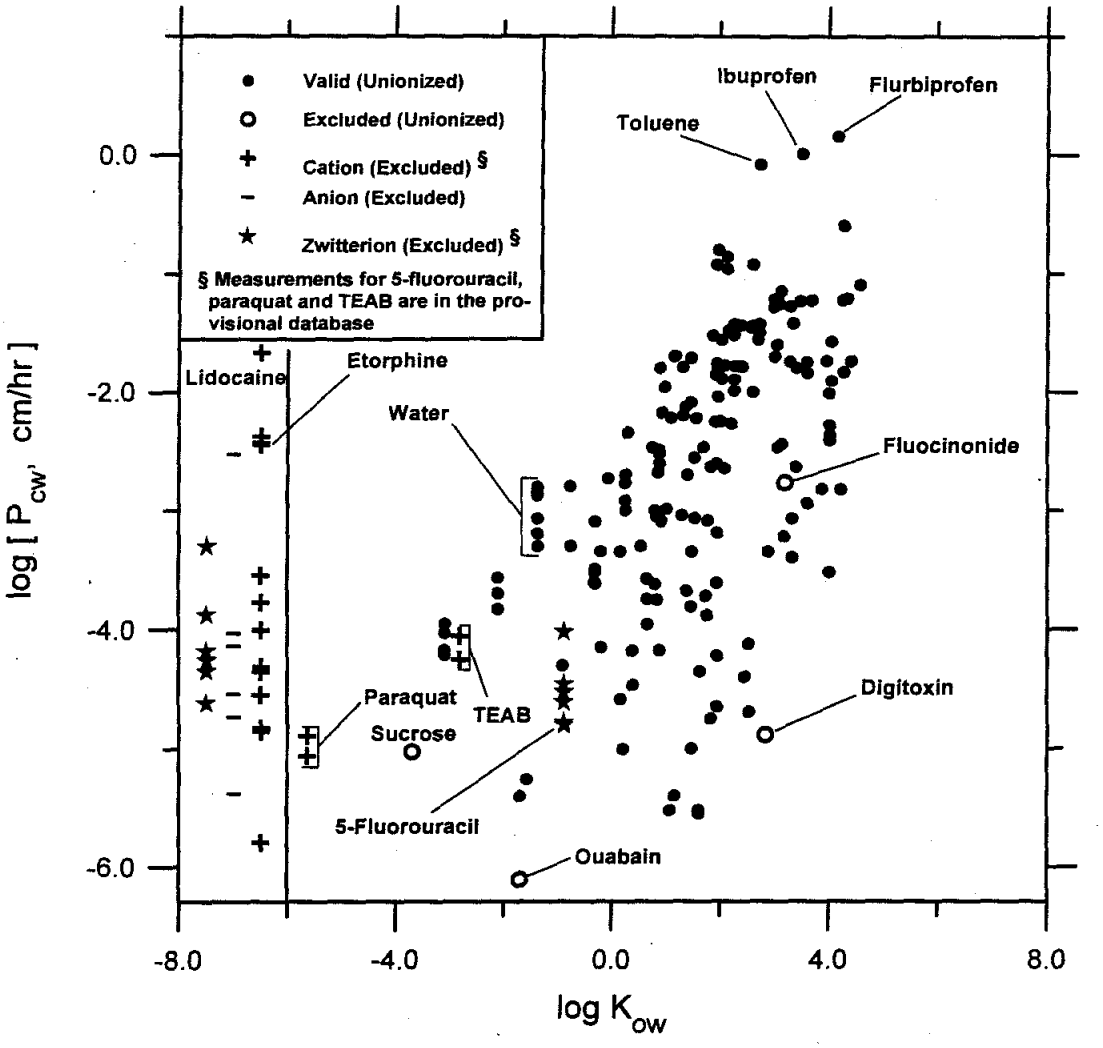

Figure 5.2 Permeability coefficients of the fully-validated database and several values from the excluded and provisional databases plotted as a function of K_{ow}. Ionic species, without an appropriate $\log K_{ow}$, are plotted to the left of all other measurements at an assigned $\log K_{ow}$ (cations at $\log K_{ow} = -6.5$, anions at $\log K_{ow} = -7.0$, and zwitterions at $\log K_{ow} = -7.5$). Paraquat, tetraethylammonium (TEAB), and 5-fluorouracil are plotted at $\log K_{ow}$ reported for these ionized species.

The three largest permeability coefficients, for toluene, ibuprofen, and flurbiprofen are also identified. These permeability coefficients are much larger than expected, particularly after adjustment for ionization, but we can not identify an explanation other than unusually large random experimental error, so they were not excluded.

Permeability coefficients for water are separately labeled to serve as a benchmark for assessing the penetration of other compounds. Ionic species, without an appropriate $\log K_{ow}$, are plotted to the left of all other measurements Cations are plotted at a $\log K_{ow}$ = -6.5; anions, at $\log K_{ow}$ = -7.0; and zwitterions at $\log K_{ow}$ = -7.5. Paraquat, tetraethylammonium (TEAB), and 5-fluorouracil, are shown and separately labeled because valid $\log K_{ow}$ are available for these ions. Other excluded measurements, which we believe should not follow even the qualitative trends of the data (due to nonaqueous vehicle effects, vehicle depletion, etc.), are not shown, but are listed in the excluded database (Table 5A.3) for reference. The measurement for hydroxypregnenolone, from the provisional database, is not shown because a suitable K_{ow} for this compound is not available.

Figure 5.2 shows that permeability coefficients in the fully-validated database vary by approximately six orders of magnitude. These permeability coefficients are quite well correlated with $\log K_{ow}$ and have a nearly linear dependence over the entire range shown. A portion of the difference among measurements that is not explained by $\log K_{ow}$ can be explained by differences in MW as will be shown later. The permeability coefficients for ionic species, although apparently quite variable, are generally lower than for unionized lipophilic species. In this database, permeability coefficients for cations are larger than permeability coefficients for either anions or zwitterions. However, many more measurements are necessary to determine whether this trend persists.

5.4.2. Comparison with the Flynn Permeability Coefficient Database

The fully-validated database contains 170 measured permeability coefficients, for 127 compounds, encompassing many different organic compound structural classes. The entire fully-validated database is broad in the sense of $\log K_{ow}$ (-3.10 [mannitol] < $\log K_{ow}$ < 4.57 [decanol]; mean = 1.66, median = 1.94, standard deviation = 1.75) and MW (18.0 [water] < MW 584.6 [ouabain]; mean = 201.9, median = 160.25, standard deviation = 129.11) and should be useful for predicting permeability coefficients for a diverse set of organic compounds. Permeability coefficients for large MW compounds (MW > 600) are not represented in the database and can not be accurately estimated. The fully-validated database has measurements for 13 hydrophilic ($\log K_{ow} < 0.0$) compounds (2,3-butanediol, caffeine, ethanol, 2-ethoxy ethanol, α-hydroxyphenyl acetamide, mannitol, methanol, N-nitrosodiethanolamine, ouabain, p-phenylenediamine, scopolamine, urea, and water).

By contrast, Flynn (Flynn, 1990) assembled 97 human skin (unvalidated) permeability coefficients for 94 compounds with a relatively broad range of $\log K_{ow}$ (-2.25 [sucrose] < $\log K_{ow}$ < 5.49 [HC-21-yl octanoate]; mean = 2.05, median = 2.03, standard deviation = 1.40) and MW (18 [water] < MW < 765 [digitoxin]; mean = 238.4, median = 184.2, standard deviation = 148.8). These statistics are based on $\log K_{ow}$ reported by Flynn (Flynn, 1990), for all but four compounds (chlorpheniramine, diethylcarbamazine, N-nitrosodiethanolamine, and ouabain). Values of K_{ow} for these compounds were either obtained from Hansch *et al.* (Hansch *et al.*, 1995) (chlorpheniramine, ouabain) or calculated using Daylight software (PCModels, 1995) (diethylcarbamazine, N-nitrosodiethanolamine). Three *in vivo* measurements are included (benzene, styrene, toluene). There are nine hydrophilic ($\log K_{ow} < 0.0$) compounds in the Flynn database (2,3-butanediol, ethanol, 2-ethoxy ethanol, methanol, N-nitrosodiethanolamine, ouabain,

sucrose, scopolamine, water). Only four (N-nitrosodiethanolamine, ouabain, sucrose, water) of those compounds have a $\log K_{ow} \leq -1$.

Fully-validated permeability coefficients which existed in the Flynn database are contained within brackets in Table 5A.1. Updated values (PCModels, 1995) have replaced the n-alcohol permeability coefficients appearing in the Flynn database (Scheuplein and Blank, 1971). (The only practical difference is that a lower permeability coefficient for propanol (1.2×10^{-3} cm/hr) replaced the value (1.4×10^{-3} cm/hr) included in the Flynn database.) *In vivo* measurements included in the Flynn database (Dutkiewicz and Tyras, 1967; Dutkiewicz and Tyras, 1969), for three compounds (ethylbenzene, styrene, toluene), were excluded from the current analysis. The measured permeability coefficient of hydrocortisone (Hadgraft and Ridout, 1987), determined from a 5% ethanol vehicle, and the permeability of naproxen, determined from an aqueous gel vehicle, were placed in the excluded database. Likewise, etorphine was excluded since Jolicoeur and others (Jolicoeur *et al.*, 1992) identified it as being very different from quite similar compounds, and much different in hairless mouse skin. Digitoxin was excluded since it was likely determined in the unsteady-state. Subsequent investigations for the compounds morphine (Roy *et al.*, 1994) and fentanyl and sufentanil (Roy and Flynn, 1990) were considered in addition to the prior investigation (Roy and Flynn, 1989) cited in the Flynn database. Various compounds from the Flynn database were excluded because they were more than 90% ionized.

5.4.3. Data Analysis with the Conventional Correlation

Multiple linear regressions in this section and sections that follow were performed by standard procedures using JMP (SAS Institute, 1995). The fully-validated permeability data was correlated with Eq. (5.11) with the result:

$$\begin{aligned} &\log P_{cw}\ [\text{cm / hr}] = -2.44(0.12) + 0.514(0.04)\log K_{ow} - 0.0050(0.0005)\ \text{MW} \\ &(n = 170,\ r^2 = 0.551,\ r^2(\text{adj.}) = 0.546,\ \text{RMSE} = 0.803,\ \text{F} - \text{Ratio} = 102.6) \end{aligned} \tag{5.27}$$

This equation is based on almost twice as many measurements as contained in the Flynn database, with roughly half again as many new compounds. Uncertainties in brackets are given as the standard error of the coefficients. Equation (5.27) shows that approximately 55.1% of the variability in the 170 permeability measurements can be explained by variation in $\log K_{ow}$ and MW. The r^2(adj.) statistic is analogous to r^2 but allows for more relevant comparisons between models with different numbers of fitted parameters (JMP User's Guide, (SAS Institute, 1995)). Specifically, $(1 - r^2)$ = error sum of squares/total sum of squares and $(1 - r^2(\text{adj.}) = (1 - r^2)(n - 1)/(n - p)$ where n = # of data points and p = # of parameters. RMSE is the root mean square error of the model, which is zero when the model perfectly correlates the data. When presented in an equation, F-Ratio is the model F-Ratio (sum of squares for the model divided by the degrees of freedom for the model) / (sum of squares for the error divided by the degrees of freedom for the error), and when presented for a term such as the MW or $\log K_{ow}$, the F-Ratio is the effect F-Ratio (sum of squares for the effect divided by the degrees of freedom for the effect) / (sum of squares for the error divided by the degrees of freedom for the error). The model F-ratio = 1 when there is zero correlation with the parameters and is large for correlations with good predictive power. Because the number of fitted parameters is in the denominator of the F-Ratio, changes in the model F-Ratio with an increase in the number of parameters should reflect the effect on predictive power relative to the number of fitted parameters. Thus, a correlation with a larger number of parameters might give a higher r^2 but a lower F-Ratio than a correlation with fewer parameters. This would indicate that the improvement in predictive power (as indicated by a larger r^2) was not as large per parameter as for the equation with fewer parameters. Equation (5.27) should reasonably estimate the skin permeability coefficient for a wide range of organic compounds.

One test of validity, for a quantitative structure-activity relationship, is the ability to predict similar regression coefficients when different subsets of data are analyzed; we

can compare Eq. (5.27) with those derived in Chapter 4 for this purpose. In Chapter 4 we developed a correlation from the fraction of the Flynn database which satisfies the validation criteria of this chapter (i.e., Eq. (4.7)):

$$\log P_{cw}\ [\text{cm / hr}] = -2.76(0.20) + 0.52(0.06)\log K_{ow} - 0.0041(0.0006)\ \text{MW}$$
$$(n = 84,\quad r^2 = 0.537,\ r^2(\text{adj.}) = 0.526,\ \text{RMSE} = 0.820,\ \text{F} - \text{Ratio} = 47.0) \qquad (4.7)$$

The coefficients are quite similar when uncertainty is considered. It is not surprising that the r^2 is as low for Eq. (5.27) as that for the equation based on the Flynn database, since, with more data collected by different research groups there are more potential sources of variation. Without eliminating these sources of variability it is likely that correlations based on a larger (but equivalently validated) set of data will provide more meaningful coefficients.

Importantly, correlations should not be used for estimating permeability coefficients for compounds that are very different from those used to develop the database. That is, appropriate bounds on $\log K_{ow}$ and MW need to be set for all permeability correlations. In the fully-validated database, 95% of the chemicals have $-2.83 \leq \log K_{ow} \leq 4.27$ with equal percentages higher and lower than these bounds. Likewise, 80% of the chemicals have $-1.3 \leq \log K_{ow} \leq 3.85$. The more conservative lower bound (i.e., $\log K_{ow} \geq -1.3$) and the less conservative upper bound (i.e., $\log K_{ow} \geq 4.27$) are chosen. A reasonable lower bound for MW is that of water (i.e., the lowest MW chemical in the database, MW = 18). Permeability coefficients for water are of high quality and there are many permeability coefficients for chemicals with slightly higher MW. In the fully-validated database, 10% of chemicals have MW ≥ 392 and 2.5% have MW ≥ 500. The less conservative bound (i.e., MW ≤ 500) is chosen. Generally, the correlations developed from the entire fully-validated database will provide a reasonable estimate of P_{cw} for aqueous organic compounds with lipophilicities in the range ($-1.3 < \log K_{ow} < 4.3$) and molecular weights in the range ($18 < \text{MW} < 500$).

Equation (5.27), based on a very conventional analysis of permeability data, will be useful for examining effects in the database not accounted for by $\log K_{ow}$ and MW. Figures 5.3 –5.10 show permeability coefficients from the fully-validated database compared to those predicted by Eq. (5.27) in various ways, to examine the factors that influence permeability coefficients.

5.4.4. Trends in the Fully-Validated Database

Permeability measurements in the fully-validated database but not in the Flynn database, and those in both the Flynn database and the fully-validated database are compared with predictions using Eq. (5.27) in Figure 5.3 at different $\log K_{ow}$. The $\log K_{ow}$ from Table 5A.1, which were only different from the $\log K_{ow}$ reported by Flynn (Flynn, 1990) for a few chemicals (see Appendix 4A for details), were used to make this plot. Measurements above the upper dashed line are underestimated by more than an order of magnitude and those below the lower dashed line are overestimated by more than an order of magnitude (these measurements are identified by ↑ and ↓, respectively in Table 5A.1). The overall uncertainty appears similar in both sets of data. Figure 5.3 shows that more valid hydrophilic measurements are available in the fully-validated database than in the validated portion of the Flynn database.

Figure 5.4 compares the fully-validated permeability coefficients and predictions against the year that the measurements were collected. There is no systematic trend over the period of time shown. Several of the trends are better explained by Figure 5.7 (which, we will show later, reveals the (sometimes) systematic discrepancies between laboratories and research groups). This chart indicates that uncertainty in measuring permeability coefficients is nearly the same in recent measurements as in those measured as early as 1964. This plot also shows that measurements of dermal absorption have been made for several decades and still continue until today. The last decade has been a particularly active period of research in dermal absorption.

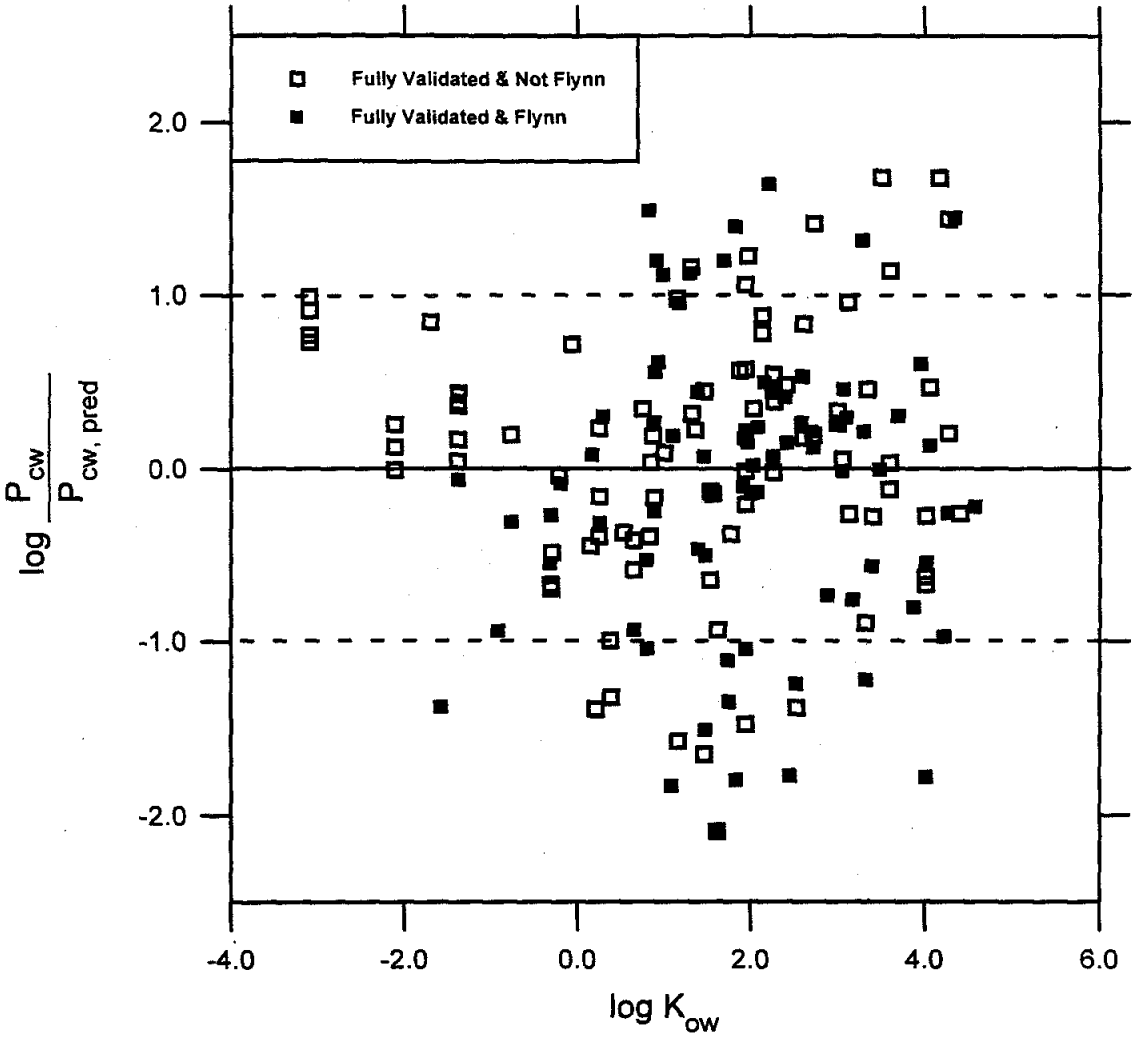

Figure 5.3 A comparison of permeability coefficients in the fully-validated database (P_{cw}) with predictions ($P_{cw,pred}$) from a correlation developed from the fully-validated database, Eq. (5.27).

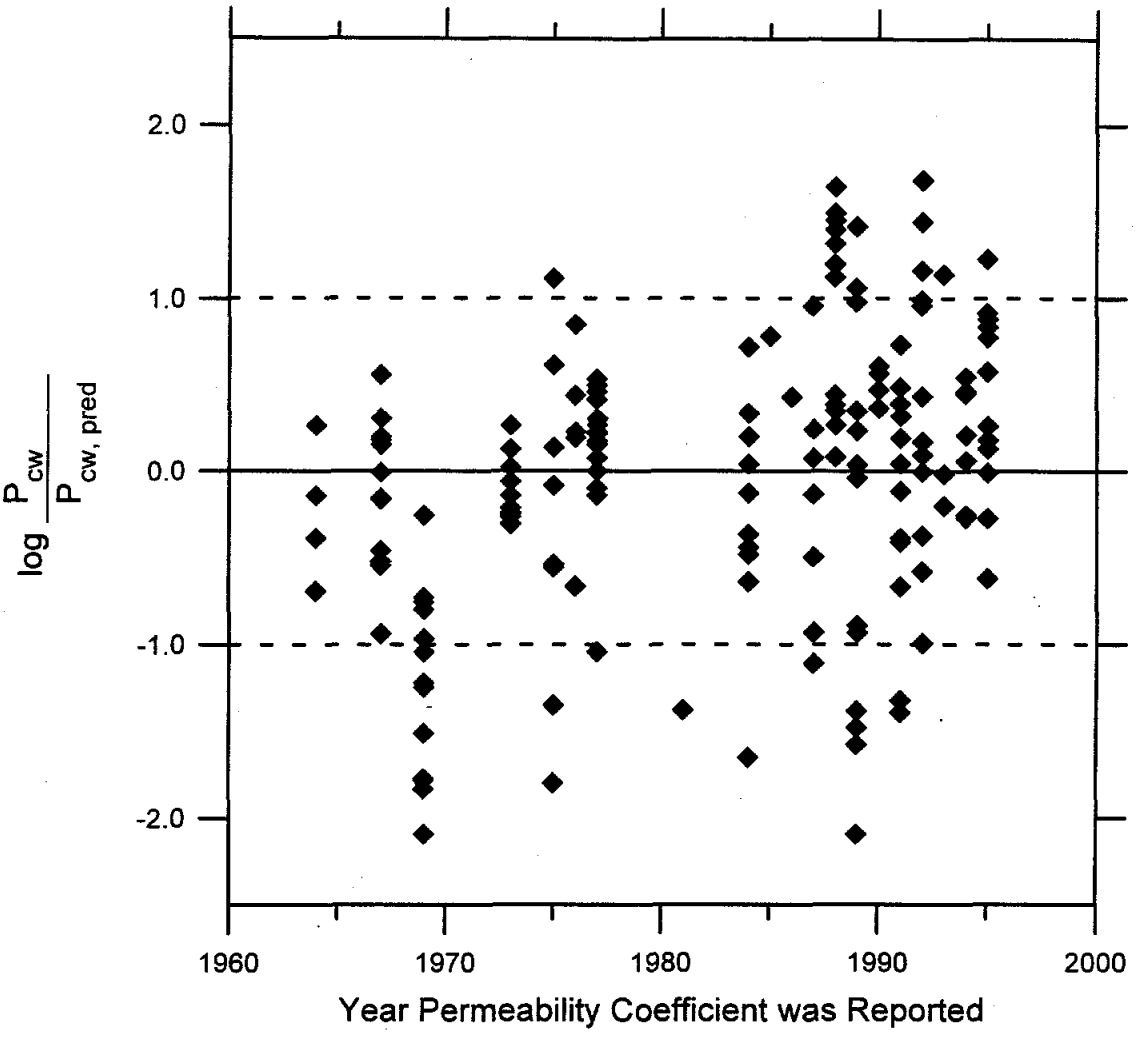

Figure 5.4 A comparison of permeability coefficients in the fully-validated database (P_{cw}) to those predicted ($P_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (5.27), as a function of the year in which the data were published.

Figure 5.5 identifies the effects of temperature and ionization on the ratio of fully-validated measurements to predictions (using Eq. (5.27)). Measurements made at temperatures less than 30°C are overestimated (on average), and measurements made at temperatures greater than 30°C are underestimated (on average). This is the anticipated effect based on most theories, including free-volume theory. To better quantify the effect of temperature, average residuals ($\log P_{cw} - \log P_{cw,pred}$) have been calculated. The average residuals are -0.256 and +0.366 when $T \leq 30°C$ and $T > 30°C$, respectively. When $T \leq 30°C$ (average is 26.7°C) permeability coefficients are overestimated by an average factor of 1.8. When $T > 30°C$ (average is 36.1°C) permeability coefficients are underestimated by an average factor of 2.3. The average temperature for all permeability coefficient measurements shown in Figure 5.4 is 30.6°C. The increase of SC permeability coefficients by a factor of two resulting from approximately a 5°C temperature change is in agreement with experimental evidence of the effect of temperature (Scheuplein and Blank, 1971). These results provide encouraging evidence that a large database of SC permeability coefficients can predict physically realistic trends that are difficult to determine from individual measurements.

Figure 5.5 contains ionization-unadjusted (dashes) and adjusted (solid or open squares) permeability coefficients, for partly ionized compounds, connected to one another by vertical line segments. These results show that adjusting for ionization always increases the calculated permeability coefficient. Several adjustments were inconsequential. The three most underpredicted permeability coefficients (for flurbiprofen, ibuprofen, and indomethacin), measured by Morimoto and colleagues (Morimoto *et al.*, 1992), are in better agreement with Eq. (5.27) before adjustment for fraction unionized. There was no indication in the publication that the reported permeability values had been adjusted for the fraction ionized. We were unable to confirm this with the investigators.

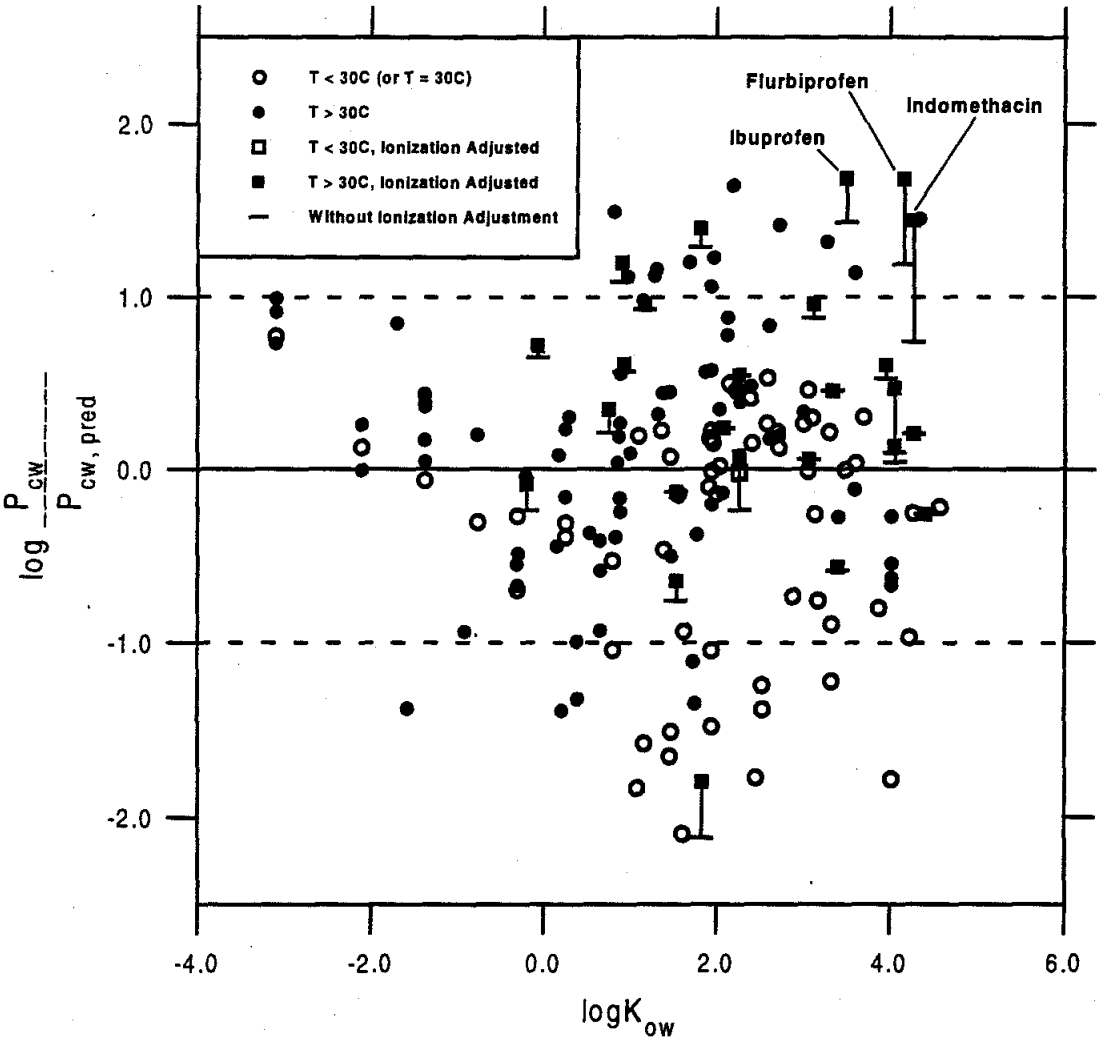

Figure 5.5 A comparison of permeability coefficients in the fully-validated database (P_{cw}) to those predicted ($P_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (5.27), with different temperatures and levels of ionization designated.

Figure 5.6 identifies the measurements in the data base from different investigations of the same compound. Multiple measurements of a single compound are indicated with dashes which are connected by a vertical line. Because p-cresol and hydrocortisone have similar K_{ow}, the replicate measurements for these compounds overlap and the p-cresol data are separately identified. The database has 66 replicate measurements for 23 different compounds. Several coefficients disagree by more than an order of magnitude, and some by two orders of magnitude. These results should change our understanding of predictive permeability correlations in two important ways: (1) we must learn to accept that very accurate predictions can not be made if measurements as variable as these appear to be are used in the development of the correlation, and (2) correlations will provide much more certain estimates on average than individual measurements (i.e., when there are differences between measurements and a judiciously developed predictive correlation the weight of the evidence strongly favors the correlation). The multiple permeability coefficients of water are in close agreement. This may indicate that skin is consistently permeable to water or that researchers select experimental results which are consistent with expected permeability values for water.

Figure 5.7 shows that measurements made in particular laboratories can be systematically different from measurements made in different laboratories. The permeability coefficients which are shown have already been adjusted for the effects of ionization. One would expect, assuming that MW and $\log K_{ow}$ effects were dominant, that permeability coefficients measured in all studies would be randomly scattered about the line of perfect estimation (i.e., $\log P_{cw}/P_{cw,\,pred} = 0.0$). Permeability coefficients for the steroids measured by Scheuplein and others (Scheuplein *et al.*, 1969) are always overestimated by Eq. (5.27). Likewise, for a similar group of chemicals, Siddiqui *et al.* (Siddiqui *et al.*, 1989) report permeability coefficients which are smaller than predicted for six of the seven chemicals studied. Conversely, the penetration of slightly modified

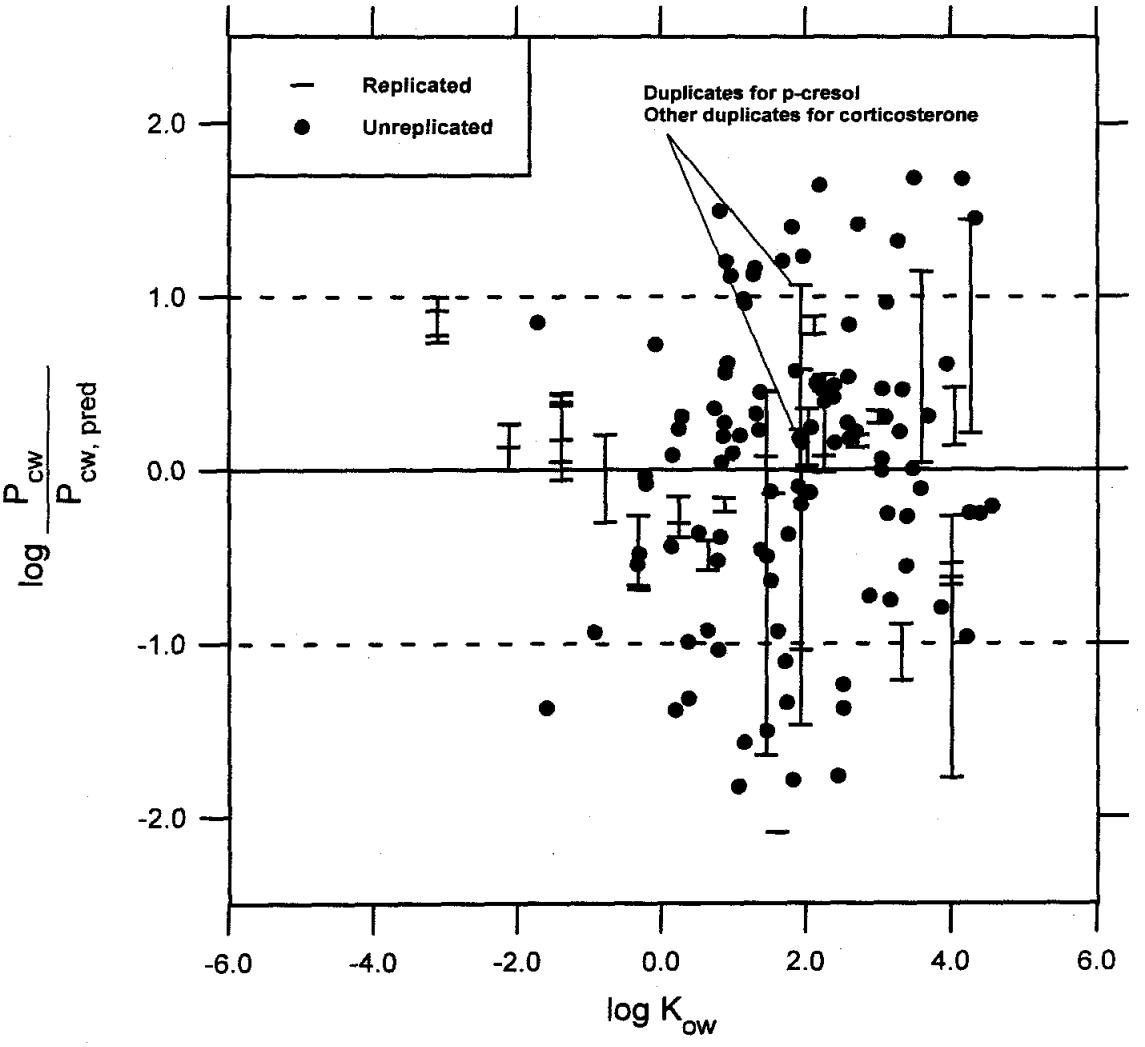

Figure 5.6 A comparison of permeability coefficients in the fully-validated database (P_{cw}) to those predicted ($P_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (5.27), with replicated (multiple permeability coefficients measured for the same compound) measurements designated.

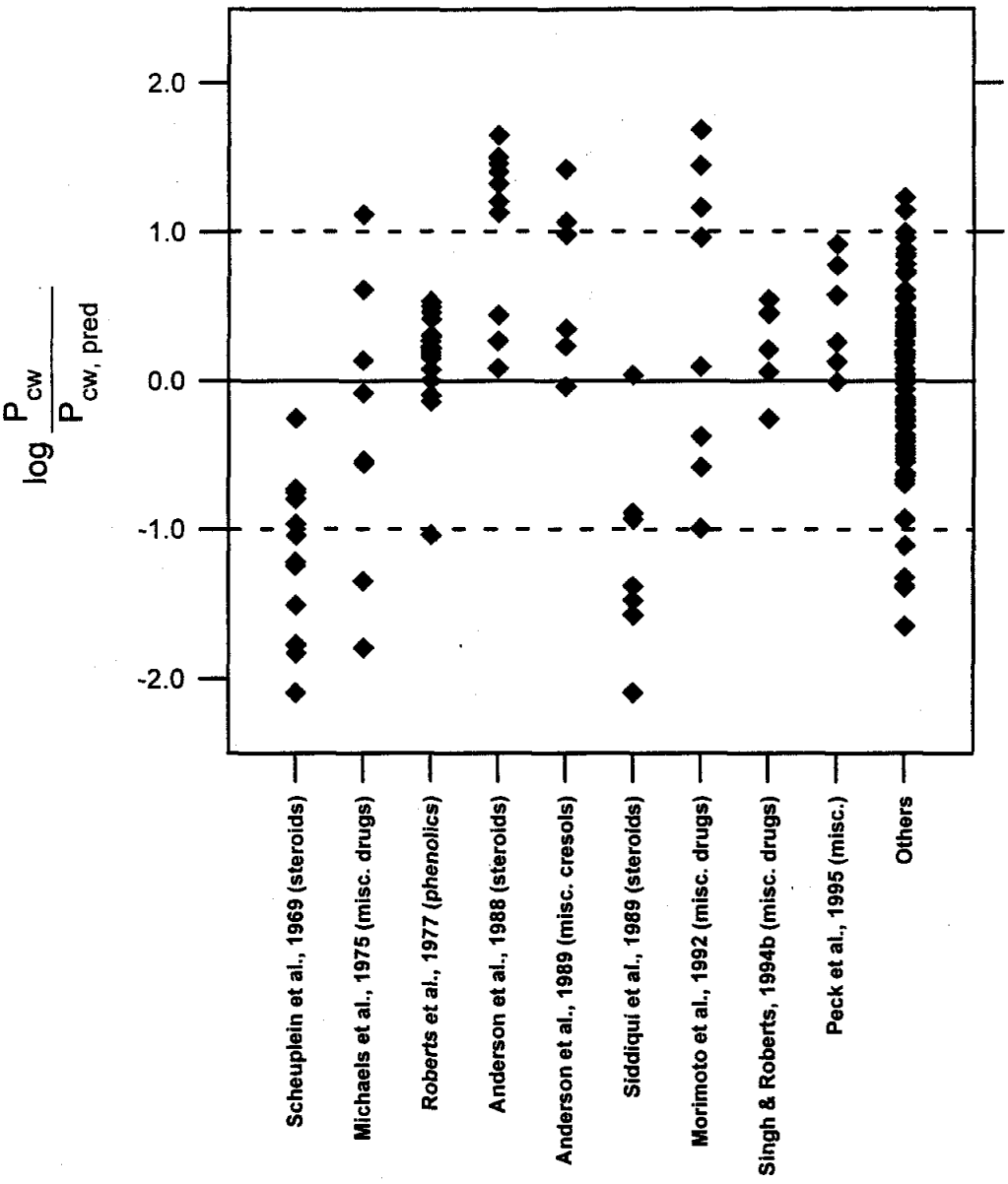

Figure 5.7 A comparison of permeability coefficients in the fully-validated database (P_{cw}) to those predicted ($P_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (5.27), as reported in the prominent investigations.

hydrocortisone steroids measured by Anderson and colleagues (Anderson *et al.*, 1988) are systematically underestimated. These are structurally very similar compounds. Anderson and colleagues also report higher than expected permeability coefficients for several cresols (Anderson and Raykar, 1989) compared to other fully-validated measurements. In light of these two studies, it is possible that the skin used in the Anderson investigations was uncommonly permeable, or that the experimental procedure used by Anderson and colleagues lead to higher than normal permeabilities.

The investigation of Morimoto and colleagues (Morimoto *et al.*, 1992) also had some poorly estimated measurements, although not systematically high or low. After extensive study of their data and procedures we have not been able to identify a cause. Permeability coefficients for the hydrophilic compounds measured by Peck and others (Peck *et al.*, 1995) are always overestimated, but hydrophilic compounds may not be optimally represented by this correlation (which is based predominantly on compounds with $\log K_{ow} > 0$). The remaining permeability coefficients (labeled others) show the anticipated scatter. This plot shows that at least some of the variability not resolved by permeability correlations such as Eq. (5.27) may arise from systematic differences in measurement between laboratories. Whether this is due to skin properties or an unidentified experimental protocol is unknown. The analysis we present in Chapter 6 shows that systematic differences also occur in measurement of SC-water partition coefficients (K_{cw}). Interestingly, when permeability coefficients and partition coefficients were measured by the same research group, systematic differences were usually in the same direction. Elucidation of potential experimental causes for these results would be useful.

Figure 5.8 shows the prediction of the fully-validated permeability coefficients measured in isolated SC, whole epidermis (EPID), and split or full-thickness (SPLIT/FULL) skin as a function of $\log K_{ow}$. The skin type for some measurements was

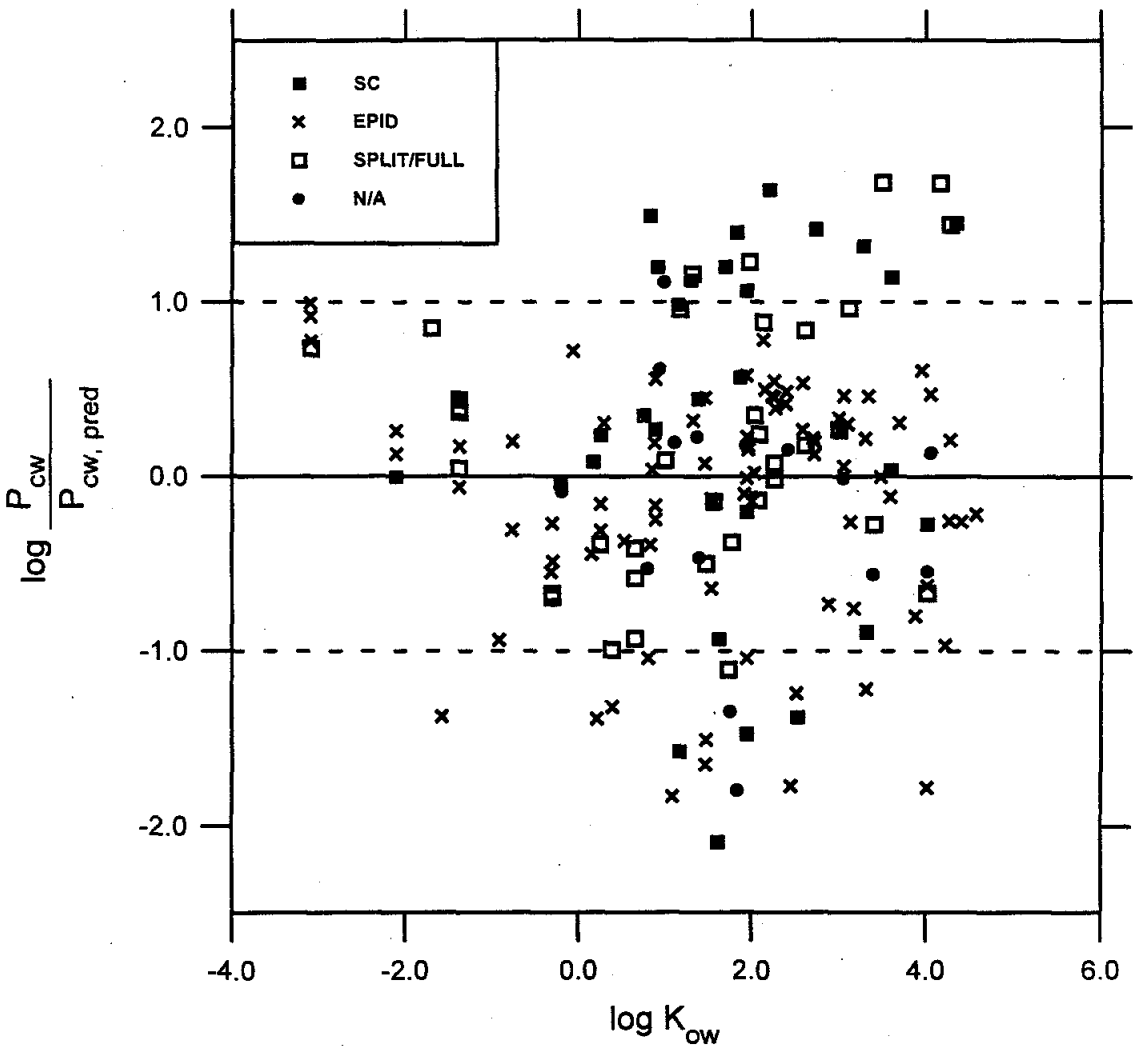

Figure 5.8 A comparison of permeability coefficients in the fully-validated database (P_{cw}) to those predicted ($P_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (5.27), with the skin layers present designated: isolated stratum corneum (SC), intact epidermis (EPID), split or full-thickness skin (SPLIT/FULL), or not specified (N/A).

not specified (N/A) as indicated in Fig. 5.8. More of the permeability coefficients measured with isolated SC are underestimated by the correlation (21 lie above the $\log P_{cw}/P_{cw,\,pred} = 0.0$ line) than overestimated by the correlation (9 lie below the $\log P_{cw}/P_{cw,\,pred} = 0.0$ line). The average residual, $\log(P_{cw}/P_{cw,pred})$, for 30 measurements is 0.298. This would be expected if the extra barriers presented by the viable epidermis (EPID and SPLIT/FULL measurements) and dermis (for SPLIT/FULL measurements) were significant relative to other uncertainties and effects, or if the isolated SC membranes were susceptible to damage during preparation. Measurements using the whole epidermis (EPID) and including dermis (SPLIT/FULL) each showed equal measurements above and below $\log(P_{cw}/P_{cw,pred}) = 0$ (14 measurements for each EPID and SPLIT/FULL above and below). The average residual, $\log(P_{cw}/P_{cw,pred})$, was determined to be –0.148 for 89 measurements plotted as EPID and 0.193 for 36 measurements plotted as SPIT/FULL.

Figure 5.9 shows the ratio of measured to predicted permeability coefficients for surgically removed skin from live humans (labeled patient) and skin removed postmortem (cadaver) as a function of MW of the penetrating compound. The surgically removed skin was obtained by surgical procedures on living human beings. Most commonly, patient skin was from the abdomen or breast (female), although this variable has not been noted. Usually, patient skin was used fresh, or stored frozen for varying periods of time. Cadaver skin is subject to harsh cleaning procedures prior to removal, which might compromise the integrity of the transport barrier. However, the data shown in Fig. 5.9 indicate that on average cadaver skin provided a more resistive barrier to absorption. The average values of $\log(P_{cw}/P_{cw,pred})$ calculated for the 119 measurements using cadaver skin is –0.206 and the average value for 32 measurements using skin excised from live humans is 0.311. It is not clear whether these results can be separated from other factors, such as whether or not the skin was frozen and laboratory effects

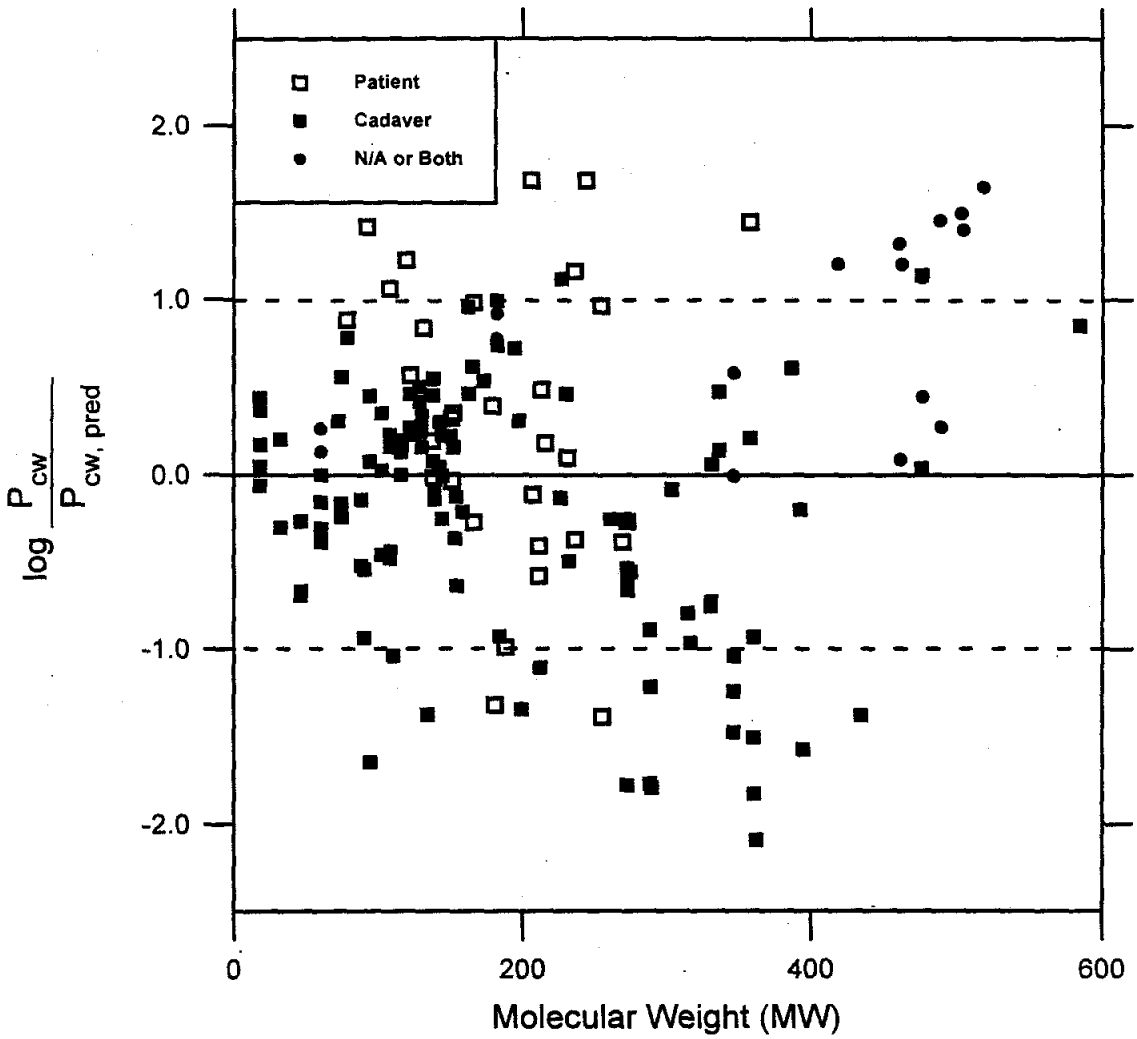

Figure 5.9 A comparison of permeability coefficients in the fully-validated database (P_{cw}) to those predicted ($P_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (5.27), as a function of MW with the skin source designated: excised from living donors (patient), excised from cadavers (cadaver), and not specified (N/A) or an average permeability coefficient from measurements with both patient and cadaver skin (both).

(many of the cadaver results are for steroid measurements by Scheuplein *et al.* (Scheuplein *et al.*, 1969) and Siddiqui *et al.* (Siddiqui *et al.*, 1989)). The skin type for some measurements either were not specified (N/A) or were averages of data from both patient and cadaver and are identified as such on Fig. 5.9.

Figure 5.10 shows the ratio of measured to predicted permeability coefficients as a function of the pH at which the chemical's permeability coefficient was measured. Permeability coefficients for partially ionized chemicals have been adjusted (i.e., divided by the fraction unionized when $f_{ui} > 0.1$). Consequently, all data shown in Fig. 5.10 should represent the permeability coefficient for the unionized species. Measurements for chemicals with no dissociation (labeled ND in Table 5A.1) are grouped at pH = 7 when the pH was not specified. One group was treated differently. Our understanding is that the permeability coefficients for several carboxylic acids (Scheuplein, 1967) were measured at a low enough pH to be unionized. However, we are uninformed of the actual pH and so these measurements are placed at pH = 4. Singh and Roberts (Singh and Roberts, 1994b) reported that their permeability coefficients were measured at the pH when they were 50% ionized (i.e., pH = pK_a). However, they did not list pH or pK_a values for any of the chemicals they studied. Lacking these values, pK_a values for each chemical were calculated using SPARC. Values for compounds whose permeability coefficient was calculated using a range of pH measurements (i.e., ethyl nicotinate, methyl nicotinate, salicylic acid) were placed at the pH corresponding to the midpoint of the pH interval given in Table 5A.1.

Although the data scatter around $\log(P_{cw}/P_{cw,pred}) = 0$, there may be a slight trend. The trend, in Figure 5.10, seems to indicate that permeability coefficients measured from an acidic aqueous vehicle are higher (on average) than permeability coefficients measured from basic aqueous solution. The compounds studied at low pH (acidic solution) are primarily carboxylic acids, and the compounds studied at high pH (basic solution) are

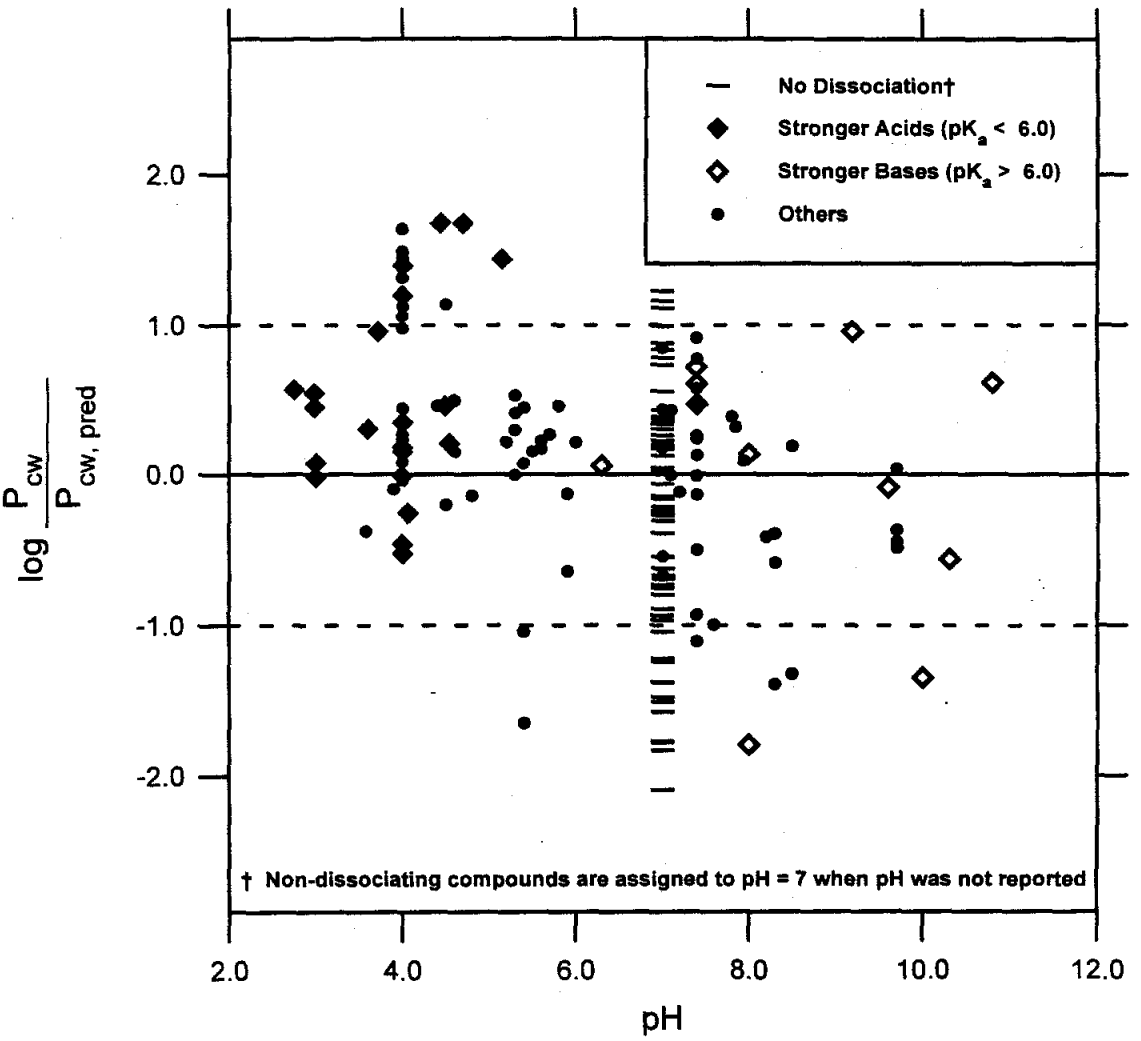

Figure 5.10 A comparison of permeability coefficients in the fully-validated database (P_{cw}) to those predicted ($P_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (5.27), as a function of the pH of the vehicle. Measurements were plotted at pH = 7 when pH was not reported for compounds that do not dissociate.

primarily nitrogen bases. To quantitatively explore the possibility that acid-base interactions (between the skin and the penetrating molecule) cause this weak trend, we distinguished the stronger acids ($pK_a < 6.0$), and the stronger bases ($pK_a > 6.0$), from non-dissociating compounds or other acids ($pK_a > 6.0$) or bases ($pK_a < 6.0$). It appears that the pH more than a chemicals acidity or basicity is responsible for the trend. We neglected the measurements arbitrarily plotted at pH = 7 and regressed the residual $\log(P_{cw}/P_{cw,pred})$ on pH for the 105 remaining measurements. The line determined, $\log(P_{cw}/P_{cw,pred}) = 1.06 - 0.146*pH$, had a slope that was statistically different from zero at the 95% level of confidence, and reasonably described the data ($r^2 = 0.165$, r^2(adj.) = 0.157, RMSE = 0.66, Model F-Ratio = 20.4). The data in Figure 5.10 suggest that acidic conditions may weakly enhance the permeability of the SC in some way. However, more data are needed to confirm this conclusion.

In summary, the data shown in Figures 5.3-5.10 quantify the uncertainty in permeability coefficient measurements and attributes a portion of the uncertainty to several factors affecting SC permeability coefficients. From Figure 5.3, the 84 validated Flynn database permeability coefficients and the 86 newly assembled permeability coefficients have nearly the same amount of uncertainty. Based on results shown in Figures 5.4-5.10, several factors have no effect on the permeability coefficient: (1) permeability coefficients measured as early as 1960 appear to be of equal quality as more recently measured permeability coefficients, (2) additional resistances to penetration by the viable epidermis and dermis appear to have minimal effect on the measured permeability coefficients for chemicals in the validated database, and (3) no consistent differences between skin from living and recently dead subjects. Temperature, ionization, and solution acidity do effect the permeability coefficient and explain a portion of the overall uncertainty. Also, the permeability coefficients collected using isolated SC membranes are frequently high, indicative that the SC membrane may be

difficult to prepare without introducing damage. However, these effects do not explain the differences between replicate permeability coefficients measured for the same compound, which often surpass an order of magnitude. Further, there are unexplained systematic differences between permeability coefficients measured for a group of compounds in a particular laboratory.

Next, we use the fully-validated database to quantitatively examine (1) effects of temperature, (2) the use of MW as a representation for molecular volume, (3) the existence of a different mechanism of absorption for hydrophilic chemicals, and (4) the use of LSER parameters to model SC permeability coefficients.

5.4.5. Effect of Temperature

In Chapter 4 we found that permeability coefficients from the Flynn database (Flynn, 1990) increased on average with increasing temperature. Correlations that incorporated temperature were able to explain more of the variability in permeability coefficients (see Chapter 4). In this section, we investigate the fully-validated database for effects of temperature to explain the apparent trend in Figure 5.5.

The absolute temperature at which P_{cw} was measured was used to reduce the variability of SC permeability coefficients according to Eq. (5.13). Equation (5.13) was fit to the fully-validated database:

$$\log P_{cw}\ [\mathrm{cm/hr}] = -2.412(0.116) + 0.520(0.04)\log K_{ow} - 1.58(0.16)\left(\frac{MW}{T}\right) \quad (5.28)$$

$$(n = 170,\ r^2 = 0.564,\ r^2(\mathrm{adj.}) = 0.559,\ \mathrm{RMSE} = 0.792,\ F-\mathrm{Ratio} = 108.2)$$

Approximately 56.4% of the variability in $\log P_{cw}$ can be explained by variation in $\log K_{ow}$ and MW/T compared to 55.1% when T was not included (i.e., Eq. (5.27)). Consequently, adding temperature to the correlation of permeability coefficients provides a minor improvement. This may arise because temperature only weakly influences individual permeability coefficients, altering them by a factor somewhere between 2 - 5, however,

many measurements are influenced by temperature, so, incorporation of temperature reduces variability in P_{cw} and improves the model.

5.4.6. Effect of Differences in Penetrant Liquid Density

As shown in Eq. (5.10) the diffusivity of a chemical in the SC depends upon the molecular volume of the chemical. Molecular volume is usually replaced by MW. In this section MW is made to more closely resemble MV by making adjustments with an experimental liquid density for the chemical (previously discussed in Section 5.3.3).

Experimental reference liquid densities (ρ_{ref}) and the temperatures at which they were measured (T_{ref}) (Lide, 1996), melting point temperatures (T_m) (Lide, 1996), and critical temperatures (T_c) (Reid *et al.*, 1987) for some compounds from the fully-validated permeability coefficient database are summarized in Table 5A.6. Critical temperatures are not known for some of the compounds for which the reference liquid densities, reference temperatures, and melting point temperatures are known.

The models developed in Section 5.3.3 require the coefficient of thermal expansion, K_T, which is often unknown. If $K_T(T_{ref} - T)$ is small, then

$$\begin{aligned} \exp\left[-K_T(T_{ref} - T)\right] &\approx 1 - K_T(T_{ref} - T) \\ &= 1 - K_T T_c\left(\frac{T_{ref}}{T_c} - \frac{T}{T_c}\right) \end{aligned} \tag{5.29}$$

With this simplification, Eq. (5.15) becomes,

$$\begin{aligned} \log P_{cw} = &\left[\log(a) + \log(D_o / L_c)\right] + f \cdot \log K_{ow} + \beta^{\#} \cdot \frac{MW}{\rho_{ref}} \\ &- \left[\beta^{\#} \cdot K_T\right] \cdot \frac{MW\,(T_{ref} - T)}{\rho_{ref}} \end{aligned} \tag{5.30}$$

and Eq. (5.16), which also includes temperature effects on permeability coefficients, becomes,

$$\log P_{cw} = \left[\log(a) + \log(D_o / L_c)\right] + f \cdot \log K_{ow} + \beta_2^{\#} \cdot \frac{MW}{T\,\rho_{ref}} - \left[\beta_2^{\#} \cdot K_T\right] \cdot \frac{MW\,(T_{ref} - T)}{T\,\rho_{ref}} \tag{5.31}$$

If we assume K_T is approximately constant, then $\beta^{\#} K_T$ can be replaced by an additional parameter which is found by data regression.

Based on the concept of corresponding states, $K_T T_c$ may be more constant than K_T alone. Incorporating Eq. (5.29) into equations which assume $K_T T_c$ is constant (i.e., Eqs. (5.18) and (5.19)), yields respectively:

$$\log P_{cw} = \left[\log(a) + \log(D_o / L_c)\right] + f \cdot \log K_{ow} + \beta^{\#} \cdot \frac{MW}{\rho_{ref}} - \left[\beta^{\#} \cdot K_T T_c\right] \cdot \frac{MW}{\rho_{ref}} \left(\frac{T_{ref}}{T_c} - \frac{T}{T_c}\right) \tag{5.32}$$

and

$$\log P_{cw} = \left[\log(a) + \log(D_o / L_c)\right] + f \cdot \log K_{ow} + \beta_2^{\#} \cdot \frac{MW}{T\,\rho_{ref}} - \left[\beta_2^{\#} \cdot K_T T_c\right] \cdot \frac{MW}{T\,\rho_{ref}} \left(\frac{T_{ref}}{T_c} - \frac{T}{T_c}\right) \tag{5.33}$$

In this case, ($\beta^{\#} K_T T_c$) or ($\beta_2^{\#} K_T T_c$) can be treated as additional regression parameters.

The next several equations were developed using chemicals from the fully-validated database for which reference liquid densities, reference temperatures, and melting point temperatures are readily available. In each regression, the number of data points included is specified as n. First, we assume that K_T is approximately constant for all organic liquids (i.e., using equations in the form of Eqs. (5.30) and (5.31). Assuming no further temperature adjustment (i.e., Eq. (5.30)), we obtain:

$$\log P_{cw}\ [\text{cm/hr}] = -1.74(0.25) + 0.85(0.10)\log K_{ow} - 0.016(0.004)\frac{MW}{\rho_{ref}} - 9.0(4.0)x10^{-5}\frac{MW\,(T_{ref} - T)}{\rho_{ref}} \tag{5.34}$$

$$(n = 53,\quad r^2 = 0.755,\ r^2(\text{adj.}) = 0.740,\ \text{RMSE} = 0.47,\ F - \text{Ratio} = 50.3)$$

Analyzing with Eq. (5.31) which includes a separate effect of temperature on the permeability coefficient, we obtain:

$$\log P_{cw}\ [\text{cm/hr}] = -1.75(0.24) + 0.84(0.10)\log K_{ow} - 4.74(1.06)\frac{MW}{T\,\rho_{ref}} - 0.024(0.012)\frac{MW\,(T_{ref} - T)}{T\,\rho_{ref}} \tag{5.35}$$

$$(n = 53,\quad r^2 = 0.761,\ r^2(\text{adj.}) = 0.746,\ \text{RMSE} = 0.47,\ F - \text{Ratio} = 51.9)$$

Analysis of the same database using the conventional correlation produced the following result:

$$\log P_{cw}\ [\text{cm/hr}] = -1.89(0.26) + 0.81(0.11)\log K_{ow} - 0.014(0.004)\,MW \tag{5.36}$$

$$(n = 53,\quad r^2 = 0.716,\ r^2(\text{adj.}) = 0.704,\ \text{RMSE} = 0.51,\ F - \text{Ratio} = 62.9)$$

Although the predictive power of Eq. (5.34) is greater than that of Eq. (5.36), as indicated by larger r^2, the predictive power per parameter is not as large. When temperature is included (i.e., Eq. (5.35)), the overall predictive power and predictive power per parameter both increase, although only slightly. Next we analyze assuming that the product $K_T T_c$ is constant, which we have shown, is more true.

As shown in Section 5.3.3, $K_T T_c$ has approximately half the variability of K_T. However, regressions which assume $K_T T_c$ is approximately constant (Eqs. (5.32) and (5.33)) require that T_c be known for each compound. Unfortunately, T_c was not found for a few chemicals included in the last regression, and consequently, the database for regression of Eqs. (5.32) and (5.33) is slightly smaller than that for Eqs. (5.30) and (5.31). When only liquid density is included, we obtain:

$$\log P_{cw}\,[\mathrm{cm/hr}] = -1.926(0.33) + 0.747(0.15)\log K_{ow} - 0.0131(0.005)\frac{MW}{\rho_{ref}}$$
$$-0.167(0.091)\frac{MW}{\rho_{ref}}\left(\frac{T_{ref}}{T_c} - \frac{T}{T_c}\right) \tag{5.37}$$
$$(n = 44,\quad r^2 = 0.720,\ r^2(\mathrm{adj.}) = 0.700,\ \mathrm{RMSE} = 0.50,\ F-\mathrm{Ratio} = 34.4)$$

Analyzing the same data with Eq. (5.33) which incorporates temperature effects on the permeability coefficient produces:

$$\log P_{cw}\,[\mathrm{cm/hr}] = -1.910(0.31) + 0.755(0.14)\log K_{ow} - 4.01(1.47)\frac{MW}{\rho_{ref}\,T}$$
$$-47.36(27.36)\frac{MW}{\rho_{ref}\,T}\left(\frac{T_{ref}}{T_c} - \frac{T}{T_c}\right) \tag{5.38}$$
$$(n = 44,\quad r^2 = 0.727,\ r^2(\mathrm{adj.}) = 0.707,\ \mathrm{RMSE} = 0.50,\ F-\mathrm{Ratio} = 35.5)$$

Analyzing the same set of data with the conventional correlation, we obtain:

$$\log P_{cw}\,[\mathrm{cm/hr}] = -1.743(0.41) + 0.855(0.19)\log K_{ow} - 0.017(0.007)\,MW$$
$$(n = 44,\quad r^2 = 0.675,\ r^2(\mathrm{adj.}) = 0.659,\ \mathrm{RMSE} = 0.54,\ F-\mathrm{Ratio} = 42.5) \tag{5.39}$$

Here too, the predictive power of Eq. (5.37) is greater than that of Eq. (5.39), as indicated by larger r^2, but, the predictive power per parameter is not as large. When temperature is included (i.e., Eq. (5.38)), the overall predictive power and predictive power per parameter both increase, although only slightly.

In a limited test to determine whether the data are better correlated by equations which assume constant K_T or constant $K_T T_c$, the same set of 44 data points were regressed to Eqs. (5.30) and (5.31) which assume that K_T is constant. Using Eq. (5.30), we obtain:

$$\log P_{cw}\,[\mathrm{cm/hr}] = -1.90(0.32) + 0.756(0.15)\log K_{ow} - 0.013(0.005)\frac{MW}{\rho_{ref}}$$
$$- 0.00018(0.0001)\frac{MW\,(T_{ref} - T)}{\rho_{ref}} \qquad (5.40)$$

$(n = 44,\ r^2 = 0.721,\ r^2(\mathrm{adj.}) = 0.700,\ \mathrm{RMSE} = 0.50,\ \text{Model F - Ratio} = 34.4)$

Using Eq. (5.31), which includes a separate effect of temperature on the permeability coefficient, we obtain:

$$\log P_{cw}\,[\mathrm{cm/hr}] = -1.89(0.30) + 0.762(0.14)\log K_{ow} - 4.09(1.45)\frac{MW}{T\,\rho_{ref}}$$
$$- 0.052(0.030)\frac{MW\,(T_{ref} - T)}{T\,\rho_{ref}} \qquad (5.41)$$

$(n = 44,\ r^2 = 0.727,\ r^2(\mathrm{adj.}) = 0.707,\ \mathrm{RMSE} = 0.50,\ \text{Model F - Ratio} = 35.6)$

The goodness of fit parameter (r^2) for regressing the same set of 44 data points to the constant K_T equations are 0.721 for liquid density only and 0.727 when liquid density and T are both included. The statistics are not significantly different from those for Eqs. (5.37) (i.e., r^2=0.720) and (5.38) (i.e., r^2=0.727). The same conclusion comes from comparing the F-Ratios for these fits. Analysis with this limited database indicates that there is no significant improvement in incorporating the factor T_c. Since T_c are not available for many compounds, it may be practical to assume K_T is constant rather than assuming $K_T T_c$ is constant.

The correction of MW with a pseudo liquid density has been relatively easy to accomplish and has removed some of the uncertainty in the data. The reduction of unexplained uncertainty is in agreement with results reported by Kasting *et al.* when they substituted MW for MV in an analysis with less data and observed that r^2 was reduced by about 0.05 (Kasting *et al.*, 1987). We have attained essentially the same change in regression statistics without having to calculate MV.

Figure 5.11 illustrates the inadequacy of molecular weight to represent molecular volume in correlations for predicting the permeability coefficient of low and high density organic compounds. Experimental permeability coefficients for chemicals listed in Table 5A.6 are compared with predictions using a conventional correlation (i.e., Eq. (5.36) as a function of ρ_{ref}. This figure shows that permeability coefficients for lower density compounds (density < 0.8 g/mL, designated with triangles) are overestimated on average and permeability coefficients for higher density compounds (density > 1.2 g/mL, designated with ×) are underestimated on average. Clearly, MW is not entirely sufficient at representing the molar volume for compounds with significantly different liquid densities.

Figure 5.12 illustrates the effect of modifying MW by liquid density. Here the same experimental permeability coefficients shown in Fig. 5.11 are compared with predictions from Eq. (5.35) (assumes K_T is constant and accounts for temperature effects on the permeability coefficient). It is easy to see the improvement in prediction of the permeability coefficients for lower and higher density organic compounds. The effect is most pronounced for chemicals with ρ_{ref} > 1.2 g/mL. However, the number of data points is quite small and hence, it is important to treat general conclusions with some caution. Nevertheless, these results suggest that a better estimate of molecular size than MW should improve correlation of permeability coefficients.

Figures 5.13 and 5.14 compare measured permeability coefficients with predictions made using the conventional correlation, Eq. (5.39) (Figure 5.13), and the correlation assuming $K_T T_c$ is constant, (5.38) (Figure 5.14). The results are essentially identical with those in Figures 5.11 and 5.12. Although the number of data points in Fig. 5.13 are slightly fewer than Fig. 5.11 (because T_c was not found for all chemicals listed in Table 5A.6). There is essentially no noticeable difference in the two approaches using liquid density to adjust MW.

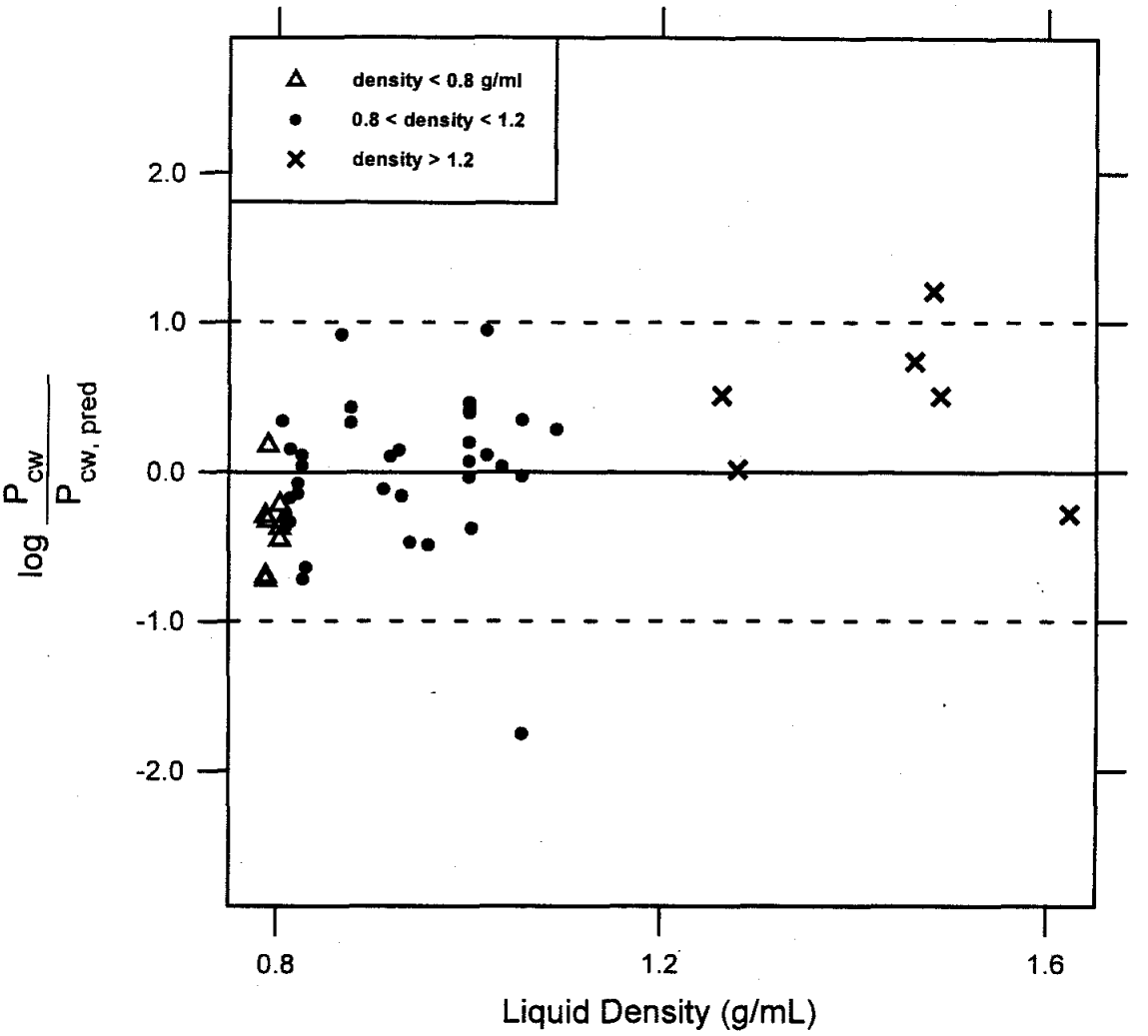

Figure 5.11 A comparison of permeability coefficients of the subset of compounds from the fully-validated database (P_{cw}) for which experimental liquid densities are available to those predicted ($P_{cw,pred}$) by a correlation with MW and $\log K_{ow}$, Eq. (5.37) developed from that subset of permeability coefficients as a function of liquid density at T_{ref} (see Table 5A.6).

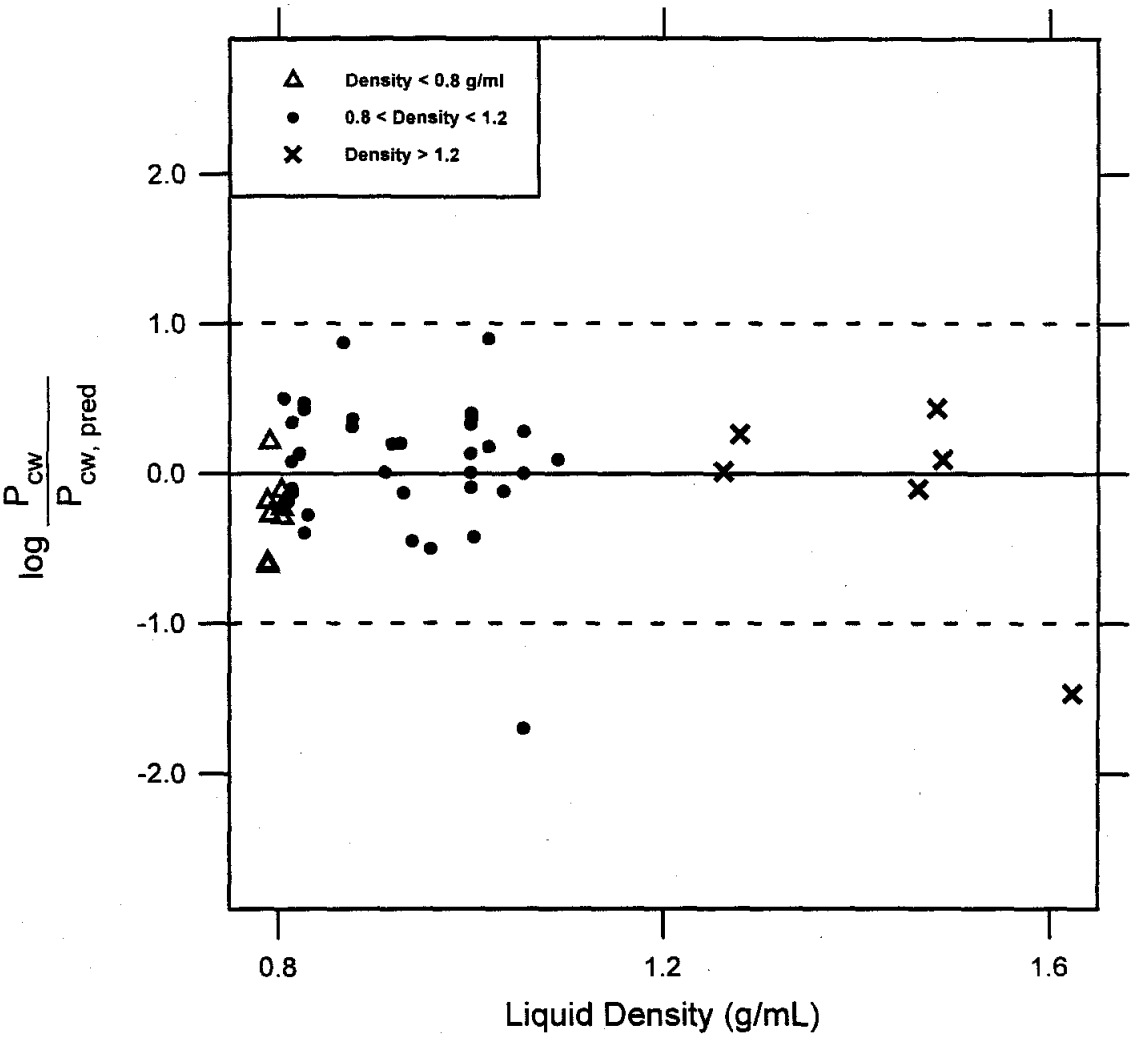

Figure 5.12 A comparison of permeability coefficients of the subset of compounds from the fully-validated database (P_{cw}) for which experimental liquid densities are available to those predicted ($P_{cw,pred}$) by a correlation with MW and $\log K_{ow}$, liquid density, and temperature, Eq. (5.36) developed from that subset of permeability coefficients as a function of liquid density at T_{ref} (see Table 5A.6).

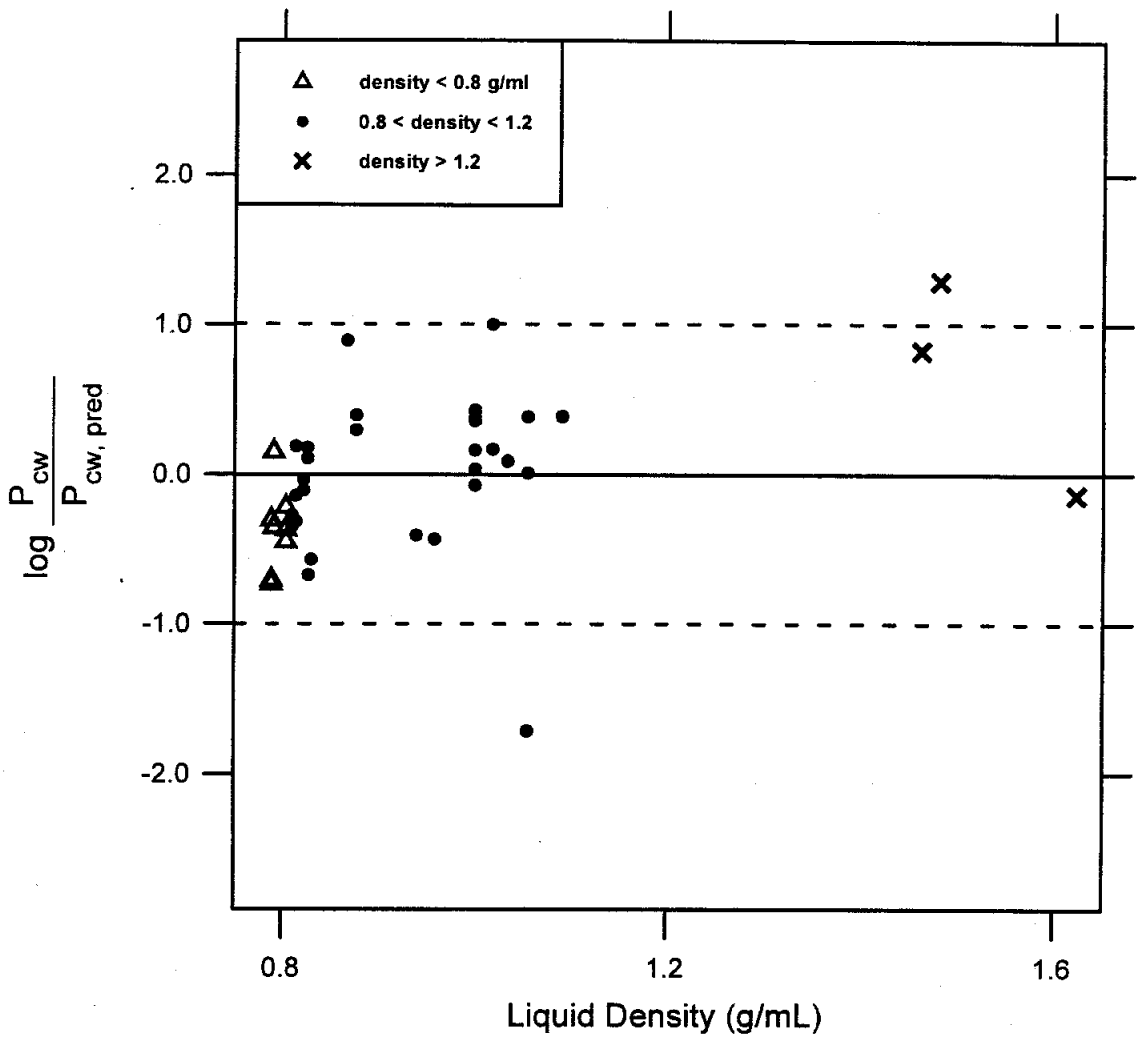

Figure 5.13 A comparison of permeability coefficients of the subset of compounds from the fully-validated database (P_{cw}) for which experimental liquid densities and critical temperatures are available to those predicted ($P_{cw,pred}$) by a correlation with MW and $\log K_{ow}$, Eq. (5.40) developed from that subset of permeability coefficients as a function of liquid density at T_{ref} (see Table 5A.6).

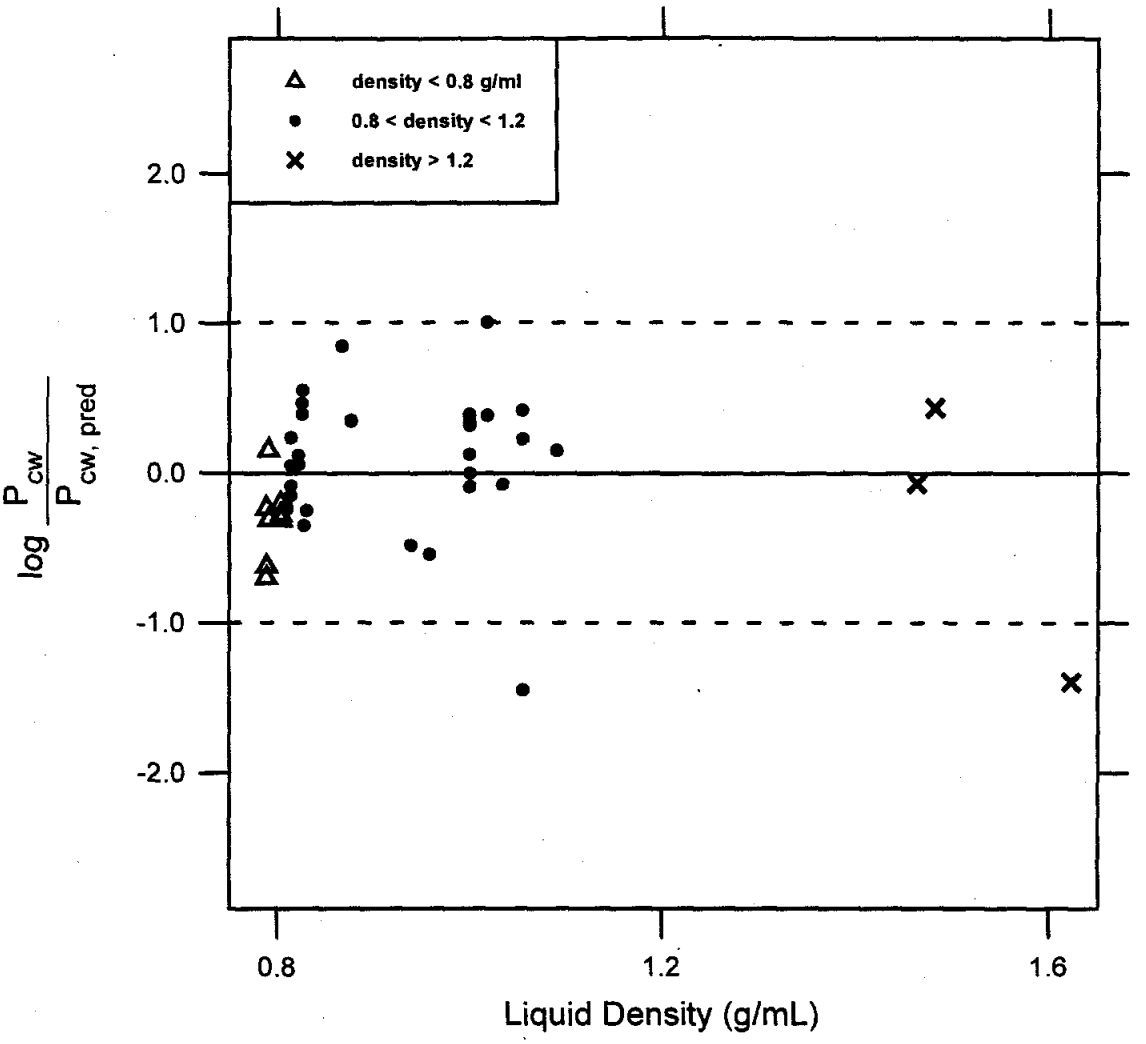

Figure 5.14 A comparison of permeability coefficients of the subset of compounds from the fully-validated database (P_{cw}) for which experimental liquid densities and critical temperatures are available to those predicted ($P_{cw,pred}$) by a correlation with MW, $\log K_{ow}$, liquid density, and temperature, Eq. (5.39) developed from that subset of permeability coefficients as a function of liquid density at T_{ref} (see Table 5A.6).

5.4.7. Analysis with the Two-Mechanism Model

Several authors have argued that hydrophilic chemicals may penetrate skin by a different mechanism than more lipophilic chemicals. Specifically, based on data like those shown in Figure 5.2, they have hypothesized that the permeability coefficient for hydrophilic chemicals depends less strongly on K_{ow} than it does for more lipophilic chemicals. Here we statistically analyze the data in Figure 5.2 to determine if a two-mechanism model is supported by the data. This analysis faces two difficulties: (1) permeability coefficient measurements of hydrophilic chemicals are often more variable than for lipophilic chemicals of similar size, and (2) there are very few measured permeability coefficients for hydrophilic compounds.

First, we arbitrarily divide the data in the fully-validated database into a group of lipophilic ($\log K_{ow} > 0.0$) and hydrophilic ($\log K_{ow} \leq 0.0$) chemicals. Each of these two groups were separately regressed to an equation of the form given by Eq. (5.11). The resulting equations are provided in Table 5.1 along with a description of the database analyzed (n = number of data points and m = number of different chemicals) and regression statistics. Uncertainties in brackets are given as the standard error of the coefficients. Coefficients that are not meaningfully different from zero, at the 95% confidence level, are indicated with an asterisk. When it was determined that a coefficient was not statistically different from zero, the regression was repeated with this term deleted. The hydrophilic fraction of the fully-validated database were analyzed with and without the inclusion of permeability coefficients for three chemicals (5-fluorouracil, paraquat, and TEAB) from the provisional database.

The regressions from Table 5.1 do support the hypothesis that penetration of hydrophilic and lipophilic compounds may be mechanistically different. Indeed, regressions of the permeability coefficient data for hydrophilic and lipophilic compounds apparently depend differently upon K_{ow}. Lipophilic compounds have a stronger

Table 5.1 Analysis of the Hydrophilic ($\log K_{ow} \leq 0.0$) and Lipophilic ($\log K_{ow} > 0.0$) Fractions of the Fully-Validated (FV) and Provisional Database Permeability Coefficients with the Conventional Correlation

Data Reference	Permeability Correlation[a,b] (cm/hr)	r^2	n[d] (m[e])
Lipophilic[c] fraction of the FV Database	$\log P_{cw} = -2.591(0.179) + 0.615(0.062) \log K_{ow} - 0.00538(0.00059)\,MW$ ($r^2 = 0.514$, $r^2(adj.) = 0.507$, RMSE = 0.833, F – Ratio = 73.6)	0.51	142 (114)
Hydrophilic[c] fraction of the FV Database	$\log P_{cw} = -2.917(0.168) + 0.179(0.097) \log K^*_{ow} - 0.00392(0.00077)\,MW$ ($r^2 = 0.569$, $r^2(adj.) = 0.534$, RMSE = 0.477, F – Ratio = 16.5)	0.57	28 (13)
	$\log P_{cw} = -3.134(0.125) - 0.00414(0.00080)\,MW$ ($r^2 = 0.510$, $r^2(adj.) = 0.491$, RMSE = 0.498, F – Ratio = 27.1)	0.51	28 (13)
Hydrophilic[c] fraction of the FV Database & TEAB, Paraquat, and 5-FU	$\log P_{cw} = -3.126(0.153) + 0.110(0.072) \log K^*_{ow} - 0.00430(0.00086)\,MW$ ($r^2 = 0.513$, $r^2(adj.) = 0.485$, RMSE = 0.546, F – Ratio = 18.4)	0.51	38 (16)
	$\log P_{cw} = -3.243(0.135) - 0.00476(0.00083)\,MW$ ($r^2 = 0.480$, $r^2(adj.) = 0.466$, RMSE = 0.555, F – Ratio = 33.3)	0.48	38 (16)

[a] The uncertainties expressed within parenthesis are reported as standard error in the coefficients.
[b] Coefficients indicated with an asterisk (*) are not meaningfully different from zero at the 95% confidence level.
[c] The lipophilic fraction is arbitrarily defined as $\log K_{ow} > 0.0$; the hydrophilic fraction is defined as $\log K_{ow} \leq 0.0$.
[d] Number of data points
[e] Number of different chemicals

dependence upon $\log K_{ow}$ (around 0.615) than hydrophilic compounds (around 0.11-0.18 and frequently not meaningfully different from zero at the 95% confidence level). Permeability coefficients for hydrophilic compounds do depend upon MW, although somewhat less than for the lipophilic fraction (the absolute value of the coefficient multiplying MW is approximately 0.004-0.0045 for the hydrophilic chemicals compared to about 0.0054 for lipophilic chemicals). The leading coefficient of the regressions is significantly smaller (i.e., coefficients are more negative) for hydrophilic compounds (approximately = -3) compared to that for lipophilic chemicals (approximately = -2.6). This could arise if hydrophilic species were confined to the polar regions of the lipid bilayer material, meaning that the area available for their penetration is smaller than that for lipophilic compounds which penetrate through the larger nonpolar region of the lipid bilayer. Since this coefficient physically represents D_c/L_c for a molecule of MW = 0, it would seem that hydrophilic chemicals may diffuse more slowly than lipophilic chemicals. The hydrophilic fraction in this database is small, and these hypothesis need to be tested using a larger number of measurements for hydrophilic chemicals.

The Table 5.1 correlations are informative, but, the model does not force a smooth transition between P_{cw} for hydrophilic and lipophilic compounds. Also, the analysis summarized in Table 5.1 arbitrarily assumed that $\log K_{ow} = 0.0$ divided chemicals penetrating by hydrophilic and lipophilic mechanisms. We also analyzed the fully-validated database using Eqs. (5.22) and (5.24) which require the equations for hydrophilic and lipophilic chemicals to intersect at $K_{ow}^{\#}$, the transition between hydrophilic and lipophilic mechanisms. These equations assume that the only difference between hydrophilic and lipophilic chemicals is in the effect of K_{ow}. That is, the effect of molecular size (i.e., MW) is the same for both hydrophilic and lipophilic chemicals.

The fully-validated database was analyzed with Eq. (5.22) for different values of $\log K_{ow}^{\#}$ (i.e., $\log K_{ow}^{\#}$ = -1, -0.75, -0.5, -0.25, 0.0, 0.25, 0.5, 0.75, and 1.0). The best fit occurred when $\log K_{ow}^{\#}$ = -0.5:

$$\log P_{cw}\,[\mathrm{cm/hr}] = -2.9(0.2) + 0.11(0.14)\log K_{ow}^{*} + 0.5(0.17)(\log K_{ow} + 0.5)\delta - 0.0052(0.0005)\,\mathrm{MW} \tag{5.42}$$

$$(n = 170,\ r^2 = 0.573,\ r^2(\mathrm{adj.}) = 0.566,\ \mathrm{RMSE} = 0.786,\ F-\mathrm{Ratio} = 74.4)$$

the asterisk indicates that the coefficient 0.11 multiplying $\log K_{ow}$ is not meaningfully different from zero at the 95% level of confidence. This result is based on only twenty measurements for six chemicals with $\log K_{ow}^{\#} \leq$ -0.5. According to Eqs. (5.20) and (5.21), the coefficient multiplying $\log K_{ow}$ is 0.11 (= b) for hydrophilic chemicals compared to 0.61 (= b + c) for lipophilic chemicals. The coefficient multiplying MW (d) is -0.0052 (assumed the same for both pathways), and (a = -2.90) and [(a - c· $\log K_{ow}^{\#}$) = -2.84] are the intercepts for the hydrophilic and lipophilic compounds, respectively.

We also analyzed the fully-validated database assuming that permeability coefficients for hydrophilic chemicals do not depend upon K_{ow} at all (Eq. (5.24)):

$$\log P_{cw}\,[\mathrm{cm/hr}] = -3.01(0.13) + 0.635(0.05)(\log K_{ow} + 0.5)\delta - 0.0052(0.0005)\,\mathrm{MW} \tag{5.43}$$

$$(n = 170,\ r^2 = 0.572,\ r^2(\mathrm{adj.}) = 0.567,\ \mathrm{RMSE} = 0.785,\ F-\mathrm{Ratio} = 111.5)$$

According to Eq. (5.24), (a = -3.01) is the average $\log P_{cw}$ for the hydrophilic chemicals, and (c = 0.635) is the slope and [(a - c· $\log K_{ow}^{\#}$) = -2.69] the intercept of the linear regression for lipophilic compounds.

Equation (5.42) showed that hydrophilic chemicals did not have a meaningful dependence on $\log K_{ow}$, so the statistics decreased only slightly when K_{ow} dependence was removed for the hydrophilic chemicals (i.e., Eq. (5.43)). This result is consistent with the premise that hydrophilic chemicals penetrate by a different mechanism, but the data are

limited and these findings must be substantiated by more measurements that are of better quality.

5.4.8. Analysis with a Linear Solvation-Energy Relationship

The LSER model, Eq. (5.26), has been used to analyze chemicals from the fully-validated database for which solvatochromic parameters could be found. These chemicals, identified in Table 5A.1 with an asterisk left of the chemical name, represent a low MW fraction of the fully-validated database. The solvatochromic parameters, all from Abraham *et al.* (Abraham *et al.*, 1994), were calculated by averaging multiple normalized solvent effects on a variety of chemical properties involving many varied types of indicators (Abraham *et al.*, 1994). These experimentally determined descriptors are now available for well over 1000 solutes (Abraham *et al.*, 1994). Table 5A.5 contains the LSER parameters used.

The LSER database consists of 65 fully-validated permeability coefficients for 43 different chemicals and is larger than other known databases analyzed with the LSER model (e.g., (Abraham *et al.*, 1995)). For purposes of analysis, the entire LSER database is divided into lipophilic ($\log K_{ow} > 0.0$) and hydrophilic ($\log K_{ow} \leq 0.0$) fractions. Various regressions of the entire LSER database, the lipophilic fraction, and the hydrophilic fraction of the fully-validated database are listed in Table 5.2, along with a description of the database analyzed (n = number of data points and m = number of different chemicals) and the r^2 statistic. Generally, each of the three databases is analyzed with the conventional correlation first, followed by analysis with the full LSER model and then by a reduced LSER model which includes only the parameters which are meaningful at the 95% level of confidence. The number of data points (n) and the number of different chemicals (m) included in the regression are listed. Coefficients that are not meaningfully different from zero, at the 95% confidence level, are indicated with an asterisk. Uncertainties in brackets are given as the standard error of the coefficients.

Analysis of the entire LSER database is presented first in Table 5.2. The regression fit on all LSER parameters gives a better fit to the data ($r^2 = 0.76$) than a fit to the conventional correlation ($r^2 = 0.68$). However, for this set of data, only the hydrogen bond basicity (β) and molecular volume (V_x) are statistically significant predictors of permeability. Regression on only these two parameters gave a comparable fit ($r^2 = 0.70$) to the fit with the conventional correlation ($r^2 = 0.68$). Interestingly, the fit is much poorer when the LSER database is analyzed with β and MW ($r^2 = 0.56$) than when analyzed with β and V_x ($r^2 = 0.70$), indicating that for the LSER database MW is not a good substitute for V_x. Regression on the two most significant parameters, β and $\log K_{ow}$, produces a minimal improvement of fit ($r^2 = 0.72$) compared to when β and V_x are used ($r^2 = 0.70$), indicating probably that β and $\log K_{ow}$ contain similar chemical information.

Regressions of the lipophilic fraction of the LSER database are listed next in Table 5.2. The regressions to the entire LSER database and the lipophilic fraction are not meaningfully different, but this is probably because the lipophilic fraction dominates the LSER database. The hydrogen bond basicity, β, and calculated volume, V_x, are meaningful and the hydrogen bond acidity, α, is marginally significant at the 95% confidence level for analysis of the lipophilic chemicals. The fit on β and V_x alone give coefficients that are not statistically different from the fit of the entire LSER database, although the fit is poorer ($r^2 = 0.70$ for the LSER database compared to $r^2 = 0.52$ for the lipophilic fraction). This fit on β and V_x is comparable in quality to the fit of the conventional correlation to the same data ($r^2 = 0.54$).

The hydrophilic chemicals were analyzed with the conventional correlation and neither MW or $\log K_{ow}$ were found to be significant. No terms in the LSER model were significant when at least two terms (i.e., any two of α, β, π or V_x) were included in analysis. V_x was significant when it was included alone in the LSER model, and that fit was able to represent as much variability in permeability coefficients for the hydrophilic

Table 5.2 Comparison of Regressions of a Subset of the Fully-Validated (FV) Database with LSER Parameters and with $\log K_{ow}$ and MW

Data Reference	Permeability Correlation[a,b] (cm/hr)	r^2	n^f (m^g)
Entire LSER Database[c]	$\log P_{cw} = -2.026(0.24) + 0.740(0.11)\log K_{ow} - 0.0114(0.004)\,MW$	0.68	65 (43)
Entire LSER Database	$\log P_{cw} = -1.67(0.23) - 0.46(0.24)\,\pi^* - 0.46(0.27)\,\alpha^* - 3.44(0.41)\,\beta + 1.76(0.19)\,V_x$	0.76	65 (43)
Entire LSER Database	$\log P_{cw} = -2.215(0.20) - 3.276(0.41)\,\beta + 1.731(0.17)\,V_x$ (r^2(adj.) = 0.691, RMSE = 0.472)	0.70	65 (43)
Entire LSER Database	$\log P_{cw} = -1.998(0.19) - 1.689(0.41)\,\beta + 0.409(0.04)\log K_{ow}$	0.72	65 (43)
Entire LSER Database	$\log P_{cw} = -2.428(0.28) - 2.070(0.50)\,\beta + 0.012(0.002)\,MW$	0.56	65 (43)
Lipophilic[d] fraction of LSER Database	$\log P_{cw} = -2.234(0.33) + 0.758(0.12)\log K_{ow} - 0.0098(0.004)\,MW$	0.54	51 (39)

Data Reference	Permeability Correlation[a,b] (cm/hr)	r^2	n^f (m^g)
Lipophilic[d] fraction of LSER Database	$\log P_{cw} = -2.04(0.32) - 0.12(0.28)\,\pi^* - 1.08(0.37)\,\alpha - 3.01(0.50)\,\beta + 2.01(0.29)\,V_x$	0.66	51 (39)
Lipophilic[d] fraction of LSER Database	$\log P_{cw} = -2.348(0.32) - 3.231(0.53)\,\beta + 1.853(0.33)\,V_x$	0.52	51 (39)
Hydrophilic[e] fraction of LSER Database	$\log P_{cw} = -3.090(0.39) - 0.18(0.23)\log K^*_{ow} - 0.00644(0.006)\,MW^*$	0.52	14 (4)
Hydrophilic[e] fraction of LSER Database	$\log P_{cw} = -3.132(0.42) - 1.550(1.75)\,\beta^* - 2.20(1.24)\,V^*_x$	0.55	14 (4)
Hydrophilic[e] fraction of LSER Database	$\log P_{cw} = -2.774(0.110) - 1.147(0.321)\,V_x$	0.51	14 (4)

[a] The uncertainties expressed within parenthesis are reported as standard error in the coefficients.
[b] Coefficients indicated with an asterisk (*) are not meaningfully different from zero at the 95% confidence level.
[c] Measurements in the fully-validated database for which LSER parameters are available (primarily low MW compounds).
[d] The lipophilic fraction is arbitrarily defined as $\log K_{ow} > 0.0$.
[e] The hydrophilic fraction is arbitrarily defined as $\log K_{ow} \leq 0.0$.
[f] Number of data points
[g] Number of different chemicals

chemicals ($r^2 = 0.51$) as when the conventional correlation was used ($r^2 = 0.52$). The leading coefficients are significantly smaller (approximately = -2.8 for the LSER model with only V_x) than the leading coefficients for the entire LSER database (approximately = -2.2 for the LSER model with β and V_x) or the lipophilic fraction of that database is analyzed (approximately = -2.3 for the LSER model with β and V_x). This could be further evidence that hydrophilic species were confined to the polar regions of the lipid bilayer material, meaning that the area available for their penetration is smaller than that for lipophilic compounds which penetrate through the larger nonpolar region of the lipid bilayer.

We conclude that regression on the independent chemical descriptors α, β, π, V_x, provides insight into the separate physicochemical effects of transport through skin, but does not greatly improve the predictive ability of the model. Importantly, the chemical diversity of the solutes used in the LSER regression are by no means optimal (much less diverse than the entire fully-validated database), which makes precise specification of the coefficients difficult. Moreover, LSER parameters are not available for, and would be difficult to determine for many of the compounds for which permeability coefficients have been measured.

The LSER database could be expanded to include new chemicals if LSER parameters were available for those chemicals. Potentially, LSER parameters for one chemical could be calculated from known LSER parameters for a second, structurally related chemical. Methods for these calculations might include group contributions, etc. Table 5.3 lists thirteen chemicals from the fully-validated database (with unknown LSER parameters) that are very similar to chemicals with known LSER parameters.

Table 5.3 Compounds structurally similar to other chemicals with known P_{cw} but unknown LSER parameters

Fully-Validated Database Chemical (with Unknown LSER Parameters)	**Similar Chemical (with known LSER Parameters)**
2-nitro-p-phenylenediamine	2-nitroaniline
2-amino-4-nitrophenol	3-nitroaniline or 4-nitroaniline
4-amino-2-nitrophenol	2-nitrophenol
4-chloro-m-phenylenediamine	4-chloroaniline
o-phenylenediamine	aniline
salicylic acid	benzoic acid
p-phenylenediamine	aniline
methyl hydroxy benzoate	methyl benzoate
chloroxylenol	4-chloro-3-methylphenol
chloroxylenol	4-chloro-3-methylphenol
2,3-butanediol	2-butanol
2,4-dichlorophenol	2-chlorophenol
4-hydroxybenzyl alcohol	benzyl alcohol

5.5. Conclusions

Overall, then, we have presented a sizable (170 measurements for 127 compounds) and diverse (MW ranging from 18 to 584, and $\log K_{ow}$ ranging from -3.1 to 4.6) database of pharmacological and toxic compounds which have been examined for certain quality criteria. Ionization can dramatically impact the magnitude of the permeability coefficients, consequently, permeability coefficients measured under conditions at which chemical was partially ionized were adjusted for ionization. Analysis of these data have shown that temperature variation in the data (between 25 and 37°C) may introduce 2-5 fold differences in the permeability measurements. Despite the application of a set of quality criteria, repeated measurements from different labs have differed by more than one order of magnitude. Identifying the cause of differences between replicate measurements is required.

The correlation based on only K_{ow} and MW is the simplest equation to provide estimates for the SC permeability coefficient. Other factors can be considered with some improvement in predictability. For example, Eq. (5.35) includes temperature and liquid density which better represents compounds with relatively low or high densities. The results of Eq. (5.35) indicate that estimates of permeability coefficients for low or high molecular weight chemicals can be improve if MW is adjusted by liquid density or by using a method that includes the molecular volume instead of MW. Based on the smaller data set, the LSER correlations do not appear to be more useful than conventional correlations, especially since the parameters are not as available. More permeability coefficient data for hydrophilic compounds is required to decide conclusively whether hydrophilic chemicals penetrate skin by a different mechanism than lipophilic chemicals.

5.6. Notation

B	=	A parameter measuring the SC/VE permeability ratio
D_c	=	Effective diffusivity of the absorbing chemical in the SC
D_e	=	Effective diffusivity of the absorbing chemical in the VE
D_o	=	Diffusion constant of hypothetical chemical having zero MV
E_a	=	Activation energy in an Arrhenius-type relationship, Eq. (5.12)
f_{ui}	=	Fraction of the total chemical dose that is unionized in the vehicle
K_a	=	Acid dissociation constant for the absorbing chemical
K_{ce}	=	Partition coefficient between the SC and VE for the absorbing chemical
K_{cw}	=	Partition coefficient between the SC and water for the absorbing chemical
K_{ew}	=	Partition coefficient between the VE and water for the absorbing chemical
K_{ow}	=	Octanol-water partition coefficient of the penetrating chemical
$K_{ow}^{\#}$	=	Value of K_{ow} at which the piecewise linear regression changes slope and distinguishes the hydrophilic compounds from the lipophilic compounds
K_T	=	Coefficient of thermal expansion
L_c	=	Effective thickness of the SC
L_e	=	Effective thickness of the VE
MV	=	Molecular volume of the absorbing chemical
MW	=	Molecular weight of the absorbing chemical
P_{cw}	=	Steady-state permeability of the SC from water
$P_{cw,\,pred}$	=	Predicted steady-state permeability of the SC from water
P_{ew}	=	Steady-state permeability of the VE from water
P_w	=	Steady-state permeability of the SC-VE composite membrane from water
pH	=	Negative logarithm of the hydrogen ion molarity $-\log_{10}[H^+]$
pK_a	=	Negative logarithm of the acidity equilibrium constant $-\log_{10}[K_a]$
R	=	Ideal gas constant
SC	=	Stratum corneum
T	=	Absolute temperature (Kelvin)
T_c	=	Critical temperature of the absorbing chemical
T_{ref}	=	Absolute temperature (Kelvin) of the liquid density measurement
V_x	=	Characteristic volume of McGowans (LSER parameter)
VE	=	Viable epidermis

Greek

α	=	Hydrogen bond acidity of the absorbing chemical (LSER parameter)
β	=	Hydrogen bond basicity of the absorbing chemical (LSER parameter)
δ	=	Indicator variable defined by Eq. (5.23)
λ	=	General linear free energy property
π	=	Dipolarity/polarizability of the absorbing chemical (LSER parameter)

ρ = Pseudo liquid density of the absorbing chemical
ρ_{ref} = Experimental liquid density of the absorbing chemical

Superscripts
H = Hydrophilic chemicals
L = Lipophilic chemicals
* = Regression constants not statistically different from zero at 95% confidence
# = Distinguish regression constants (different meaning when used with K_{ow})

5.7. References

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5.8. Appendix 5A: Tables of Permeability Coefficients, Regression Parameters and Intermediate Calculations (Calculation of Natural pH and Adjustment of pK_a for Temperature)

Table 5A.1 The Fully-Validated Database

Table 5A.2 The Provisional Database

Table 5A.3 The Excluded Database

Table 5A.4 Estimates of the Fraction of the Unionized Species at the Experimental Temperature (T)

Table 5A.5 LSER Parameters and Permeability Coefficients for Compounds in the LSER Database

Table 5A.6 Parameters to Modify Molecular Weight with an Experimental Liquid Density

Table 5A.1 The Fully-Validated Database

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{ow} (rep)[c]	P_{ow} (adj)	↑↓[d]	f_u[e]	pH[f]	Skin[g]	Reference
	[Aldosterone]	1.08	360.4	26	3.00E-06	3.00E-06	↓	1	ND	EPID	Scheuplein et al., 1969
	2-Amino-4-Nitrophenol	1.53	154.1	32	6.60E-04	8.59E-04		0.78	5.9	EPID	Bronaugh & Congdon, 1984
	4-Amino-2-Nitrophenol	1.53	154.1	32	2.80E-03	2.80E-03		0.98	5.9	EPID	Bronaugh & Congdon, 1984
	Aminopyrine	1.00	231.3	37	[1.02E-03]	1.02E-03		1	7.94	FULL	Morimoto et al., 1992
	[Amylobarbital]	2.07	226.3	30	2.27E-03	2.27E-03		1	7.4	FULL	Hadgraft & Ridout, 1987
	Antipyrine	0.38	188.2	37	[6.58E-05]	6.58E-05		1	7.6	FULL	Morimoto et al., 1992
	Atrazine	2.61	215.7	37	1.00E-02	1.00E-02		1	7[h]	SPLIT	Nakai et al., 1995
	[Atropine]	1.83	289.4	30	8.60E-06	1.39E-05	↓	0.62	8	N/A	Michaels et al., 1975
	[Barbital]	0.65	184.2	30	1.10E-04	1.10E-04		1	7.4	FULL	Hadgraft & Ridout, 1987
*	Benzene	2.13	78.1	31	1.11E-01	1.11E-01		1	ND	EPID	Blank & McAuliffe, 1985
*	Benzene	2.13	78.1	37	1.40E-01	1.40E-01		1	ND	SPLIT	Nakai et al., 1995
*	Benzoic acid	1.87	122.1	35	3.00E-02	3.00E-02		1	2.75	SC	Parry et al., 1990
*	[Benzyl alcohol]	1.10	108.1	25[h]	6.00E-03	6.00E-03		1	ND	N/A	Roberts, 1976
	Betamethasone	1.94	392.5	37	2.44E-04	2.44E-04		1	4.5	SC[i]	Kubota & Maibach, 1993
	Betamethasone-17-valerate	3.60	476.0	37	1.46E-02	1.46E-02	↑	1	4.5	SC[i]	Kubota & Maibach, 1993
	Betamethasone-17-valerate	3.60	476.0	25[h]	1.15E-03	1.15E-03		1	ND	SC[h]	Siddiqui et al., 1989
*	[p-Bromophenol]	2.59	173.0	25	3.61E-02	3.61E-02		1	[5.3]	EPID	Roberts et al., 1977
	[2,3-Butanediol]	-0.92	90.1	30	5.00E-05[j]	5.00E-05		1	ND	EPID	Blank et al., 1967
*	[Butanoic acid]	0.79	88.1	N/A	1.00E-03	1.00E-03		1[k]	N/A	N/A	Scheuplein, 1967
*	[Butanol]	0.88	74.1	25	2.50E-03	2.50E-03		1	ND	EPID	Scheuplein & Blank, 1973
*	Butanol	0.88	74.1	30	3.00E-03	3.00E-03		1	ND	EPID	Blank et al., 1967
*	[2-Butanone]	0.29	72.1	30	4.50E-03	4.50E-03		1	ND	EPID	Blank et al., 1967
	[Butobarbitone]	1.73	212.2	30	1.90E-04	1.90E-04	↓	1	7.4	FULL	Hadgraft & Ridout, 1987
	Caffeine	-0.07	194.2	30	1.60E-03	1.80E-03		0.89	7.4[h]	EPID[h]	Southwell et al., 1984
	4-Chloro-m-phenylenediamine	0.85	142.6	32	2.10E-03	2.10E-03		1	9.7	EPID	Bronaugh & Congdon, 1984
*	[Chlorocresol]	3.10	142.6	25	5.50E-02	5.50E-02		1	[5.3]	EPID	Roberts et al., 1977

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	↑↓[d]	f_{ui}[e]	pH[f]	Skin[g]	Reference
*	Chloroform	1.97	119.4	37	1.60E-01	1.60E-01	↑	1	ND	SPLIT	Nakai et al., 1995
*	[o-Chlorophenol]	2.15	128.6	25	3.31E-02	3.31E-02		1	[4.6]	EPID	Roberts et al., 1977
*	[p-Chlorophenol]	2.39	128.6	25	3.63E-02	3.63E-02		1	[5.3]	EPID	Roberts et al., 1977
	[Chloroxylenol]	[3.48]	115.5	25	5.90E-02	5.90E-02		1	[5.3]	EPID	Roberts et al., 1977
	[Chlorpheniramine]	3.39	274.8	30	2.20E-03	2.27E-03		0.97	10.3	N/A	Michaels et al., 1975
	[Cortexolone]	2.52	346.5	26	7.50E-05	7.50E-05	↓	1	ND	EPID	Scheuplein et al., 1969
	[Cortexone]	2.88	330.5	26	4.50E-04	4.50E-04		1	ND	EPID	Scheuplein et al., 1969
	Corticosterone	1.94	346.5	27	[6.47E-04]	6.47E-04		1	7.4	EPID	Peck et al., 1995
	Corticosterone	1.94	346.5	39	[2.49E-03]	2.49E-03		1	7.4	EPID	Peck et al., 1995
	[Corticosterone]	1.94	346.5	26	6.00E-05	6.00E-05	↓	1	ND	EPID	Scheuplein et al., 1969
	Corticosterone	1.94	346.5	25[h]	2.24E-05	2.24E-05	↓	1	ND	SC[h]	Siddiqui et al., 1989
	[Cortisone]	1.47	360.5	26	1.00E-05	1.00E-05	↓	1	ND	EPID	Scheuplein et al., 1969
*	[m-Cresol]	1.96	108.1	25	1.52E-02	1.52E-02		1	[5.5]	EPID	Roberts et al., 1977
*	[o-Cresol]	1.95	108.1	25	1.57E-02	1.57E-02		1	[5.6]	EPID	Roberts et al., 1977
*	p-Cresol	1.94	108.1	37	1.20E-01	1.20E-01	↑	1	4	SC	Anderson et al., 1989
*	[p-Cresol]	1.94	108.1	25	1.75E-02	1.75E-02		1	[5.6]	EPID	Roberts et al., 1977
	Cyclobarbitone	1.77	236.3	37	[8.14E-04]	8.14E-04		1	3.58	FULL	Morimoto et al., 1992
*	[Decanol]	4.57	158.3	25	8.00E-02	8.00E-02		1	ND	EPID	Scheuplein & Blank, 1973
	[2,4-Dichlorophenol]	3.06	163.0	25	6.01E-02	6.01E-02		1	[4.4]	EPID	Roberts et al., 1977
	Diclofenac	4.40	260.7	37	1.82E-02	1.82E-02		0.50	pK_a[l]	EPID	Singh & Roberts, 1994b
	[Diethylcarbamazine]	[1.75]	199.3	30	1.30E-04	1.30E-04	↓	1	10	N/A	Michaels et al., 1975
	[Ephedrine]	0.93	165.2	30	6.00E-03	6.38E-03		0.94	10.8	N/A	Michaels et al., 1975
	β-Estradiol	4.01	272.4	30	3.89E-03	3.89E-03		1	7	FULL	Galey et al., 1976
	[β-Estradiol]	4.01	272.4	30	5.20E-03	5.20E-03		1	7	N/A	Michaels et al., 1975
	[β-Estradiol]	4.01	272.4	26	3.00E-04	3.00E-04	↓	1	ND	EPID	Scheuplein et al., 1969
	β-Estradiol	4.01	272.4	37	9.70E-03	9.70E-03		1	ND	SC[m]	Liu et al., 1994
	β-Estradiol	4.01	272.4	32	4.31E-03[n]	4.31E-03		1	ND	EPID	Megrab et al., 1995

	COMPOUND[a]	$logK_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	↑↓[d]	f_{ul}[e]	pH[f]	Skin[g]	Reference
	[Estriol]	2.45	288.4	26	4.00E-05	4.00E-05	↓	1	ND	EPID	Scheuplein et al., 1969
	[Estrone]	3.13	270.4	26	3.60E-03	3.60E-03		1	ND	EPID	Scheuplein et al., 1969
*	Ethanol	-0.31	46.0	22	3.00E-04	3.00E-04		1	ND	FULL	Blank, 1964
*	[Ethanol]	-0.31	46.0	25	8.00E-04	8.00E-04		1	ND	EPID	Scheuplein & Blank, 1973
*	Ethanol	-0.31	46.0	30	3.17E-04	3.17E-04		1	ND	FULL	Scott et al., 1991
*	[2-Ethoxy ethanol]	-0.32	90.1	30	2.50E-04	2.50E-04		1	ND	EPID	Blank et al., 1967
*	[Ethyl ether]	0.89	74.1	30	1.60E-02	1.60E-02		1	ND	EPID	Blank et al., 1967
*	[p-Ethylphenol]	2.58	122.2	25	3.49E-02	3.49E-02		1	[5.7]	EPID	Roberts et al., 1977
	[Fentanyl]	4.05	336.5	30	1.00E-02	1.14E-02		0.88	8	N/A	Michaels et al., 1975
	Fentanyl	4.05	336.5	37	1.13E-02	1.55E-02		0.73	7.4	EPID	Roy & Flynn, 1990
	Flurbiprofen	4.16	244.3	37	[4.62E-01]	1.43E+00	↑	0.32	4.7	FULL	Morimoto et al., 1992
*	[Heptanoic acid]	[2.41]	130.2	N/A	2.00E-02	2.00E-02[k]		1[k]	N/A	N/A	Scheuplein, 1967
*	Heptanol	2.72	116.0	30	3.76E-02	3.76E-02		1	ND	EPID	Blank et al., 1967
*	[Heptanol]	2.72	116.0	25	3.20E-02	3.20E-02		1	ND	EPID	Scheuplein & Blank, 1973
*	[Hexanoic acid]	1.92	116.2	N/A	1.40E-02	1.40E-02[k]		1[k]	N/A	N/A	Scheuplein, 1967
*	Hexanol	2.03	102.2	31	2.77E-02	2.77E-02		1	ND	SPLIT	Bond & Barry, 1988
*	[Hexanol]	2.03	102.2	25	1.30E-02	1.30E-02		1	ND	EPID	Scheuplein & Blank, 1973
	[Hydrocortisone (HC)]	1.61	362.5	26	3.00E-06	3.00E-06	↓	1	ND	EPID	Scheuplein et al., 1969
	Hydrocortisone (HC)	1.61	362.5	25[h]	2.82E-06	2.82E-06	↓	1	ND	SC[h]	Siddiqui et al., 1989
	[HC-yl-succinamate]	[0.17]	461.6	37	2.60E-05	2.60E-05		1	4	SC	Anderson et al., 1988
	[HC-yl-N,N-dimethyl succinamate]	[0.88]	489.6	37	6.70E-05	6.70E-05		1	4	SC	Anderson et al., 1988
	[HC-yl-hemipimelate]	[1.82]	504.6	37	1.80E-03	2.31E-03	↑	0.78	4	SC	Anderson et al., 1988
	[HC-yl-hemisuccinate]	[0.91]	462.5	37	6.30E-04	8.10E-04	↑	0.78	4	SC	Anderson et al., 1988
	[HC-yl-hexanoate]	[3.28]	460.6	37	1.80E-02	1.80E-02	↑	1	4	SC	Anderson et al., 1988
	[HC-yl-6-hydroxy hexanoate]	[1.29]	476.6	37	9.10E-04	9.10E-04	↑	1	4	SC	Anderson et al., 1988
	[HC-yl-octanoate]	[4.34]	488.7	37	6.20E-02	6.20E-02	↑	1	4	SC	Anderson et al., 1988
	[HC-yl-pimelamate]	[0.82]	503.6	37	8.90E-04	8.90E-04	↑	1	4	SC	Anderson et al., 1988

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	↑↓[d]	f_{ui}[e]	pH[f]	Skin[g]	Reference
	[HC-yl-priopionate]	[1.69]	418.5	37	3.40E-03	3.40E-03	↑	1	4	SC	Anderson et al., 1988
	4-Hydroxybenzyl alcohol	0.25	124.1	37	2.00E-03	2.00E-03		1	4	SC	Anderson et al., 1989
	α-(4-Hydroxyphenyl) acetamide	[-0.21]	151.2	37	4.50E-04	4.50E-04		1	4	SC	Anderson et al., 1989
	4-Hydroxyphenyl acetic acid	0.75	152.1	37	2.50E-03	3.41E-03		0.73	4	SC	Anderson et al., 1989
	[17α-Hydroxyprogesterone]	3.17	330.5	26	6.00E-04	6.00E-04		1	ND	EPID	Scheuplein et al., 1969
	Ibuprofen	3.50	206.3	37	[5.70E-01]	1.02E+00	↑	0.56	4.44	FULL	Morimoto et al., 1992
	Indomethacin	4.27	357.8	37	[5.05E-02]	2.54E-01	↑	0.20	5.15	FULL	Morimoto et al., 1992
	Indomethacin	4.27	357.8	37	1.48E-02	1.48E-02		0.50	pK_a[i]	EPID	Singh & Roberts, 1994b
*	[Isoquinoline]	2.08	129.2	30	1.67E-02	1.68E-02		0.99	7.4	FULL	Hadgraft & Ridout, 1987
	Isosorbide dinitrate	1.31	236.1	37	[1.63E-02]	1.63E-02	↑	1	ND	FULL	Morimoto et al., 1992
	Ketoprofen	3.12	254.3	37	[5.89E-02]	7.10E-02		0.83	3.72	FULL	Morimoto et al., 1992
	Mannitol	-3.10	182.2	30	[1.11E-04]	1.11E-04	↑	1	ND	EPID	Dick & Scott, 1992
	Mannitol	-3.10	182.2	27	6.71E-05	6.71E-05		1	7.4	EPID	Peck et al., 1995
	Mannitol	-3.10	182.2	39	9.30E-05	9.30E-05		1	7.4	EPID	Peck et al., 1995
	Mannitol	-3.10	182.2	30	6.10E-05	6.10E-05		1	ND	FULL	Scott et al., 1991
*	[Methanol]	-0.77	32.0	25	5.00E-04	5.00E-04		1	ND	EPID	Scheuplein & Blank, 1973
*	Methanol	-0.77	32.0	30	1.60E-03	1.60E-03		1	ND	EPID[h]	Southwell et al., 1984
	Methyl 4-hydroxy phenylacetate	[1.15]	166.0	37	2.00E-02	2.00E-02		1	4	SC	Anderson et al., 1989
	[Methyl-4-hydroxy benzoate]	1.96	152.1	25	9.12E-03	9.12E-03		1	[4.6]	EPID	Roberts et al., 1977
	[Methyl-HC-yl-pimelate]	[2.20]	518.6	37	5.40E-03	5.40E-03	↑	1	4	SC	Anderson et al., 1988
	[Methyl-HC-yl-succinate]	[1.38]	476.6	37	2.10E-04	2.10E-04		1	4	SC	Anderson et al., 1988
*	[β-Naphthol]	2.70	144.2	25	2.79E-02	2.79E-02		1	[5.2]	EPID	Roberts et al., 1977
	Naproxen	3.34	230.3	37	3.82E-02	3.82E-02		0.50	pK_a[i]	EPID	Singh & Roberts, 1994b
	Nicorandil	[0.65]	211.2	37	2.66E-04	2.66E-04		1	[8.0]	FULL	Sato et al., 1991
	Nicorandil	[0.65]	211.2	37	[1.79E-04]	1.79E-04		1	[8.0]	FULL	Morimoto et al., 1992
	Nicotinate, benzyl	2.40	213.2	37	1.62E-02	1.62E-02		1	[7.3]	EPID	Dal Pozzo et al., 1991
	Nicotinate, butyl	2.27	179.1	37	1.66E-02	1.66E-02		1	[7.7]	EPID	Dal Pozzo et al., 1991

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	↑↓[d]	f_{ul}[e]	pH[f]	Skin[g]	Reference
	Nicotinate, ethyl	1.32	151.0	37	6.34E-03	6.34E-03		1	[7.3, 8.3]	EPID	Dal Pozzo et al., 1991
	Nicotinate, hexyl	3.59	207.2	37	1.79E-02	1.79E-02		1	[7.2]	EPID	Dal Pozzo et al., 1991
	Nicotinate, methyl	0.87	137.1	37	3.25E-03	3.25E-03		1	[8.3, 8.5]	EPID	Dal Pozzo et al., 1991
	Nicotinate, PG	[0.39]	181.0	37	3.40E-05	3.40E-05	↓	1	[8.3]	EPID	Dal Pozzo et al., 1991
	Nicotinate, TEG-Me	[0.83]	269.1	37	1.77E-04	1.77E-04		1	[8.1]	EPID	Dal Pozzo et al., 1991
	Nicotinate, TEG-OH	[0.21]	255.1	37	9.90E-06	9.90E-06	↓	1	[8.1]	EPID	Dal Pozzo et al., 1991
	[Nicotine]	1.17	162.2	30	1.90E-02	1.98E-02		0.96	9.2	FULL	Hadgraft & Ridout, 1987
	2-Nitro-p-phenylenediamine	0.53	153.1	32	5.00E-04	5.00E-04		1	9.7	EPID	Bronaugh & Congdon, 1984
	[Nitroglycerine]	[0.98]	227.1	30	1.10E-02	1.10E-02	↑	1	ND	N/A	Michaels et al., 1975
*	[m-Nitrophenol]	2.00	139.1	25	5.64E-03	5.64E-03		1	[4.8]	EPID	Roberts et al., 1977
*	[p-Nitrophenol]	1.91	139.1	25	5.58E-03	5.58E-03		1	[3.9]	EPID	Roberts et al., 1977
	[N-Nitrosodiethanolamine]	[-1.58]	134.1	32	5.50E-06	5.50E-06	↓	1	ND	EPID	Bronaugh et al., 1981
*	[Nonanol]	4.26	144.0	25	6.00E-02	6.00E-02		1	ND	EPID	Scheuplein & Blank, 1973
*	[Octanoic acid]	3.05	144.2	N/A	2.50E-02	2.50E-02[k]		1[k]	N/A	N/A	Scheuplein, 1967
*	Octanol	3.00	130.2	22	5.20E-02	5.20E-02		1	ND	FULL	Blank, 1964
*	[Octanol]	3.00	130.2	25	5.20E-02	5.20E-02		1	ND	EPID	Scheuplein & Blank, 1973
*	Octanol	3.00	130.2	30	6.10E-02	6.10E-02		1	ND	EPID[h]	Southwell et al., 1984
	Ouabain	-1.70	584.6	30	3.96E-06	3.96E-06		1	7	FULL	Galey et al., 1976
*	[Pentanoic acid]	1.39	102.1	N/A	2.00E-03	2.00E-03[k]		1[k]	N/A	N/A	Scheuplein, 1967
*	Pentanol	1.56	88.0	22	6.00E-03	6.00E-03		1	ND	FULL	Blank, 1964
*	[Pentanol]	1.56	88.0	25	6.00E-03	6.00E-03		1	ND	EPID	Scheuplein & Blank, 1973
	[Phenobarbitone]	1.47	232.2	30	4.50E-04	4.50E-04		1	7.4	FULL	Hadgraft & Ridout, 1987
*	[Phenol]	1.46	94.1	25	8.22E-03	8.22E-03		1	[5.4]	EPID	Roberts et al., 1977
*	Phenol	1.46	94.1	37	1.95E-02	1.95E-02		1	N/A	EPID	Singh & Roberts, 1994b
*	Phenol	1.46	94.1	22	1.55E-04	1.55E-04	↓	1	[5.4]	EPID[h]	Southwell et al., 1984
*	2-Phenylethanol	1.36	122.2	25[h]	7.50E-03	7.50E-03		1	ND	N/A	Roberts, 1976
	o-Phenylenediamine	0.15	108.1	32	4.50E-04	4.50E-04		1	9.7	EPID	Bronaugh & Congdon, 1984

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	↑↓[d]	f_{ul}[e]	pH[f]	Skin[g]	Reference
	p-Phenylenediamine	-0.30	108.1	32	2.40E-04	2.40E-04		1	9.7	EPID	Bronaugh & Congdon, 1984
	Piroxicam	3.06	331.4	37	3.40E-03	3.40E-03[o]		0.50	pK_a[l]	EPID	Singh & Roberts, 1994b
	Prednisolone	1.62	360.4	25[h]	4.47E-05	4.47E-05		1	ND	SC[h]	Siddiqui et al., 1989
	[Pregnenolone]	4.22	316.5	26	1.50E-03	1.50E-03		1	ND	EPID	Scheuplein et al., 1969
	[Progesterone]	3.87	314.5	26	1.50E-03	1.50E-03		1	ND	EPID	Scheuplein et al., 1969
*	Propanol	0.25	60.0	22	1.00E-03	1.00E-03		1	ND	FULL	Blank, 1964
*	Propanol	0.25	60.0	30	1.70E-03	1.70E-03		1	ND	EPID	Blank et al., 1967
*	[Propanol]	0.25	60.0	25	1.20E-03	1.20E-03		1	ND	EPID	Scheuplein & Blank, 1973
*	[Resorcinol]	0.80	110.1	25	2.40E-04	2.40E-04	↓	1	[5.4]	EPID	Roberts et al., 1977
	[Salicylic acid]	2.26	138.1	30	6.26E-03	1.28E-02		0.49	3	FULL	Hadgraft & Ridout, 1987
	Salicylic acid	2.26	138.1	25	Range	1.03E-02		(.2-.5)	2-4	FULL	Harada et al., 1993
	Salicylic acid	2.26	138.1	37	3.04E-02	3.04E-02		0.50	pK_a[l]	EPID	Singh & Roberts, 1994b
	Salicylic acid[p]	2.26	138.1	37	3.76E-02	3.76E-02		0.50	pK_a[l]	EPID	Singh & Roberts, 1994b
	[Scopolamine]	[-0.20]	303.4	30	5.00E-05	6.17E-05		0.81	9.6	N/A	Michaels et al., 1975
	[Sufentanil]	3.95	386.5	37	1.52E-02	1.60E-02		0.95	7.4	EPID	Roy & Flynn, 1990
	[Testosterone]	3.32	288.4	26	4.00E-04	4.00E-04	↓	1	ND	EPID	Scheuplein et al., 1969
	Testosterone	3.32	288.4	25[h]	8.51E-04	8.51E-04		1	ND	SC[h]	Siddiqui et al., 1989
*	Tetrachloroethylene	3.40	165.9	37	1.60E-02	1.60E-02		1	ND	SPLIT	Nakai et al., 1995
*	[Thymol]	3.30	150.2	25	5.28E-02	5.28E-02		1	[6.0]	EPID	Roberts et al., 1977
*	Toluene	2.73	92.1	37	8.30E-01	8.30E-01	↑	1	4	SC	Anderson et al., 1989
	Triamcinolone	1.16	394.5	25[h]	3.98E-06	3.98E-06	↓	1	ND	SC[h]	Siddiqui et al., 1989
	Triamcinolone acetonide	2.53	434.5	25[h]	2.02E-05	2.02E-05	↓	1	ND	SC[h]	Siddiqui et al., 1989
*	Trichloroethylene	2.61	131.4	37	1.20E-01	1.20E-01		1	ND	SPLIT	Nakai et al., 1995
	[2,4,6-Trichlorophenol]	3.69	197.5	25	5.94E-02	5.94E-02		1	[3.6]	EPID	Roberts et al., 1977
	Urea	-2.11	60.1	37	1.48E-04	1.48E-04		1	7.1[h]	SC[h]	Barber et al., 1992
	Urea	-2.11	60.1	27	2.01E-04	2.01E-04		1	7.4	EPID	Peck et al., 1995
	Urea	-2.11	60.1	39	2.72E-04	2.72E-04		1	7.4	EPID	Peck et al., 1995

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	↑↓[d]	f_{ul}[e]	pH[f]	Skin[g]	Reference
*	Water	-1.38	18.0	30[h]	1.56E-03	1.56E-03		1	7.1	SC[h]	Barber et al., 1992
*	Water	-1.38	18.0	31	1.40E-03	1.40E-03		1	ND	SPLIT	Bond & Barry, 1988
*	Water	-1.38	18.0	32	1.55E-03	1.55E-03		1	ND	EPID	Bronaugh et al, 1986
*	Water	-1.38	18.0	30	[8.54E-04]	8.54E-04		1	ND	EPID	Dick & Scott, 1992
*	Water	-1.38	18.0	30	1.58E-03	1.58E-03		1	7	FULL	Galey et al., 1976
*	Water	-1.38	18.0	31	1.34E-03	1.34E-03		1	ND	SPLIT	Rigg and Barry, 1990
*	[Water]	-1.38	18.0	25	5.00E-04	5.00E-04		1	ND	EPID	Scheuplein & Blank, 1973
*	Water	-1.38	18.0	30	6.39E-04	6.39E-04		1	ND	FULL	Scott et al., 1991
*	[3,4-Xylenol]	2.23	122.2	25	3.60E-02	3.60E-02		1	[5.8]	EPID	Roberts et al., 1977

a. Compounds contained within brackets (e.g. [Aldosterone]) also appeared in the Flynn database. Those indicated with an asterisk to the left (e.g. benzene) were used in the LSER analysis.
b. Reported $\log K_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for chloroxylenol [3.48]), in which case they were calculated (Daylight, 1995).
c. Permeability coefficients contained within brackets were digitized from figures in the reference.
d. (↓) indicates a measurement which is more than one order of magnitude lower than predicted by Eq. (5.28). (↑) indicates a measurement which is more than one order of magnitude higher than predicted by Eq. (5.28).
e. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 5A.4.
f. Reported solution pH unless contained within brackets (e.g. for p-bromophenol [5.3]) in which case the pH was calculated from the reported concentration and calculated pKa values (see Table 5A.4). Chemicals that are undissociated are indicated by ND when no pH was reported.
g. Type of skin used in the study: isolated stratum corneum (SC), epidermal membranes (EPID), split (SPLIT) or full-thickness skin (FULL).
h. Information that was obtained through personal communication with authors
i. SC permeability coefficients were reported for betamethasone (BMS) and BMS 17-Valerate. The SC permeability coefficient was calculated from separate permeability measurements for split thickness skin (epidermis and part of dermis) and dermis using a multilaminate model.
j. The permeability coefficient was reported as an upper bound. Value not treated differently in the analysis.
k. Scheuplein et al. did not report pH of solution. However, we assume that the permeability coefficients for carboxylic acids were measured at a pH assuring the chemicals were 100% unionized
l. Permeability coefficient equals twice that measured when pH was set at the pK_a, although the pK_a was not reported. pK_a values calculated using SPARC are listed in Table 5A.4. These may be different from the pH values used experimentally.
m. The SC permeability coefficient was calculated from the difference of the inverse permeability coefficients of the epidermis (SC+VE) and the viable epidermis alone.
n. Calculated from the flux provided and a concentration that was determined by personal communication with (Barry, 1996).

o. The permeability of piroxicam (Singh & Roberts, 1994b) is taken to be 0.0034 cm/hr rather than 0.034 cm/hr which is twice the 50% ionized value and is consistent with their Fig. 2

p. According to Singh and Roberts (1994b), the salt diethylamine salicylate is ionized under the experimental conditions. Consequently, the free base form, salicylic acid, was the penetrating species.

Table 5A.2 The Provisional Database

COMPOUND[a]	$logK_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	f_{ui}[d]	pH[e]	Skin[f]	Reference
5-Fluorouracil (+ - + -)	-0.89	130.1	31	9.51E-05	<0.1	4.75[g]	SPLIT	Bond & Barry, 1988
5-Fluorouracil (+ - + -)	-0.89	130.1	32	3.00E-05	<0.1	4.75[g]	EPID	Cornwell & Barry, 1994
5-Fluorouracil (+ - + -)	-0.89	130.1	37	[1.58E-05]	<0.1	4.66	FULL	Morimoto et al., 1992
5-Fluorouracil (+ - + -)	-0.89	130.1	31	3.48E-05	<0.1	4.75[g]	SPLIT	Rigg & Barry, 1990
5-Fluorouracil (+ - + -)	-0.89	130.1	31	1.66E-05	<0.1	4.75[g]	SPLIT	Rigg & Barry, 1990
5-Fluorouracil (+ - + -)	-0.89	130.1	32	2.46E-05	<0.1	4.75[g]	EPID	Williams & Barry, 1991
[Hydroxypregnenolone]	N/A	N/A	26	6.00E-04	1	ND	EPID	Scheuplein et al., 1969
[Ouabain][i]	-1.70	584.6	30	7.80E-07	1	7	N/A	Michaels et al., 1975
Paraquat (Dichloride)[h] (+ +)	[-5.65]	257.3	30	[1.28E-05]	<0.1	ND	EPID	Dick & Scott, 1992
Paraquat Dichloride (+ +)	[-5.65]	257.3	30	8.70E-06	<0.1	ND	FULL	Scott et al., 1991
Tetraethylammonium Bromide (+)	-2.82	210.2	27	[5.58E-05]	<0.1	7.4	EPID	Peck et al., 1995
Tetraethylammonium Bromide (+)	-2.82	210.2	39	[8.82E-05]	<0.1	7.4	EPID	Peck et al., 1995

a. Compounds contained within brackets (e.g. [Hydroxypregnenolone] also appeared in the Flynn database. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, 5-fluorouracil with two positive and two negative charges is indicated by (+ - + -).

b. Reported $logK_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for paraquat dichloride [-5.65]), in which case they were calculated (Daylight, 1995).

c. Permeability coefficients contained within brackets are digitized from figures in the reference.

d. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as shown in Table 5A.4.

e. Reported solution pH unless contained within brackets in which case the pH was calculated from the reported concentration and calculated pK_a values (as given in Table 5A.4). Compounds that essentially do not dissociate are indicated by ND when no pH was reported.

f. Type of skin: isolated stratum corneum (SC), epidermal membranes (EPID), split (SPLIT) or full-thickness (FULL) skin.

g. Information obtained through personal communication from Barry (1996).

h. Corresponding anion was not specified, but given it was dichloride in Scott et al. (Scott et al., 1991) it is likely that paraquat was applied as the dichloride salt in this measurement as well.

i. No information was provided to determine whether P_{cw} for ouabain (MW=584) was measured at steady state. We have found that the P_{cw} is large for a chemical with oubain's properties, indicating that steady-state might have been attained. Based on this analysis, the permeability coefficient for ouabain could be moved to the fully-validated database

Table 5A.3 The Excluded Database

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{ow} (rep)[c]	f_{ui}[d]	pH[e]	Skin[f]	Reference
Amphetamine (+)	1.76	135.2	30	1.40E-05	<0.1	7	FULL	Galey et al., 1976
Aniline[g]	0.90	93.1	30	2.24E-02	1	[7.6]	SPLIT	Barry et al., 1985
Anisole[g]	2.11	108.1	30	7.37E-02	1	ND	SPLIT	Barry et al., 1985
Aspartic acid (- + -)	N/A	133.1	37	9.36E-05	<0.1	7.3	FULL	Sznitowska et al., 1993
Aspartic acid (- + and - + -)	N/A	133.1	37	1.33E-04	<0.1	3.4	FULL	Sznitowska et al., 1993
Benzaldehyde[g]	1.48	106.1	30	6.08E-02	1	ND	SPLIT	Barry et al., 1985
Benzyl Alcohol[g]	1.10	108.1	30	1.69E-02	1	ND	SPLIT	Barry et al., 1985
Chromone-2-Carboxylic Acid I (-)	N/A	288.0	37	7.30E-05	<0.1	7	EPID	Swarbrick et al., 1984
Chromone-2-Carboxylic Acid II (-)	N/A	272.0	37	2.90E-05	<0.1	7	EPID	Swarbrick et al., 1984
Chromone-2-Carboxylic Acid III (-)	N/A	230.0	37	1.86E-05	<0.1	7	EPID	Swarbrick et al., 1984
Chromone-2-Carboxylic Acid IV (-)	N/A	306.0	37	4.20E-06	<0.1	7	EPID	Swarbrick et al., 1984
Cobalt Chloride[h]	N/A	N/A	N/A	4.00E-04	<0.1	N/A	N/A	Wahlberg, 1965a
[Codeine] (+)	1.14	299.4	37	4.90E-05	<0.1	7.4	EPID	Roy and Flynn, 1989
Coumarin[i]	1.39	146.1	37	9.10E-03	1	7.4	FULL	Ritschel et al., 1989
Diclofenac (-)	4.40	260.7	37	[3.00E-03]	<0.1	7.96	FULL	Morimoto et al., 1992
[Digitoxin][j]	2.83	764.9	30	1.30E-05	1	7	N/A	Michaels et al., 1975
Dopamine (+)	[-0.05]	153.2	37	[9.90E-05]	<0.1	3.26	FULL	Morimoto et al., 1992
[Etorphine][k] (+)	[1.41]	411.5	37	3.60E-03	<0.1	7.3	N/A	Jolicoeur et al., 1992
[Fluocinonide][l]	3.19	494.6	37	1.70E-03	1	4	SC	Anderson et al., 1988
Formaldehyde[m]	0.35	30.0	30	4.51E-04	1	7.4	FULL	Loden, 1986
Griseofulvin[i]	2.18	352.8	37	1.30E-03	1	7.4	FULL	Ritschel et al., 1989
Histidine (+ - +)	-3.56	155.2	37	4.46E-05	<0.1	5	FULL	Sznitowska et al., 1993
Histidine (- +)	-3.56	155.2	37	5.54E-05	<0.1	7.3	FULL	Sznitowska et al., 1993
Histidine (- +)	-3.56	155.2	37	4.50E-05	<0.1	8.5	FULL	Sznitowska et al., 1993

COMPOUND[a]	$logK_{ow}$[b]	MW	T (C)	P_{ow} (rep)[c]	f_{bi}[d]	pH[e]	Skin[f]	Reference
[Hydrocortisone][n]	1.61	362.5	30	1.19E-04	1	7.4	FULL	Hadgraft and Ridout, 1987
[Hydromorphone] (+)	[0.55]	285.3	37	1.50E-05	<0.1	7.4	EPID	Roy and Flynn, 1989
Isoprenaline (+)	[0.08]	211.2	37	[2.84E-05]	<0.1	2.75	FULL	Morimoto et al., 1992
Ketorolac acid[o] (-)	[1.77]	255.3	32	1.30E-02	<0.1	2.1	SPLIT	Roy et al., 1995
Lead Acetate[h]	N/A	N/A	N/A	4.20E-06	<0.1	N/A	N/A	Moore et al., 1980
Levodopa (- +)	-2.74	197.0	37	[6.60E-05]	<0.1	5.42	FULL	Morimoto et al., 1992
Lidocaine (+)	2.26	234.3	37	[2.17E-02]	<0.1	6.82	FULL	Morimoto et al., 1992
Lidocaine (+)	2.26	234.3	37	4.20E-03	<0.1	7.4	EPID	Singh & Roberts, 1994a
Lysine (+ - +)	-3.05	146.2	37	1.69E-04	<0.1	7.3	FULL	Sznitowska et al., 1993
Lysine (- +) and (+ - +)	-3.05	146.2	37	4.97E-04	<0.1	8.9	FULL	Sznitowska et al., 1993
[Meperidine] (+)	2.45	247.4	37	3.70E-03	<0.1	7.4	EPID	Roy and Flynn, 1989
Mercuric Chloride[h]	N/A	N/A	N/A	9.30E-04	<0.1	N/A	N/A	Wahlberg, 1965b
Morphine (+)	0.76	285.3	37	[2.84E-04]	<0.1	4.22	FULL	Morimoto et al., 1992
[Morphine] (+)	0.76	285.3	37	1.60E-06	<0.1	7.5	EPID	Roy et al., 1994
[Naproxen][p] (-)	3.34	230.3	N/A	4.00E-04	<0.1	6.5	FULL	Chowhan and Pritchard, 1978
Nickel Chloride[h]	N/A	N/A	N/A	1.00E-04	<0.1	N/A	N/A	Fullerton et al., 1988
Nickel Sulfate[h]	N/A	N/A	N/A	<9.00E-06	<0.1	N/A	N/A	Samitz & Katz, 1976
Nicotinate, PEG 350-Me[q]	N/A	N/A	37	1.14E-05	1	[8.0]	EPID	Dal Pozzo et al., 1991
Nicotinate, PPG 425[q]	N/A	N/A	37	9.39E-04	1	[7.9]	EPID	Dal Pozzo et al., 1991
Nicotinic acid (- +)	[0.77]	123.1	37	2.42E-05	<0.1	[3.4]	EPID	Dal Pozzo et al., 1991
2-Phenyl ethanol[g]	1.36	122.2	30	1.27E-02	1	ND	SPLIT	Barry et al., 1985
Propranolol[i] (+)	2.98	259.3	37	[1.20E-03]	<0.1	7.4	FULL	Ritschel et al., 1989
Silver Nitrate[h]	N/A	N/A	N/A	<3.50E-04	<0.1	N/A	N/A	Norgaard, 1954
Sodium Chromate[h]	N/A	N/A	N/A	2.10E-03	<0.1	N/A	N/A	Baranowska & Dutkiewicz, 1981
[Sucrose][l]	-3.70	342.3	37	9.40E-06	1	4	SC	Anderson et al., 1988

a. Compounds contained within brackets (e.g. [codeine]) also appeared in the Flynn database. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, aspartic acid with two negative charges and one positive charge is indicated by (-+-).
b. Reported $\log K_{ow}$ for the unionized compound are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for dopamine [-0.05]), in which case they were calculated (Daylight, 1995).
c. Permeability coefficients contained within brackets were digitized from figures in the reference.
d. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as shown in Table 5A.4.
e. Reported solution pH unless contained within brackets (e.g., for aniline [7.6]) in which case the pH was calculated from the reported concentration and calculated pKa values (see Table 5A.4). Compounds that are undissociated are indicated by ND when no pH was reported.
f. Type of skin used in the study: isolated stratum corneum (SC), epidermal membranes (EPID), split-thickness (SPLIT), or full-thickness skin (FULL).
g. Ethanol was in the receptor chamber and it is likely that the skin barrier was compromised.
h. Inorganic species that was not considered further.
i. The concentration of this penetrant depleted during this experiment.
j. It was likely that the permeability coefficient was not measured at steady state.
k. Not included in the fully-validated database because (1) $f_{ui} < 0.1$, and (2) the reported permeability coefficient was higher than that of similar compounds in the study (see Appendix 5B).
l. Anderson (1995) suggested that the permeability coefficient for fluocinonide and sucrose should not be included in the fully-validated database.
m. Formaldehyde was applied in a solution of formalin, containing methanol, which may have compromised the skin barrier.
n. Ethanol was in the donor solution, and likely altered the skin permeability.
o. The skin may have been damaged at this pH.
p. Naproxen was delivered in an aqueous gel vehicle.
q. These permeability coefficients refer to penetration of several compounds from a polydisperse mixture.

Table 5A.4 Estimates of the Fraction of the Species Unionized at the Experimental Temperature (T)

COMPOUND	$pK_a(25)$[a]	T (°C)	ΔH (kcal/mol)[b]	$pK_a(T)$[c]	C_w (mol/L)[d]	pH[e]	f_{ul}[f]	Reference
2-Amino-4-nitrophenol (N → -)	6.84		5	6.75				
2-Amino-4-nitrophenol (- → ±)	6.07	32	7.5	5.94		5.9	0.78[g]	Bronaugh & Congdon, 1984
2-Amino-4-nitrophenol (N → +)	2.7		7.5	2.57				
4-Amino-2-nitrophenol (N → -)	7.74		5	7.65				
4-Amino-2-nitrophenol (- → ±)	4.34	32	7.5	4.21		5.9	0.98[g]	Bronaugh & Congdon, 1984
4-Amino-2-nitrophenol (N → +)	2.94		7.5	2.81				
Aniline	4.8	30	7.5	4.71	3.60E-04	[7.6]	1	Barry et al., 1985
Atropine	7.91	30	10	7.79		8.0	0.62	Michaels et al., 1975
p-Bromophenol	9.4	25	5	9.40	0.058[h]	[5.3]	1	Roberts et al., 1977
Caffeine	6.54	30	5	6.48		7.4	0.89	Southwell et al., 1984
Chlorocresol	9.55	25	5	9.55	0.070[h]	[5.3]	1	Roberts et al., 1977
o-Chlorophenol	8.25	25	5	8.25	0.078[h]	[4.6]	1	Roberts et al., 1977
p-Chlorophenol	9.43	25	5	9.43	0.078[h]	[5.3]	1	Roberts et al., 1977
Chloroxylenol	9.68	25	5	9.68	0.086[h]	[5.3]	1	Roberts et al., 1977
Chlorpheniramine	8.95	30	10	8.83		10.3	0.97	Michaels et al., 1975
m-Cresol	10.13	25	5	10.13	0.092[h]	[5.5]	1	Roberts et al., 1977
o-Cresol	10.33	25	5	10.33	0.092[h]	[5.6]	1	Roberts et al., 1977
p-Cresol	10.31	25	5	10.31	0.092[h]	[5.6]	1	Roberts et al., 1977
2,4-Dichlorophenol	7.67	25	5	7.67	0.061[h]	[4.4]	1	Roberts et al., 1977
Diclofenac	4.07	37	0	4.50		pK_a	0.50	Singh & Roberts 1994b
Ephedrine	9.73	30	10	9.61		10.8	0.94	Michaels et al., 1975
p-Ethylphenol	10.29	25	5	10.29	0.082[h]	[5.7]	1	Roberts et al., 1977
Fentanyl	7.25	30	10	7.13		8.0	0.88	Michaels et al., 1975
Fentanyl	7.25	37	10	6.96		7.4	0.73	Roy & Flynn, 1990
Indomethacin	4.54	37	0	4.5		pK_a	0.50	Singh & Roberts 1994b
Isoquinoline	5.29	30	5	5.23		7.4	0.99	Hadgraft & Ridout, 1987
Methyl-4-hydroxy benzoate	7.93	25	5	7.93	0.066[h]	[4.6]	1	Roberts et al., 1977
β-Naphthol	9.34	25	5	9.34	0.069[h]	[5.2]	1	Roberts et al., 1977
Naproxen	4.49	37	0	4.5		pK_a	0.50	Singh & Roberts 1994b

COMPOUND	$pK_a(25)$[a]	T (°C)	ΔH (kcal/mol)[b]	$pK_a(T)$[c]	C_w (mol/L)[d]	pH[e]	f_u[f]	Reference
Nicorandil	2.87	37	5	2.73	0.188	[8.0]	1	Morimoto et al., 1992
Nicorandil	2.87	37	5	2.73	0.188[i]	[8.0]	1	Sato et al., 1991
Nicotinate, benzyl	2.98	37	5	2.84	0.004	[7.3]	1	Dal Pozzo et al., 1991
Nicotinate, butyl	3.17	37	5	3.03	0.021	[7.7]	1	Dal Pozzo et al., 1991
Nicotinate, ethyl	3.17	37	5	3.03	0.003	[7.3]	1	Dal Pozzo et al., 1991
Nicotinate, ethyl	3.17	37	5	3.03	0.331	[8.3]	1	Dal Pozzo et al., 1991
Nicotinate, hexyl	3.17	37	5	3.03	0.001	[7.2]	1	Dal Pozzo et al., 1991
Nicotinate, methyl	3.17	37	5	3.03	0.365	[8.3]	1	Dal Pozzo et al., 1991
Nicotinate,methyl	3.17	37	5	3.03	0.730	[8.5]	1	Dal Pozzo et al., 1991
Nicotinate, PEG 350-Me	2.82	37	5	2.68	0.212	[8.0]	1	Dal Pozzo et al., 1991
Nicotinate, PG	3.05	37	5	2.91	0.552	[8.3]	1	Dal Pozzo et al., 1991
Nicotinate, PPG 425	3.05	37	5	2.91	0.063	[7.9]	1	Dal Pozzo et al., 1991
Nicotinate, TEG-Me-	2.82	37	5	2.68	0.372	[8.1]	1	Dal Pozzo et al., 1991
Nicotinate, TEG-OH-	2.78	37	5	2.64	0.392	[8.1]	1	Dal Pozzo et al., 1991
Nicotine	7.89	30	10	7.77		9.2	0.96	Hadgraft & Ridout, 1987
2-Nicotinic acid (N → -)	3.43	37	0	3.43	0.168	[3.4]	< 0.1	Dal Pozzo et al., 1991
2-Nicotinic acid (± → -)	4.79		5	4.65				
2-Nicotinic acid (+ → N)	3.4		5	3.26				
m-Nitrophenol	8.39	25	5	8.39	0.072[h]	[4.8]	1	Roberts et al., 1977
p-Nitrophenol	6.83	25	5	6.83	0.072[h]	[3.9]	1	Roberts et al., 1977
Phenol	10	25	5	10.00	0.106[h]	[5.4]	1	Roberts et al., 1977
Phenol	10	37	5	10		pK_a	0.50	Singh & Roberts 1994b
Phenol	10	22	5	10.04	0.106	[5.4]	1	Southwell et al., 1984
Piroxicam	N/A	37	N/A	N/A		pK_a	0.50	Singh & Roberts 1994b
Resorcinol	9.86	25	5	9.86	0.091[h]	[5.4]	1	Roberts et al., 1977
Salicylic acid	4.5	37	0	4.5		pK_a	0.50	Singh & Roberts 1994b
Salicylic acid	4.5	37	0	4.5		pK_a	0.50	Singh & Roberts 1994b
Scopolamine	9.09	30	10	8.97		9.6	0.81	Michaels et al., 1975
Sufentanil	6.44	37	10	6.15		7.4	0.95	Roy & Flynn, 1990
Thymol	10.82	25	5	10.82	0.066[h]	[6.0]	1	Roberts et al., 1977
2,4,6-Trichlorophenol	5.94	25	5	5.94	0.051[h]	[3.6]	1	Roberts et al., 1977

COMPOUND	$pK_a(25)$[a]	T (°C)	ΔH (kcal/mol)[b]	$pK_a(T)$[c]	C_w (mol/L)[d]	pH[e]	f_{ul}[f]	Reference
3,4-Xylenol	10.44	25	5	10.44	0.082[h]	[5.8]	1	Roberts et al., 1977

a. pK_a values calculated in SPARC at 25C using methods described in Section 5.2.1.
b. These heats of ionization are approximate values obtained from the literature (Sober, 1968). See also Section 5.2.1.
c. Calculated using an integrated form of the van't Hoff equation, Eq. (5.2).
d. Solution concentration provided only when it was needed to calculate the pH.
e. The pH was reported in the original paper, unless contained within brackets, in which case it was calculated from $pK_a(T)$ and the solution concentration assuming that pH was 7.0 prior to chemical addition.
f. The fraction unionized was calculated using Eq. (5.1) when one pK_a is dominant. Otherwise it was determined using a more rigorous solution of simultaneous equilibrium (as discussed in Section 5.2.1).
g. The fraction unionized for this compound with multiple pK_a was calculated in SPARC at T = 25C.
h. The concentrations were consistently dilute (circa 1% (w/v)), but not reported. We have used a concentration of 1% (w/v) to calculate the pH and the fraction unionized.
i. The concentration of saturated nicorandil solution at 37C was reported by Morimoto et al., 1992.

Table 5A.5 LSER Parameters[a] and Permeability Coefficients for Chemicals in the LSER Database

COMPOUND	π	α	β	V_X [cm^3/mol/100][b]	T (C)	P_{cw} (adj)[c]	Permeability Reference
Benzene	0.52	0	0.14	0.716	31	1.11E-01	Blank & McAuliffe, 1985
Benzene	0.52	0	0.14	0.716	37	1.40E-01	Nakai et al., 1995
Benzoic Acid	0.9	0.59	0.4	0.932	35	3.00E-02	Parry et al., 1990
[Benzyl alcohol]	0.87	0.33	0.56	0.916	25	6.00E-03	Roberts, 1976
[p-Bromophenol]	1.17	0.67	0.2	0.95	25	3.61E-02	Roberts et al., 1977
[Butanoic acid]	0.62	0.6	0.45	0.747	N/A	1.00E-03	Scheuplein, 1967
[Butanol]	0.42	0.37	0.48	0.731	25	2.50E-03	Scheuplein & Blank, 1973
Butanol	0.42	0.37	0.48	0.731	30	3.00E-03	Blank et al., 1967
[2-Butanone]	0.67	0.03	0.48	0.69	30	4.50E-03	Blank et al., 1967
[Chlorocresol]	1.02	0.65	0.22	1.038	25	5.50E-02	Roberts et al., 1977
Chloroform	0.49	0.15	0.02	0.617	37	1.60E-01	Nakai et al., 1995
[o-Chlorophenol]	0.88	0.32	0.31	0.898	25	3.31E-02	Roberts et al., 1977
[p-Chlorophenol]	1.08	0.67	0.2	0.898	25	3.63E-02	Roberts et al., 1977
[m-Cresol]	0.88	0.57	0.34	0.916	25	1.52E-02	Roberts et al., 1977
[o-Cresol]	0.86	0.52	0.3	0.916	25	1.57E-02	Roberts et al., 1977
p-Cresol	0.87	0.57	0.31	0.916	37	1.20E-01	Anderson et al., 1989
[p-Cresol]	0.87	0.57	0.31	0.916	25	1.75E-02	Roberts et al., 1977
[Decanol]	0.42	0.37	0.48	1.576	25	8.00E-02	Scheuplein & Blank, 1973
Ethanol	0.42	0.37	0.48	0.449	22	3.00E-04	Blank, 1964
[Ethanol]	0.42	0.37	0.48	0.449	25	8.00E-04	Scheuplein & Blank, 1973
Ethanol	0.42	0.37	0.48	0.449	30	3.17E-04	Scott et al., 1991
[2-Ethoxyethanol]	0.5	0.3	0.83	0.79	30	2.50E-04	Blank et al., 1967
[Ethyl ether]	0.25	0	0.45	0.731	30	1.60E-02	Blank et al., 1967
[p-Ethylphenol]	0.9	0.55	0.36	1.057	25	3.49E-02	Roberts et al., 1977
[Heptanoic acid]	0.6	0.6	0.45	1.169	N/A	2.00E-02	Scheuplein, 1967

COMPOUND	π	α	β	V_X [cm^3/mol/100][b]	T (C)	P_{cw} (adj)[c]	Permeability Reference
Heptanol	0.42	0.37	0.48	1.154	30	3.76E-02	Blank et al., 1967
[Heptanol]	0.42	0.37	0.48	1.154	25	3.20E-02	Scheuplein & Blank, 1973
[Hexanoic acid]	0.6	0.6	0.45	1.028	N/A	1.40E-02	Scheuplein, 1967
Hexanol	0.42	0.37	0.48	1.013	31	2.77E-02	Bond & Barry, 1988
[Hexanol]	0.42	0.37	0.48	1.013	25	1.30E-02	Scheuplein & Blank, 1973
[Isoquinoline]	0.92	0	0.44	1.044	30	1.68E-02	Hadgraft & Ridout, 1987
[Methanol]	0.44	0.43	0.47	0.308	25	5.00E-04	Scheuplein & Blank, 1973
Methanol	0.44	0.43	0.47	0.308	30	1.60E-03	Southwell et al., 1984
[Beta-Naphthol]	1.08	0.61	0.4	1.144	25	2.79E-02	Roberts et al., 1977
[m-Nitrophenol]	1.57	0.79	0.23	0.949	25	5.64E-03	Roberts et al., 1977
[p-Nitrophenol]	1.72	0.82	0.26	0.949	25	5.58E-03	Roberts et al., 1977
[Nonanol]	0.42	0.37	0.48	1.435	25	6.00E-02	Scheuplein & Blank, 1973
[Octanoic acid]	0.6	0.6	0.45	1.31	N/A	2.50E-02	Scheuplein, 1967
Octanol	0.42	0.37	0.48	1.295	22	5.20E-02	Blank, 1964
[Octanol]	0.42	0.37	0.48	1.295	25	5.20E-02	Scheuplein & Blank, 1973
Octanol	0.42	0.37	0.48	1.295	30	6.10E-02	Southwell et al., 1984
[Pentanoic acid]	0.6	0.6	0.45	0.888	N/A	2.00E-03	Scheuplein, 1967
Pentanol	0.42	0.37	0.48	0.872	22	6.00E-03	Blank, 1964
[Pentanol]	0.42	0.37	0.48	0.872	25	6.00E-03	Scheuplein & Blank, 1973
[Phenol]	0.89	0.6	0.3	0.775	25	8.22E-03	Roberts et al., 1977
Phenol	0.89	0.6	0.3	0.775	37	1.95E-02	Singh & Roberts, 1994b
Phenol	0.89	0.6	0.3	0.775	22	1.55E-04	Southwell et al., 1984
2-Phenylethanol	0.91	0.3	0.64	1.057	25	7.50E-03	Roberts, 1976
Propanol	0.42	0.37	0.48	0.59	22	1.00E-03	Blank, 1964
Propanol	0.42	0.37	0.48	0.59	30	1.70E-03	Blank et al., 1967
[Propanol]	0.42	0.37	0.48	0.59	25	1.20E-03	Scheuplein & Blank, 1973

COMPOUND	π	α	β	V_X [cm^3/mol/100][b]	T (C)	P_{cw} (adj)[c]	Permeability Reference
[Resorcinol]	1	1.1	0.58	0.834	25	2.40E-04	Roberts et al., 1977
Tetrachloroethylene	0.44	0	0	0.837	37	1.60E-02	Nakai et al., 1995
[Thymol]	0.6	0.27	0.35	1.34	25	5.28E-02	Roberts et al., 1977
Toluene	0.52	0	0.14	0.857	37	8.30E-01	Anderson et al., 1989
Trichloroethylene	0.37	0.08	0.03	0.524	37	1.20E-01	Nakai et al., 1995
Water	0.45	0.82	0.35	0.167	30	1.56E-03	Barber et al., 1992
Water	0.45	0.82	0.35	0.167	31	1.40E-03	Bond & Barry, 1988
Water	0.45	0.82	0.35	0.167	32	1.55E-03	Bronaugh et al, 1986
Water	0.45	0.82	0.35	0.167	30	8.54E-04	Dick & Scott, 1992
Water	0.45	0.82	0.35	0.167	30	1.58E-03	Galey et al., 1976
Water	0.45	0.82	0.35	0.167	31	1.34E-03	Rigg & Barry, 1990
[Water]	0.45	0.82	0.35	0.167	25	5.00E-04	Scheuplein & Blank, 1973
Water	0.45	0.82	0.35	0.167	30	6.39E-04	Scott et al., 1991
[3,4-Xylenol]	0.86	0.56	0.39	1.057	25	3.60E-02	Roberts et al., 1977

a. All LSER parameters reported by Abraham and colleagues (Abraham *et al*., 1994b)

b. The reported V_X have been normalized by a factor of 100 to make the values comparable in magnitude to the other LSER parameters.

c. Permeability coefficients are from Table 5A.1

Table 5A.6 Parameters to Modify Molecular Weight with an Experimental Liquid Density

	COMPOUND[a]	$\log K_{ow}$[b]	MW	ρ (g/ml)[c]	T_{ref}[d] (C)	T_m[e] (C)	T_c[f] (C)	P_{cw} (adj)	T[g] (C)	Permeability Reference
*	Benzene	2.13	78.1	0.8765	20	6	562	1.11E-01	31	Blank & McAuliffe, 1985
*	Benzene	2.13	78.1	0.8765	20	6	562	1.40E-01	37	Nakai et al., 1995
	[2,3-Butanediol]	-0.92	90.1	1.0030	20	8	N/A	5.00E-05	30	Blank et al., 1967
*	[Butanoic acid]	0.79	88.1	0.9580	20	-6	628	1.00E-03	25	Scheuplein, 1967
*	[Butanol]	0.88	74.1	0.8100	20	-90	563	2.50E-03	25	Scheuplein & Blank, 1973
*	Butanol	0.88	74.1	0.8100	20	-90	563	3.00E-03	30	Blank et al., 1967
*	[2-Butanone]	0.29	72.1	0.8054	20	-87	N/A	4.50E-03	30	Blank et al., 1967
*	Chloroform	1.97	119.4	1.4830	20	-64	536	1.60E-01	37	Nakai et al., 1995
*	[o-Chlorophenol]	2.15	128.6	1.2634	20	10	N/A	3.31E-02	25	Roberts et al., 1977
*	[m-Cresol]	1.96	108.1	1.0340	20	12	706	1.52E-02	25	Roberts et al., 1977
*	p-Cresol	1.94	108.1	1.0185	40	36	705	1.20E-01	37	Anderson et al., 1989
*	[p-Cresol]	1.94	108.1	1.0185	40	36	705	1.75E-02	25	Roberts et al., 1977
*	[Decanol]	4.57	158.3	0.8300	20	7	687	8.00E-02	25	Scheuplein & Blank, 1973
*	Ethanol	-0.31	46.0	0.7890	20	-114	514	3.00E-04	22	Blank, 1964
*	[Ethanol]	-0.31	46.0	0.7890	20	-114	514	8.00E-04	25	Scheuplein & Blank, 1973
*	Ethanol	-0.31	46.0	0.7890	20	-114	514	3.17E-04	30	Scott et al., 1991
*	[2-Ethoxy ethanol]	-0.32	90.1	0.9300	20	-70	N/A	2.50E-04	30	Blank et al., 1967
*	[Heptanoic acid]	[2.41]	130.2	0.9180	20	-8	N/A	2.00E-02	25	Scheuplein, 1967
*	Heptanol	2.72	116.0	0.8220	20	-34	633	3.76E-02	30	Blank et al., 1967
*	[Heptanol]	2.72	116.0	0.8220	20	-34	633	3.20E-02	25	Scheuplein & Blank, 1973
*	[Hexanoic acid]	1.92	116.2	0.9270	20	-3	N/A	1.40E-02	25	Scheuplein, 1967
*	Hexanol	2.03	102.2	0.8140	20	-45	611	2.77E-02	31	Bond & Barry, 1988
*	[Hexanol]	2.03	102.2	0.8140	20	-45	611	1.30E-02	25	Scheuplein & Blank, 1973

	COMPOUND[a]	$\log K_{ow}$[b]	MW	ρ (g/ml)[c]	T_{ref}[d] (C)	T_m[e] (C)	T_c[f] (C)	P_{cw} (adj)	T[g] (C)	Permeability Reference
*	[Isoquinoline]	2.08	129.2	1.0910	30	27	803	1.68E-02	30	Hadgraft & Ridout, 1987
*	[Methanol]	-0.77	32.0	0.7910	20	-98	513	5.00E-04	25	Scheuplein & Blank, 1973
*	Methanol	-0.77	32.0	0.7910	20	-98	513	1.60E-03	30	Southwell et al., 1984
*	[m-Nitrophenol]	2.00	139.1	1.2800	100	97	N/A	5.64E-03	25	Roberts et al., 1977
*	[Nonanol]	4.26	144.0	0.8270	20	-5	671	6.00E-02	25	Scheuplein & Blank, 1973
*	[Octanoic acid]	3.05	144.2	0.9110	20	16	N/A	2.50E-02	25	Scheuplein, 1967
*	Octanol	3.00	130.2	0.8260	25	-16	653	5.20E-02	22	Blank, 1964
*	[Octanol]	3.00	130.2	0.8260	25	-16	653	5.20E-02	25	Scheuplein & Blank, 1973
*	Octanol	3.00	130.2	0.8260	25	-16	653	6.10E-02	30	Southwell et al., 1984
*	[Pentanoic acid]	1.39	102.1	0.9390	20	-34	651	2.00E-03	25	Scheuplein, 1967
*	Pentanol	1.56	88.0	0.8140	20	-79	588	6.00E-03	22	Blank, 1964
*	[Pentanol]	1.56	88.0	0.8140	20	-79	588	6.00E-03	25	Scheuplein & Blank, 1973
*	[Phenol]	1.46	94.1	1.0550	45	41	694	8.22E-03	25	Roberts et al., 1977
*	Phenol	1.46	94.1	1.0550	45	41	694	1.95E-02	37	Singh & Roberts, 1994b
*	Phenol	1.46	94.1	1.0550	45	41	694	1.55E-04	22	Southwell et al., 1984
*	Propanol	0.25	60.0	0.8040	20	-126	537	1.00E-03	22	Blank, 1964
*	Propanol	0.25	60.0	0.8040	20	-126	537	1.70E-03	30	Blank et al., 1967
*	[Propanol]	0.25	60.0	0.8040	20	-126	537	1.20E-03	25	Scheuplein & Blank, 1973
*	Tetrachloroethylene	3.40	165.9	1.6230	20	-22	620	1.60E-02	37	Nakai et al., 1995
*	Toluene	2.73	92.1	0.8670	20	-95	592	8.30E-01	37	Anderson et al., 1989
*	Trichloroethylene	2.61	131.4	1.4640	20	-85	572	1.20E-01	37	Nakai et al., 1995
	[2,4,6-Trichlorophenol]	3.69	197.5	1.4900	75	69	N/A	5.94E-02	25	Roberts et al., 1977
*	Water	-1.38	18.0	0.9957	30	0	647	1.56E-03	30	Barber et al., 1992
*	Water	-1.38	18.0	0.9957	30	0	647	1.40E-03	31	Bond & Barry, 1988

	COMPOUND[a]	$\log K_{ow}$[b]	MW	ρ (g/ml)[c]	T_{ref}[d] (C)	T_m[e] (C)	T_c[f] (C)	P_{cw} (adj)	T^g (C)	Permeability Reference
*	Water	-1.38	18.0	0.9957	30	0	647	1.55E-03	32	Bronaugh et al, 1986
*	Water	-1.38	18.0	0.9957	30	0	647	8.54E-04	30	Dick & Scott, 1992
*	Water	-1.38	18.0	0.9957	30	0	647	1.58E-03	30	Galey et al., 1976
*	Water	-1.38	18.0	0.9957	30	0	647	1.34E-03	31	Rigg and Barry, 1990
*	[Water]	-1.38	18.0	0.9957	30	0	647	5.00E-04	25	Scheuplein & Blank, 1973
*	Water	-1.38	18.0	0.9957	30	0	647	6.39E-04	30	Scott et al., 1991

a. Compounds contained within brackets (e.g. [Aldosterone]) also appeared in the Flynn database. Those indicated with an asterisk to the left (e.g. benzene) were used in developing the LSER correlations.

b. Reported $\log K_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for chloroxylenol [3.48]), in which case they were calculated (Daylight, 1995).

c. Density of the pure compound reported in the Handbook of Chemistry and Physics (Lide, 1996).

d. Temperature use to measure the density (Lide, 1996).

e. Melting point temperature (Lide, 1996) used to confirm that density values are for the liquid.

f. Critical temperature of chemical (Reid et al., 1987). Chemicals for which T_c was not found are designated with N/A.

g. Temperature of the reported permeability coefficient.

5.9. Appendix 5B: Documentation of Permeability Coefficients

This appendix contains specific documentation about the permeability coefficient data included in the fully-validated provisional and excluded databases. Details are arranged alphabetically by the last name of the first author of the investigation.

As stated previously, all data were evaluated with respect to the requirement that they be measured at steady state. Preferably, experimental evidence of steady state (e.g., demonstration that absorption is linear in time) was demonstrated. Sometimes steady-state had not been demonstrated experimentally, or, if it had been demonstrated, the results were not provided. Approximate estimates of the time required have been reported by Cleek and Bunge (Cleek and Bunge, 1993) at several different MW: chemicals with MW = 50 require 30 min to reach steady state; chemicals with MW = 100 require one hour; and chemicals with a higher MW = 330 require 24 hours to reach steady state. Polarity is also important. Roberts *et al.* showed that hydrophilic chemicals with low MW require longer times to reach steady state (Roberts *et al.*, 1977). Judgment was necessary in applying these trends to validation of the permeability coefficient measurements on the basis of steady-state.

Anderson et al., 1988

The measured permeability coefficients of fluocinonide and sucrose through isolated SC were taken directly from Table I, while permeability coefficients, for the remaining hydrocortisone esters, also through isolated SC, were taken from Table II. Identical values were included in the Flynn database (Flynn, 1990). All chemicals studied were at least 10% unionized in the vehicle (pH = 4). Although the exposure time was not precisely specified, we suspect that the values are at steady state (familiarity with authors). Figure 1 indicates that permeability coefficients, for several of the chemicals studied, are constant between 20 and 100-hours, indicating that excessive exposure times were not required for steady state to be achieved. Several different permeability coefficient values are reported for the hydrocortisone esters, but Anderson (Anderson, 1995) recommended that the Table II permeability coefficients be used. Also, Anderson (Anderson, 1995) noted a discrepancy between the permeability coefficient of fluocinonide in the experimental notebook (1.7×10^{-2} cm/hr) and that reported in their Table I (1.7×10^{-3} cm/hr) (Anderson *et al.*, 1988) and recommended that the permeability coefficient of fluocinonide be excluded. Anderson also recommended excluding their reported permeability coefficients of sucrose. He suggested that subsequent studies in the laboratory by Peck (Peck *et al.*, 1995) explored the permeability of polar permeants in

much greater depth and solved some of the problems leading to variability in permeability coefficients for these compounds.

Anderson et al., 1989

The reported permeability coefficients were taken directly from Table II. The structure for compound 1a in Table I is correct and the name should be, more correctly, given as α-(4-hydroxyphenyl)acetamide (Anderson, 1995). α-(4-hydroxyphenyl)acetamide, 4-hydroxybenzyl alcohol, methyl 4-hydroxy phenylacetate, p-cresol, 4-hydroxyphenyl acetic acid, and toluene were more than 10% unionized in the vehicle (pH = 4). The exposure times used in this study, were not provided, but we believe that steady state was obtained (familiarity with authors, appear to be rapidly penetrating chemicals). While the measurement for toluene is based on skin from only one donor, all other compounds were measured on skin from at least two donors (Anderson, 1995).

Barber et al., 1992

The human permeability coefficients of water and urea were taken without modification from Table 1. Urea was more than 10% unionized in the vehicle. The exposure time was 8-hours, which was sufficient for steady state to be obtained. We learned the following from personal communication with Barber (Barber, 1996): (1) the temperature used in the water permeation studies was 30 °C, (2) urea was delivered in a vehicle with pH = 7.1, (3) the membrane was isolated SC, and (4) the authors are not aware of any inaccuracies in the document.

Barry et al., 1985

Permeability coefficients for the five compounds were calculated directly from the saturated solution flux and solubility measurements provided in Table 2. Anisole, benzaldehyde, benzyl alcohol, and 2-phenyl ethanol were unionized, and aniline was more than 10% unionized in the vehicle. The exposure time was 9-hours, which was likely sufficient time for steady state to be obtained. These data were excluded because the skin barrier may have been damaged by contact with 50% aqueous ethanol in the receptor chamber.

Blank, 1964

The permeability coefficients of ethanol, propanol, pentanol, and octanol were taken from Table I. The exposure time was not provided, but the discussion in the results section indicates that they did examine the data for linearity (which was observed within 24-hours for aqueous vehicles and within 48-hours for nonpolar vehicles), indicating that steady state was achieved.

Blank et al., 1967

Permeability coefficients for ethyl ether, 2-butanone, 1-butanol, 2-ethoxyethanol, and 2,3-butanediol are taken from Table 1. Except for 2,3-butanediol Blank *et al.* report permeability coefficients as a range of values. Values included in the fully-validated database represent the midpoint of the values. Blank *et al.* report that the permeability coefficient for 2,3-butanediol was $< 0.5 \times 10^{-4}$ cm/hr (perhaps this value should be moved to the excluded database).

Blank and McAuliffe, 1985

The permeability coefficient of benzene was taken from Table I without adjustment. The exposure time was four hours. As demonstrated in Figure 1, this was sufficient time to reach steady state.

Bond and Barry, 1988a

The permeability coefficients of hexanol and water were taken unaltered from the first paragraph of the discussion. The exposure time was 6-hours, which was sufficient for steady state to be attained.

Bond and Barry, 1988b

The permeability coefficient of 5-fluorouracil was taken unaltered from the reported human measurement in Table 1. The exposure time was 60-hours, which was sufficient for steady state to occur, as shown in Figure 1. From personal communication (Barry, 1996) we learned that the pH of the saturated 5-fluorouracil solution was 4.75 at which they believe 5-fluorouracil would be unionized. However, based on calculations made using SPARC, the dominant species at this pH is a zwitterion. SPARC calculates that 5-fluorouracil is ionized at all pH.

Bronaugh et al., 1981

The permeability coefficient of N-nitrosodiethanolamine was taken directly from Table I. It is likely that this is the same value included (although incorrectly referenced) in the Flynn database(Flynn, 1990). Figure 2 indicates that steady-state penetration occurred between 20-40 hours.

Bronaugh and Congdon, 1984

The permeabilities of hair dyes were taken unaltered from Table I. Permeability coefficients from pure water were used for 2-amino-4-nitrophenol and 4-amino-2-nitrophenol since these compounds were mostly ionized in the borate buffer (pH 9.7) used to investigate the other chemicals. At these pH values, all other chemicals were more than 10% unionized in the vehicle. The exposure time was not specified but Bronaugh (Bronaugh, 1996) indicated that the exposure times were between 6-8 hours and that the vehicle did not evaporate.

Bronaugh et al., 1986

A permeability coefficient for water was taken as the average of values presented in Table II. This value is not meaningfully different from values reported in other tables in the same paper. The exposure time was 5-hours, which was sufficient for steady state to become established for water.

Chowhan and Pritchard, 1978

The permeability coefficient of naproxen was calculated by averaging the mean flux from the two experiments reported in Table I and dividing by the concentration which was assumed to be 5000μg/mL (not specified precisely). This procedure was also used by Flynn (Flynn, 1990) as described in that paper (but incorrectly referenced to the compound 2-naphthol rather than naproxen). Naproxen was more than 90% ionized in the vehicle (pH = 6.5). The issue of steady-state was never addressed. This value was excluded both because it was more than 90% ionized, and because the compound was applied to skin in an aqueous gel vehicle.

Cornwell and Barry, 1994

The permeability coefficient of 5-fluorouracil was calculated as the n-weighted arithmetic mean (that is, average values were multiplied by the number of samples) of the

data in Table 2. This is despite suggestion by the authors that a geometric mean would be more representative of the data distribution. Further work will be necessary to determine whether geometric means are more representative and should be used in developing correlations. The drug 5-fluorouracil existed as a zwitterion in the saturated aqueous solution, without a measurable level of unionized species (see discussion under (Bond and Barry, 1988a)). The exposure time was 36-hours and as Figure 3 illustrates, steady-state penetration was attained. According to Barry (Barry, 1996) the pH of saturated 5-fluorouracil solutions were 4.75.

Dal Pozzo et al., 1991

Permeability coefficients for the nicotinic acid derivatives were calculated from flux and concentration values reported in Table II. Two of the compounds (PEG 350 methyl nicotinate, and PPG 425 nicotinate) were a polydispersed mixture and so were excluded from the fully-validated database. No flux measurement was given for MEG-methyl nicotinate. Flux of methyl nicotinate and ethyl nicotinate were measured at two concentrations each. The two calculated permeability coefficients for methyl nicotinate were averaged as were the two permeability coefficients for ethyl nicotinate. All compounds were essentially unionized in the vehicle. The exposure time was 5 hours, which was deemed to be long enough to satisfactorily approximate steady state for these chemicals.

Dick and Scott, 1992

Permeability coefficient values for water, mannitol and paraquat were digitized from Figure 4. There is a discrepancy between values shown in Figure 4 and Table 1 which appear to be an error in the typesetting of Table 1. We were unsuccessful at contacting the authors, so we included the values we thought were most likely correct. Water and mannitol were not ionized in the unbuffered vehicle. Paraquat (corresponding anion not specified) exists naturally as a divalent cation and was placed in the provisional database. Paraquat was likely applied as a dichloride salt, since this was the form used by Scott *et al.* (Scott *et al.*, 1991). The exposure times were not specified but, steady-state was likely obtained for these hydrophilic chemicals.

Galey et al., 1976

Permeabilities for the chemicals investigated by Galey and others were converted directly from values (in cm/sec) reported in Table II. Based on pK_a values calculated in SPARC, water, ouabain, and estradiol were significantly less than 90% ionized in the vehicle (pH = 7), while amphetamine was more than 90% ionized in the vehicle at the

same pH. No quantitative indication of exposure time was given, although the authors, who have had extensive experience with membrane transport, report no time dependency in measured permeability coefficients.

Hadgraft and Ridout, 1987

The permeability coefficients for chemicals investigated by Hadgraft and others were calculated from resistances reported in Table 4 ($P_w = 0.36/R_s$ cm/s). The same conversions were performed by Flynn (Flynn, 1990) to incorporate these chemicals into the Flynn database. All the chemicals studied (barbitone, nicotine, phenobarbitone, hydrocortisone, butobarbitone, amylobarbitone, isoquinoline, salicylic acid) were at least 10% unionized in the vehicle (at the reported measured pH). The authors suggest that steady-state flux values were reported, and a typical plot for phenobarbitone (Figure 3) shows that data between 70 and 120-hours of exposure were used to determine the permeability coefficient. The permeability coefficient for hydrocortisone was not included in the fully-validated database because the vehicle contained 5% aqueous ethanol, which may have altered the skin.

Harada et al., 1993

The permeability coefficient of salicylic acid was obtained by averaging the mean flux measurements (made at different pH values), through human breast skin, that are reported in Table 1 and dividing by a concentration of 500μg/mL. Flux values for human neck skin were not used. At the most acidic extreme (i.e., pH = 2.0), the skin may have been damaged and this measurement was not included. The average flux at each of the pH values at which the compound was < 90% ionized (i.e., 3.0, 3.5), were equally weighted to obtain an overall average. (At pH = 3, an average flux was calculated from two flux which were reported.) The exposure time, reported as 72 hours, was sufficient (as shown in Figure 3) to attain steady-state penetration.

Jolicoeur et al., 1992

The permeability coefficient of etorphine was taken from Table I, and also appears in Table II. The experiment was conducted at pH = 7.3 in TRIS buffer, at which the compound was more than 90% ionized based on pK_a calculated in SPARC. Data collected at times between 4-24 hours were used in determining the permeability coefficient and this probably represents steady-state penetration. It appears that this value is based on skin from only one cadaver.

Kubota and Maibach, 1993

The permeability values for betamethasone and betamethasone-17-valerate were calculated ($P_{cw} = 1/R_{sc}$) from the stratum corneum resistances (i.e., R_{sc}) reported in Table I of this reference. The authors calculated R_{sc} from permeability (diffusion cell) experiments of split-thickness skin and dermis combined with partition coefficient experiments of whole skin, dermis and stratum corneum by describing skin as a series of resistances. Both compounds were unionized. The exposure time of 72 hours was probably sufficient to attain steady-state penetration.

Liu et al., 1994

The average of the three values of the stratum corneum permeability coefficient reported in Table 4 (converted from cm/sec to cm/ hr) was used for β-estradiol (E2). The authors calculated stratum corneum permeability coefficients from permeability (diffusion cell) experiments of dermis, split-thickness skin and split-thickness skin with the stratum corneum stripped, by describing skin as a series of resistances. β-estradiol is unionized in these experiments. The permeation cell experiments were conducted for only 4 hours, which may not be long enough to achieve steady state. Because time course data were not reported in the paper, we are unable to definitively assess whether the permeability measurements are steady-state values. Despite this, we included these permeability measurements in the validated data base, judging that the measured values were not greatly different from the steady-state values.

Lodén, 1986

The permeability coefficient of formaldehyde was calculated from the rate of absorption in Table 1 using a 3.7% v/v formaldehyde solution (10% v/v solution of formalin containing 37% formaldehyde and 10-15% methanol). This value was not included in the fully-validated database because formalin may have altered the skin. The compound was nevertheless more than 10% unionized in the vehicle (assuming aqueous properties). Exposure times of 20-hours were used in these experiments, and data shown in Figure 4 indicate that steady state penetration were achieved.

Megrab et al., 1995

The permeability coefficient of β-estradiol from saturated solution was calculated from the reported flux (0.015 ± 0.004 μg/cm^2/hr) and a solubility (C_w= 3.48 μg/mL) obtained through personal communication (Barry, 1996). Neither this solubility nor the

permeability coefficient were presented in the original publication. The compound β-estradiol was not ionized. The exposure time was at least 25 hours (Barry, 1996).

Michaels et al., 1975

Permeability coefficients for the chemicals investigated by Michaels and coworkers were taken directly from Table 2. These values are also those included in the Flynn database (Flynn, 1990). All chemicals were more than 10% unionized in the aqueous solution at the specified pH). Information about steady state was never discussed in the experimental portion of the paper. Unless very long exposure times were used, unsteady-state effects are likely to reduce permeability coefficients observed for the largest molecular weight chemicals: ouabain (MW = 584.64), and digitoxin (MW = 764.92).

Morimoto et al., 1992

The reported permeability coefficients for drugs measured by Morimoto and colleagues were digitized from Figure 5 in that publication. Buffers were not used, and the chemicals came to a natural state of ionization (pH was measured and reported in Table 3). The ionization of these compounds was calculated in SPARC. Lidocaine, diclofenac sodium, morphine, isoprenaline hydrochloride, and dopamine hydrochloride were more than 90% ionized under experimental conditions. The remaining chemicals (antipyrine, nicorandil, aminopyrine, cyclobarbitone, isosorbide dinitrate, ketoprofen, indomethacin, flurbiprofen, ibuprofen) were more than 10% unionized. Additionally, 5-fluorouracil and levodopa existed in a charged, but net neutral form (i.e., they were zwitterions). For some of these chemicals, the presence of dermis (750 μm total) might have influenced (i.e., lowered) the observed permeability coefficient value, but no chemicals were excluded for this reason. The exposure time was 10-hours, which closely approximated steady-state penetration for these compounds (see Figure 2).

Nakai et al., 1995 (unpublished results)

The permeability coefficients of atrazine, benzene, chloroform, tetrachloroethylene, and trichloroethylene were obtained from the averages reported in the tables (the tables were not labeled in these unpublished results). All compounds were unionized in an unbuffered vehicle, which, according to personal communication Nakai (Nakai, 1995) had a pH of 7.0 based on pH sensitive strips. The exposure times for trichloroethylene and tetrachloroethylene was 8 hours, while that for atrazine was 24

hours. These times were sufficient to observe steady-state permeability coefficients for these chemicals.

Parry et al., 1990

The permeability coefficient of benzoic acid through isolated stratum corneum, determined with simple linear regression, was taken from Table III. This coefficient was determined with unionized benzoic acid, and no correction was necessary. The exposure time was 4-hours, and steady-state penetration was demonstrated in Figure 3.

Peck et al., 1995

The permeability coefficients at 27°C and 39°C were taken from Table 1 (urea and mannitol), Figure 4 (tetraethylammonium bromide), and Figures 5 and 8 (corticosterone). At both temperatures the permeability coefficients of corticosterone and tetraethylammonium bromide were digitized. Urea, mannitol, and corticosterone were unionized, while tetraethylammonium bromide is an ion at the experimental conditions (pH = 7.4). The exposure time was 24-hours, for which steady state was probably reached for these compounds.

Rigg and Barry, 1990

The permeability of water was taken as the n-weighted (that is, average values were multiplied by the number of samples) average of Table I values. Two separate permeability coefficients were included in the provisional database for 5-fluorouracil since different experimental protocol were involved in each. Skin was hydrated for 12 hours before the permeability coefficient of 5-fluorouracil reported in Table II was measured. Skin was not hydrated before the permeability coefficient of 5-fluorouracil reported in Table III was measured. The drug 5-fluorouracil existed as a zwitterion in the saturated aqueous solution, without a measurable level of unionized species, based on SPARC calculations. The exposure time for water (6-hours) was sufficient for steady-state penetration to be obtained. In both experiments on 5-fluorouracil, 24-hour exposure times were used to ensure steady state. From personal communication with Barry (Barry, 1996), we have determined that the pH attained by 5-fluorouracil in saturated solution was typically 4.75.

Ritschel et al., 1989

The permeability coefficients of coumarin, griseofulvin, and propranolol were taken from Table I. The vehicle volume was small enough that concentration of the penetrating chemical decreased during the experiment. Coumarin, and griseofulvin are essentially unionized in the vehicle, while propranolol is more than 90% ionized in the vehicle based on calculations in SPARC. The exposure time was 48-hours, which is sufficient time for attaining steady state. The hypodermis was removed, but the final skin thickness was not identified.

Roberts et al., 1977

The permeability coefficients of phenolic compounds were taken from Table 1 with an adjustment of dimensions from cm/min to cm/hr. Identical values appear in the Flynn database (Flynn, 1990). Personal correspondence with Roberts (Roberts, 1996) confirmed that these values are more representative than those presented in Table 3, which were measured on skin from a single donor. The pH was unspecified, but all compounds were found to be unionized in neutral (pH = 7) or slightly acidic (pH = 6) distilled water. The exposure time was not precisely specified although graphical evidence(e.g., exposure times shown in Figures 1 and 4 are 250 and 500 min, respectively).

Roy and Flynn, 1989

The permeability coefficients of morphine, hydromorphone, codeine, and meperidine are presented in Table II . The permeability coefficients of fentanyl and sufentanil in solution at various pH values are reported in Table IV. The chemicals morphine, hydromorphone, codeine, and meperidine were more than 90% ionized in the vehicle (pH = 7.4), based on SPARC calculations, so these permeability coefficients were placed in the excluded database. Only fentanyl, and sufentanil are more than 10% unionized. The exposure times used for determining the permeability coefficients can be deduced from Figures 1 and 2 as: morphine (48 hours), hydromorphone (50 hours), codeine (22 hours), fentanyl (8 hours), sufentanil (8 hours), and meperidine (8 hours). They suggest in this paper that the permeability coefficients for fentanyl and sufentanil were measured on skin that was 3-4 times less permeable to fentanyl and sufentanil than typical skins (Roy and Flynn, 1989). Permeability coefficients for fentanyl and sufentanil are reported again in a subsequent investigation by the same authors (Roy and Flynn, 1990) where they use several different skins. We included the later permeability coefficients for fentanyl and sufentanil (Roy and Flynn, 1990) in the fully-validated database and did not include the permeability coefficients reported in this reference for

fentanyl and sufentanil in any database. It is worth mentioning, Flynn (one of the authors) decided to include the Table II values of fentanyl and sufentanil from the first investigation (Roy and Flynn, 1989), rather than those of a subsequent investigation (Roy and Flynn, 1990), in the Flynn database (Flynn, 1990).

Roy and Flynn, 1990

The permeability coefficients for fentanyl and sufentanil were obtained from this reference (see also documentation on (Roy and Flynn, 1989)). Average permeability coefficients for fentanyl and sufentanil are reported in Table V for what appears to be a large number of measurements for many different skin specimens. The pH of this table is reported to be 7.4 although it appears that several measurements at pH = 8 from Table II and Table III appear to be included. When asked, Flynn was not hopeful that it would be possible to resolve the apparent discrepancy with certainty (personal communication with Flynn, 1996). We assumed that the pH of 7.4 was correct for most of the measurements and divided the average permeability coefficients from Table V by the fraction unionized at pH = 7.4. Both fentanil and sufentanil are at least 10% unionized at either pH = 7.4 or pH = 8 based on calculations in SPARC. Roy and Flynn (Roy and Flynn, 1989; Roy and Flynn, 1990) reported pK_a values (8.9 for fentanyl and 8.5 for sufentanil at 37C) which are quite different from the SPARC calculated pK_a values we used (i.e., 7.0 and 6.2 for fentanyl sufentanil after adjustment to 37°C, respectively), but, it is not clear where these values come from. The pK_a values which we have calculated in SPARC appear to be more consistent with the data than the pK_a values reported by Roy and Flynn (Roy and Flynn, 1989; Roy and Flynn, 1990), although neither are precise. Insufficient information is given to precisely know the exposure time, although the authors report that 8 hours was sufficient to reach steady state, for these compounds, (Roy and Flynn, 1989; Roy *et al.*, 1994).

Permeability coefficients measured at a range of pH for fentanyl and sufentanil and reported in Table IV are incorporated in Figure 5.1a,b in this chapter. The following table provides documentation on how the reference unionized permeability coefficients from Figure 5.1a,b are determined. Figure 5.1a compares the observed permeability coefficients (the reported ones) with unionized permeability coefficients that were determined experimentally. Those experimentally determined permeability coefficients are calculated as the average of permeability coefficients measured when more than 99% of the compound was unionized. For fentanyl the unionized permeability coefficient is 0.0339 cm/hr which is the average of permeability coefficients measured at pH = 9.04 and 9.37, and for sufentanil the unionized permeability coefficient is 0.0327 cm/hr which is the average of permeability coefficients measured at pH = 8.52, 9.04, and 9.37. Figure 5.1b compares the observed permeability coefficients with unionized permeability

coefficients that were calculated from all measurements for which at least 10% of the compound was unionized. For fentanyl and sufentanil the calculated unionized permeability coefficients of 0.029 cm/hr and 0.024 cm/hr respectively were determined by averaging all of the tabulated $P_{cw,ui}$ that are reported when the pH was between 6.02 and 9.37.

	Fentanyl			Sufentanil		
pH	f_{ui} [a]	$P_{cw,obs}$	$P_{cw,ui}$ [b]	f_{ui} [a]	$P_{cw,obs}$	$P_{cw,ui}$ [b]
2.88	$8x10^{-5}$	0.0003	N/A [c]	$5x10^{-4}$	0.00046	N/A
5.08	0.013	0.0013	N/A	0.078	0.0025	N/A
6.02	0.103	0.0051	0.0495	0.426	0.0062	0.0146
6.95	0.494	0.0071	0.0144	0.863	0.0101	0.0117
7.43	0.747	0.0127	0.0170	0.950	0.0157	0.0165
7.95	0.907	0.0224	0.0247	0.984	0.0231	0.0235
8.52	0.973	0.0276	0.0284	0.996	0.0298	0.0299
9.04	0.992	0.0349	0.0352	1.0	0.0345	0.0345
9.37	0.996	0.0329	0.0330	1.0	0.0337	0.0337

[a] Calculated using 37°C pK_a values (6.96 for fentanyl and 6.15 for sufentanil) which were calculated at 25°C in SPARC and adjusted to 37°C

[b] Calculated by dividing $P_{cw,obs}$ (observed permeability coefficient) by f_{ui}

[c] N/A means not adjusted

Roy et al., 1994

The human permeability coefficient of morphine was taken from Table 1 without alteration. This chemical was more than 90% ionized in the vehicle (pH =7.5) based on calculations in SPARC. Insufficient information is available to determine whether the permeability coefficient of morphine is based on steady or unsteady-state data. In a prior investigation (Roy and Flynn, 1989), 48-hours was used as the exposure time.

Roy et al., 1995

The permeability coefficient value for ketorolac acid is the arithmetic average of the permeability coefficients for the R- and S-enantiomers and the racemic (50:50) mixture reported in Table 2. The exposure time was at least 30-hours, which was probably sufficient to attain steady state. Ketorolac acid was more than 90% unionized in the vehicle (pH = 2.1) based on calculations in SPARC. The concentration of penetrant in the vehicle decreased during the experiment (by 12% for S-enantiomer, 14% for R-

enantiomer, and 19% for racemic mixture. These permeability coefficients were placed in the excluded database because of the extremely low pH may have altered the skin.

Sato et al., 1991

The permeability coefficient of nicorandil was taken from Table I. The experimental procedure for the permeability studies was referenced to Sato *et al.* (Sato *et al.*, 1989). Exposure times of approximately 32 hours were used which was probably sufficient to attain steady state. A saturated aqueous solution of nicorandil was used, but neither the pH or concentration were reported. Using a concentration of 0.188 mol/L (Morimoto *et al.*, 1992) for saturated solutions of nicorandil at 37 and a pK_a value calculated in SPARC, we calculated that pH = 8 for this experiment (see Table 5A.4). At pH = 8 nicorandil is unionized for the pK_a calculated in SPARC.

Scheuplein et al., 1969

Permeability coefficients were taken directly from Table 1. All compounds were unionized. Based on Figure 2, long enough times were allowed to attain steady state.

Scheuplein and Blank, 1973

Permeability coefficients were taken directly from Table 1. Exposure times were not given, but, based on the authors discussions of membrane processes in this and their many other papers, we have assumed that the measurements were probably at steady state. These chemicals (alcohols) did not ionize.

Scott et al., 1991

The permeability coefficients of water, ethanol, mannitol, and paraquat dichloride were taken as the averages reported in Table I. Paraquat was applied as the dichloride salt (paraquat exists as a divalent cation in its natural state). Except for paraquat, these compounds were more than 10% unionized in the vehicle. Paraquat is a divalent strong electrolyte which will be fully ionized. The exposure time was 6-hours, which should have been sufficient for water and ethanol to reach steady state. Mannitol and paraquat, which are larger and more polar chemicals, may require this long or longer to reach steady state. The fact that paraquat,penetrates as an ion excludes it from the fully validated database.

Siddiqui et al., 1989

Permeability coefficients of seven steroids were taken from Table I without modification. The chosen coefficients are based on a fit to the single membrane diffusion model (DM I) rather than the shunt-membrane model proposed (DM II). By personal communication (Roberts, 1996), we have confirmed that the vehicle was water, that skin samples from several donors were used to generate the data, and that the temperature at which the measurements were performed was 25°C. The steroids were unionized. The exposure time of 80-hours was adequate time to reach steady state, as evidenced by Figures 2-3.

Singh and Roberts, 1994a

The lidocaine permeability coefficient is the average of three values reported in the results and discussion section (i.e., 0.0035, 0.0050, and 0.0041 cm/hr). Lidocaine was more than 90% ionized in the vehicle (pH = 7.4) based on SPARC calculations. The exposure time was 6 hours which was probably sufficient to approach or reach steady state.

Singh and Roberts, 1994b

The permeability values, for the chemicals investigated by Singh and Roberts were taken from Table 1. As confirmed by Roberts (Roberts, 1996), the value for piroxicam was incorrectly represented in that table and the correct value is 0.0034 cm/hr which is consistent with the 50% ionized value and with Figure 2. According to the authors, diethylamine salicylate ionizes in solution, so that the conjugate base of salicylic acid is actually introduced into solution. Since the penetrating species is unionized salicylic acid, the $\log K_{ow}$ and MW for salicylic acid were used in the regressions. Permeability coefficients for all chemicals were calculated as 2 times the flux reported when the pH = pK_a (neither pH or pK_a specified in paper). Since $f_{ui} = 0.5$ at pH = pK_a, the criteria of being at least 10% unionized is met. The authors claim the data are at steady-state, although exposure times were not specified.

Southwell et al., 1984

The permeability coefficient of phenol was calculated as the n-weighted average (that is, average values were multiplied by the number of samples) of the mean flux reported in Table 1 divided by the concentration (1g/100mL). Permeability coefficients for methanol, octanol, and caffeine were taken unaltered from the steady-state diffusion

experiment results presented in Table 2. Phenol, methanol, and octanol were not ionized in solution, and caffeine was more than 10% unionized in the vehicle, which was buffered at pH = 7.4 (Barry, 1996) based on calculations in SPARC. Phenol was studied with an exposure time of 10-hours, and octanol was studied with an exposure time of 6-hours. As demonstrated in Figures 3-4, these chemicals were probably steady state. Although exposure times were not reported for methanol and caffeine, based on the authors treatment of phenol and octanol, we have assumed that the measurements for methanol and caffeine were at steady state.

Swarbrick et al., 1984

Permeability coefficients were reported by Swarbrick *et al.* at pH values of 5, 6 and 7 in Table I. At all three pH values, all four carboxylic acids were more than 90% ionized, based on calculations in SPARC, and were placed in the excluded database. The exposure time was between 48 and 60-hours.

Williams and Barry, 1991

The permeability coefficient of 5-fluorouracil was calculated as the average of the control values in Table I. The drug 5-fluorouracil existed as a zwitterion in the saturated aqueous solution, which had a pH of approximately 4.75 (Barry, 1996), based on calculations in SPARC. In this study the exposure time was 36-hours. The results plotted in Figure 3 indicate that this exposure time is sufficient to ensure steady-state penetration of the drug.

5.10. Appendix 5C: Linear Solvation-Energy Relationship (LSER) Parameters

Quantitative structure-activity relationships (QSAR) are models which relate the structural and electronic features of a molecule to its macroscopic properties. Hansch and Leo (Hansch and Leo, 1995), have found that many diverse phenomenon can be described in terms of a simplified QSAR which uses hydrophobicity alone. A more general approach for relating the hydrophobicity interaction is to analyze free energy based properties in terms of indexes of solute-solvent interactions (Cramer *et al.*, 1993; Famini and Penski, 1992). Correlations conforming to this formalism are known as Linear Solvation Energy Relationships (LSER) and they have been described well by Cramer and colleagues (Cramer *et al.*, 1993), Famini *et al.* (Famini and Penski, 1992) and Abraham and coworkers (Abraham *et al.*, 1994). There are many similarities between QSAR and LSER and indeed they both have roots in more general Linear Free-Energy Relationships (LFER).

The basic premise of LSER is that a given property can be defined by a linear relationship of two different types of terms which describe the hydrogen bonding and polarizability-dipolarity of chemical interactions. The parameters in LSER analysis, called solvatochromic parameters (because they were historically determined by measuring UV spectral shifts for select dyes), serve as markers of the exoergic solute-solvent interaction phenomenon in these separate areas. In general, a molar volume term is also added and this approach has found particular use in calculation of solubility and partition coefficients (Cramer *et al.*, 1993).

Originally developed to describe solubility of a solute in various solvents (El Tayar *et al.*, 1991; Meyer and Maurer, 1995), LSER have found more applicability in the reciprocal sense of correlating a free energy dependent property exhibited by a class of

solutes in the same solvent. In this application, the solvatochromic parameters correspond to the solutes rather than the solvent. Various solvent-dependent, free energy based properties, λ, can then be described by correlations in terms of the LSER parameters α, β, π, and V_x according to:

$$\log(\lambda) = \log(\lambda_0) + a \cdot \alpha + b \cdot \beta + c \cdot \pi + d \cdot V_x \qquad \text{C.5.1}$$

where the coefficient $\log(\lambda_0)$ and the coefficients (a, b, c and d) are determined using linear regression analysis of data for a given property and set of compounds. The solvatochromic parameters have the following physicochemical interpretation: α is the effective hydrogen-bond acidity, β is the effective hydrogen-bond basicity, π is the solute dipolarity/polarizability, V_x [units of cm^3/mol/100] is the characteristic volume of McGowans (Abraham and McGowan, 1987) calculated from molecular structure alone and is independent of intermolecular forces such as hydrogen bonding. When LSER are applied in this fashion, it is assumed that the difference in the chemical potential which drives the solute through the process, λ, results from van der Waals forces, size effects, polarizability, and the preference of a solute to act as an electron donor and an electron acceptor in hydrogen bonds.

Any site with unshared electrons is a potential hydrogen bond acceptor, although the more strongly basic and the less polarizable the acceptor site, the stronger will be the hydrogen bond. Solvents which are protic are good hydrogen donors, and those which are aprotic may or may not be good hydrogen acceptors. In general, oxygen-hydrogen and nitrogen-hydrogen bonds readily associate in hydrogen bonding, but carbon-hydrogen bonds are too weakly acidic to form hydrogen bonds. As an example, negative ions, which are good H-bond acceptors, are strongly solvated by protic solvents but are less soluble in aprotic solvents. By contrast, protic solutes will ordinarily interact by hydrogen bonding with protic solvents.

Polarizability is a measure of the ease with which the electron distribution of a molecule is distorted. It determines the attractive forces (van der Waals forces) which arise between two molecules in close proximity. The electron clouds distort and an instantaneous dipole arises, which causes attraction. In another context, polarizability measures how well a molecule can stabilize a charge or dipole by means of its dielectric effect. The polarizability term (π) can be divided by the molecular volume to put polarizability on a size-independent basis.

Unlike molar volumes, the volume term of McGowan (V_X) (Abraham and McGowan, 1987) has been calculated so that it is independent of hydrogen bonding interactions. The volume term contributes to estimation of permeability coefficients in two ways. First, as the volume of a chemical increases, its diffusion rate within the stratum corneum will decrease. Second, the solute size contributes to the partitioning of the solute between vehicle and skin due to free energies associated with cavity formation in the skin and in the aqueous vehicle (cavity formation energies in water are larger than in skin). The effects which favor partitioning into the SC are both the solute stratum corneum interactions caused by size dependent van der Waals dispersion forces and the difference in free energy costs associated with the formation of a cavity necessary to accommodate the solute in either the skin or the vehicle,.

The solvatochromic parameters used in this work, were from Abraham *et al.* (Abraham *et al.*, 1994) who determined them by averaging multiple normalized solvent effects on a variety of chemical properties involving many varied types of indicators. These experimentally determined descriptors are now available for well over 1000 solutes (Abraham *et al.*, 1994)

6. ESTIMATING HUMAN STRATUM CORNEUM-WATER PARTITION COEFFICIENTS OF ORGANIC CHEMICALS

6.1. Introduction

The long-term goal of the study of percutaneous absorption is the accurate prediction of dermal absorption in terms of physicochemical properties of penetrating molecules. In Chapter 5 we created a database of permeability coefficients and used this to improve our predictions and understanding of skin penetration. In this chapter we perform a similar investigation of the stratum corneum-water partition coefficient, another parameter which is important for characterizing dermal absorption. The stratum corneum-water partition coefficient represents the capacity of the stratum corneum (SC) for a compound relative to its aqueous concentration and is a required input parameter for some dermal absorption models. In this chapter, we describe the collection of SC-water partition coefficient measurements into a validated database and several predictive correlations developed from these data. Also, we analyze the database in ways that provide information about the mechanism by which SC-water partitioning occurs. Finally, we use the database to evaluate previously published correlations for estimation of the SC-water partition coefficient.

The quality of any predictive correlation is inextricably tied to the quality of the data with which it is trained. It is therefore important to critically review each measurement in the database of SC-water partition coefficients prior to the development of predictive correlations. In this work we investigate the criteria that are essential for

SC-water partition coefficients to be meaningful, and apply those criteria to a large database of experimentally determined SC-water partition coefficients.

6.2. Data Validation Criteria

SC-water partition coefficients are determined in relatively simple experiments. Most partition coefficients have been determined by equilibrating an accurately weighed sample of SC (often desiccated) with an aqueous solution of the absorbing compound which is often labeled with ^{14}C or ^{3}H. After sufficient time, the solute concentration in the aqueous solution and in the SC (frequently determined by liquid scintillation counting of radioactivity of the solubilized SC) are determined. The partition coefficient is calculated from the ratio of the SC and water concentrations at equilibrium. All of the partition coefficients which we review have been measured by slight variations of this protocol. We did not include partition coefficients that were calculated by fitting mathematical models to penetration data produced in diffusion cell experiments. Because these experiments are relatively easy there are a large number of SC-water partition coefficient measurements reported in the literature which can be use to develop accurate predictive correlations.

All partition coefficients analyzed in this paper were measured *in vitro* (by the partitioning technique, not the permeation technique) with human skin from aqueous solution. Prior to developing correlations, a critical review process was used to validate that a measurement met certain criteria. Every effort was made to extract data from only original references. SC-water partition coefficients reported without pertinent details are reserved for future analysis, if the missing information can be obtained from the original authors. The database is divided into three collectively exhaustive (taken together they contain all partitioning measurements we have considered) and mutually exclusive (measurements appearing in one database do not appear in others) divisions: (1) a fully-

validated database (containing the measurements used to develop correlations), (2) a provisional database (reserved for analysis once additional necessary information is obtained), and (3) an excluded database (partition coefficients that do not meet the validation criteria).

The fully-validated database contains partition coefficient values which meet six criteria: (1) the partition coefficient is based on the hydrated volume of SC or is converted to that basis, (2) the ionized state of the partitioning compound must be known and the fraction unionized, f_{ui}, must be greater than 0.9, (3) a valid $\log K_{ow}$ (either a recommended value from Hansch and colleagues (Hansch *et al.*, 1995) or else calculated using Daylight software (PCModels, 1995) which was developed from the Hansch database of validated K_{ow}) must represent the partitioning molecule (usually given for the unionized compound), (4) reasonable evidence must be provided that equilibrium was established, (5) the temperature (must be specified) must be between 20-40°C, and (6) the exposure solution can not compromise the SC (more than water does).

6.2.1. Expression of the Stratum Corneum Concentration

A review of the literature reveals that the concentration of the partitioning compound in the SC are reported in different ways producing different definitions of the SC-water partition coefficient. To be dimensionally consistent with the differential material balances (i.e., mathematical models) describing penetration of chemicals through a skin membrane, the partition coefficient when multiplied by the aqueous concentration (C_w), given as solute mass per volume of solution, should have units of solute mass per volume of fully-hydrated stratum corneum. That is, the dimensionally consistent definition of K_{cw} is:

$$K_{cw} = \frac{\text{mass of chemical / volume of hydrated SC}}{\text{mass of chemical / volume of aqueous solution}} \tag{6.1}$$

However, many researchers have reported a different partition coefficient, K'_{cw}, calculated using the dry mass of the SC as the basis. That is:

$$K'_{cw} = \frac{\text{mass of chemical / mass of dry SC}}{\text{mass of chemical / mass of aqueous solution}} \tag{6.2}$$

Partition coefficient measurements reported on a dry-mass of SC basis can be related to the dimensionally consistent coefficient (K_{cw}) through the ratio of the solution density, ρ_D, and ρ_{sc} defined as the mass of dry SC / hydrated skin volume. That is,

$$K_{cw} = K'_{cw} \frac{\rho_{sc}}{\rho_D} \tag{6.3}$$

The value of ρ_{sc} is not known precisely, although several investigations afford us a reasonable estimate of this parameter.

The most decisive measurements came from the study by Raykar and colleagues (Raykar *et al.*, 1988), who measured the water uptake of dry SC over a period of 48-72 hours. Raykar and colleagues found that an average of 2.91 grams of water absorbed per gram of dry SC over a period of 48-72 hours when the temperature was maintained at 37°C. This result is based on 73 measurements of water uptake using skin samples of various anatomical locations from at least 21 different people. To use this information to estimate ρ_{sc}, we must know the density of dry SC. Using 1.3g/mL as the density of dry SC and assuming 2.91 grams of water are absorbed per gram of dry SC, we calculate that 1 gram of dry SC should have a hydrated volume of 3.68mL (= 2.91 + 1/1.3). That is, $\rho_{sc} \approx 0.27$g/mL (=1g/3.68mL). According to Bronaugh and Congdon (Bronaugh and Congdon, 1984) the density of dry SC is 1.32g/mL (i.e., grams of dry SC per mL of dry SC) as originally reported by Scheuplein (1966). Since the Scheuplein report has a limited circulation we were not able to verify the 1.3 value for the dry SC density. However, these calculations show that ρ_{sc} is relatively insensitive to the value for the dry SC density within a reasonably expected range (i.e., 1.0 - 1.5g/mL).

Megrab and others (Megrab *et al.*, 1995) found that approximately 3.7 grams of water absorbed per gram of dry SC over a period of 48 hours leading to a ρ_{sc} value of 0.22g/mL (assuming the density of dry SC is 1.3g/mL). In their review article, Scheuplein and Blank report (Scheuplein and Blank, 1971) an even higher water absorption. They report that occluded SC slowly imbibes 5-6 times its dry weight in water. If we again assume that the dry density of SC is close to 1.3g/mL then 0.15g/mL < ρ_{sc} < 0.17g/mL. For shorter time periods, Scheuplein and Blank report (Scheuplein and Blank, 1973) that approximately 2g of water is absorbed per gram of dry SC after 24 hours of hydration (i.e., ρ_{sc} = 0.36g/mL).

Although not known precisely, the conversion factor ρ_{sc} is significantly different from unity. These results suggest that a value between 0.2g/mL and 0.3g/mL is most reasonable. We estimate that ρ_{sc} = 0.25g/mL is representative of SC which has been exposed to excess hydration for periods long enough to ensure complete hydration (i.e., at least 48 hours).

A few authors report their partition coefficient data in terms of hydrated mass of SC (referred to as wet mass in Tables 6A.1 and 6A.2). In this case, the adjustment factor is $\rho_{wet\ sc}/\rho_D$ where $\rho_{wet\ sc}$ is the mass of hydrated SC/hydrated skin volume. If the density of dry SC is 1.3g/ml and 2.91g of water absorb in 1g of dry SC, then $\rho_{wet\ sc}$ = (1g dry SC + 2.91g water) / (1g dry SC / (1.3g/mL) + 2.91g water / (1g/mL)) = 1.06g/mL. If 1g of SC can absorb 5g of water, then $\rho_{wet\ sc}$ = 1.04 g/mL. In adjusting partition coefficients based on hydrated mass of SC, we have assumed $\rho_{wet\ sc}/\rho_D \approx 1$. Based on this value, we calculate K_{cw} from K'_{cw} by multiplying K'_{cw} by 0.25 (= ρ_{sc}/ρ_D = 0.25g/mL / 1.0g/mL).

6.2.2. Effect of Ionization

SC-water partition coefficients are available for compounds which are unionized, charged, and net neutral (zwitterionic). However, the effects of chemical ionization on

the partition coefficient are not well documented experimentally except for a few isolated studies. One study measured the partitioning of ionized (anionic) and unionized lauric acid ($\log K_{ow} = 4.2$) and found that both partition into the SC although the unionized form partitioned more strongly (Smith and Anderson, 1995). Smith and Anderson found that the partition coefficient for the unionized form (5060) was approximately 70 times larger than the partition coefficient for the anionic form (72.2) (Smith and Anderson, 1995). Smith and Anderson claim that "there is abundant evidence demonstrating that carboxylic acids are effective permeability enhancers". The implication is that this permeability enhancement could be due to a partition coefficient enhancement.

Later we will examine one way to evaluate the effect of ionization, which is to compare partition coefficient data for partially ionized chemicals to the correlation developed from only nonionized chemicals. To do so, the fraction of unionized compound, f_{ui}, was determined from pK_a values calculated in SPARC and adjusted for temperature by the methods described in Chapter 5. When not reported, the pH was calculated from the solution concentration and pK_a using the general treatment of simultaneous equilibrium that was also discussed in Chapter 5. In a partition coefficient experiment the concentration can change until an equilibrium distribution is reached between the SC and the solution. Consequently, the final equilibrium concentration is the appropriate solution concentration for calculating the natural pH of the solution.

6.2.3. Selection of $\log K_{ow}$

Later in this chapter, we correlate unionized SC-water partition coefficients with $\log K_{ow}$ determined for the unionized form of the absorbing chemical following a procedure similar to that discussed in Chapter 5. The preferred $\log K_{ow}$ values (★) reported by Hansch and colleagues (Hansch *et al.*, 1995), which were "measured as or converted to the neutral form" were preferentially selected. When these recommended

values were not available, Daylight software (PCModels, 1995) was used to calculate surrogates that are consistent with the database of preferred values. Because ionic species are quite water soluble, these neutral form partition coefficients are frequently different (higher) than those measured for partially ionized chemicals. SC-water partition coefficients for all chemicals (both unionized and partially ionized compounds) are plotted at either the ★ value or a calculated value of $\log K_{ow}$ for the unionized species.

6.2.4. Equilibration Time

While a long equilibration time is desirable when measuring solvent-solvent partition coefficients, prolonged contact between skin and a solvent (i.e., the vehicle) may alter the degree to which the skin can absorb a distributing solute. Roskos and Guy suggest that a six hour equilibration time, sufficient to fully hydrate the SC (Roskos and Guy, 1989). However, more than 6 hours may be necessary for extremely hydrophilic compounds and for molecules which diffuse slowly into the SC (i.e., high molecular weight compounds). Whenever it is known, we have reported the exposure time in our database. To be validated, reasonable evidence is required to indicate that equilibrium was attained. This requirement is satisfied if the authors monitored concentration over time until there were no further changes, or by the authors using sufficient time in previous publication of similar measurements.

6.3. Correlation Development

Equations are developed in this section that will be used for later analysis of the SC-water partition coefficient database. First we develop a very simple model which has historically been used to analyze SC-water partition coefficients (i.e., the conventional correlation). Later in this analysis we will analyze the database with a linear solvation-energy relationship (LSER) model and a model which accounts for potentially different

mechanisms for partitioning into the hydrophilic and lipophilic domains of the SC. These models will provide insight into the mechanisms of partitioning into the SC.

6.3.1. Development of the Conventional Model

Solvent-solvent partition coefficients are generally correlated in terms of other solvent-solvent partition coefficients using linear free-energy relationships (Lyman *et al.*, 1982). SC-water partition coefficients are frequently analyzed in terms of octanol-water partition coefficients (El Tayar *et al.*, 1991; Roberts *et al.*, 1977) using a linear free-energy relationship of the form:

$$\log K_{cw} = a + b \cdot \log K_{ow} \tag{6.4}$$

This equation assumes that the energetics of solvation in octanol and SC are similar for compounds with a wide spectrum of hydrophobicity. Differences in cavity formation energies between the SC (which is an amorphous solid or liquid crystalline) and water, or octanol (which is liquid) and water, can be investigated with a correlation that also accounts for differences in molecular size:

$$\log K_{cw} = a + b \cdot \log K_{ow} + c \cdot MW \tag{6.5}$$

in this case represented by molecular weight, MW. In Eq. (6.5) we have allowed for $\log K_{cw}$ being linearly correlated with MW as might occur through an activated Arrhenius process.

6.3.2. Development of the Lipophilic-Hydrophilic Domain Model

Morphologically the SC is a heterogeneous membrane composed of proteinaceous and lipid domains. The protein and lipid domains in the SC are histologically revealed as a mosaic of cornified epidermal cells containing cross-linked keratin filaments and intercellular lipid-containing regions (Elias, 1981). The cellular proteins are not themselves a homogeneous domain but the differences within this milieu are small when compared with differences between the lipids and this protein phase. Researchers have

speculated that compounds partition into the protein phase of the SC as well as into the intercellular lipids (Raykar *et al.*, 1988) to extents that depend on their lipophilicity. A change in uptake mechanism can occur if the selectivity of the protein and lipid domains differ toward solutes with varying lipophilicity. Such a difference is expected since these domains have significantly different polarities.

At a simple level, a two-phase partitioning model allows for a different relationship for partitioning into the hydrophilic and lipophilic domains of the SC. Several organic solvents (e.g., octanol, and isopropylmyristate) may reasonably represent the lipid domain. Our starting place is to assume that these or other solvents can also represent partitioning into the protein domains but with a different dependence. That is, we begin with a model containing a different linear dependence on $\log K_{ow}$ for hydrophilic and lipophilic compounds (the distinction between hydrophilic and lipophilic compounds will be defined by the regression). The general form of this simple model of two-phase SC-water partitioning is:

$$\log K_{cw} = a + b \cdot \log K_{ow} + c \cdot \left(\log K_{ow} - \log K_{ow}^{\#}\right)\delta \tag{6.6}$$

where $\log K_{ow}^{\#}$ is the $\log K_{ow}$ at which the piecewise linear regression changes slope and distinguishes the hydrophilic compounds from the lipophilic compounds (in this regression), and δ is an indicator variable with the following values:

$$\delta = \begin{matrix} 1 & \text{if } \log K_{ow} \geq \log K_{ow}^{\#} \\ 0 & \text{if } \log K_{ow} < \log K_{ow}^{\#} \end{matrix} \tag{6.7}$$

For this expression, b and (b + c) are the slopes of the two regression lines, and a and (a - $c \cdot \log K_{ow}^{\#}$) are the two intercepts.

In an even simpler model, we assume that hydrophilic compounds partition into the SC at a constant value which is independent of their lipophilicity. That is,

$$\log K_{cw} = a + c \cdot \left(\log K_{ow} - \log K_{ow}^{\#}\right)\delta \tag{6.8}$$

For this expression, a is the average $\log K_{cw}$ for the hydrophilic compounds, and c is the slope and $(a - c \cdot \log K^{\#}_{ow})$ is the intercept for lipophilic compounds.

This analysis of SC-water partition coefficients in terms of two partitioning domains is simplistic. Equations (6.6) and (6.8) assume that the mechanisms for SC-water partitioning of hydrophilic and lipophilic compounds switch abruptly and completely at $\log K^{\#}_{ow}$. More sophisticated models can be developed to extend these simple models as needed and justified by the data.

6.3.3. Development of the Linear Solvation-Energy Relationship (LSER) Model

The theoretical foundations of linear solvation-energy relationships (LSER) have been described in several references (Abraham *et al.*, 1994; Cramer *et al.*, 1993; Famini and Penski, 1992). In Chapter 5, LSER parameters are discussed and used to interpret and predict SC permeability coefficients. In this chapter we use the LSER model to analyze SC-water partition coefficient data and develop predictive correlations. Similar analysis has been done before on smaller and unvalidated databases (e.g., the study of Abraham *et al.* (Abraham *et al.*, 1995)).

Linear solvation-energy relationships (LSER) have been successful at correlating free energy dependent properties exhibited by a class of solutes in the same solvent. Partitioning of organic solutes into the SC from water is a free energy-based property that can be analyzed with LSER models. The general model for analysis of SC-water partition coefficients is:

$$\log(K_{cw}) = \log(K^{o}_{cw}) + a \cdot \alpha + b \cdot \beta + c \cdot \pi + d \cdot V_x \qquad (6.9)$$

The LSER parameters (α, β, π, and V_x) are measured and tabulated for solutes rather than the solvent. The solute solvatochromic parameters have the following physicochemical interpretations: α is the effective hydrogen-bond acidity, β is the effective hydrogen-bond basicity, π is the solute dipolarity/polarizability, and V_x (units of $cm^3/mol/100$) (V_x is

conventionally divided by 100 to give it the same magnitude as the other LSER parameters) is the characteristic volume of McGowans (Abraham and McGowan, 1987) which is calculable from molecular structure alone and is independent of intermolecular forces such as hydrogen bonding.

Equation (6.9) assumes that the difference in the chemical potential causing solute to distribute from water into the SC results from differences in van Der Waals forces, size effects, polarizability, and the preference of a solute to act as an electron donor and an electron acceptor in hydrogen bonds with the vehicle and with skin. Models of SC-water partitioning are developed by regressing LSER parameters for solutes with known SC-water partitioning coefficients and thereby determining the coefficients $\log K_{cw}^{o}$ and *a* through *d* with multilinear regression analysis of data.

6.4. Results and Discussion

6.4.1. Examination of the Data

The validation criteria were applied to a collection of known partition coefficients. Details on selection of the appropriate partition coefficients (from the original publication) and their validation are given in Appendix 6B. Appendix Table 6A.1 contains all fully-validated partition coefficients which we have examined. These compounds span a fair level of diversity as measured by MW and $\log K_{ow}$. LSER parameters are available for the chemicals enclosed within brackets (e.g., [benzene]), so these measurements were included in the LSER analysis.

Table 6A.2 lists the provisional database of partition coefficients. Partition coefficients for chemicals with $f_{ui} < 0.9$ or with an unknown f_{ui} were included in the provisional database unless they were otherwise excluded. Several chemicals studied by Surber *et al.* (Surber *et al.*, 1990a; Surber *et al.*, 1992) are weak acids or bases. Unfortunately, neither the final equilibrium solution concentration or pH are reported or

could be determined by communication with the authors (Surber, 1996). In some cases, f_{ui} will be approximately 1 at the natural pH regardless of solute concentration (these are listed in Table 6A.1). For other compounds, f_{ui} is quite sensitive to the natural pH which can not be calculated without knowing the concentration. Listed in Table 6A.2, these chemicals are probably partially ionized, although to an unknown extent.

Table 6A.3 contains the excluded database of partition coefficients. Measurements for chemicals enclosed within brackets (e.g., [alachlor]) were made using sieved, powdered human stratum corneum (PHSC) using plantar calluses. These partition coefficients are excluded because they may not accurately represent partition coefficients for unpowdered, non-callus SC. This issue will be discussed more fully in Figure 6.7. The measurement for nicorandil was also excluded because it was measured using plantar callus which is potentially different from non-callus SC. Cyclosporin-A was excluded because it is much larger than the other compounds included in the database and may partition by different mechanisms than the smaller compounds. Measurements for PCB were excluded because they were measured for a polydispersed mixture of chlorinated biphenyls rather than for a single compound.

Table 6A.4 lists partition coefficients included in the LSER database (a fraction of the measurements from the Fully-Validated with known LSER parameters) and the LSER parameters for the chemical. Table 6A.5 summarizes the calculated fraction unionized and natural pH attained by aqueous solutions at known and unbuffered concentration.

Figure 6.1 shows the fully-validated database, provisional database (unadjusted for ionization), and several measurements from the excluded database (e.g., cyclosporin-A, nicorandil, and PCB) with no adjustment for basis, plotted as a function of $\log K_{ow}$. Cyclosporin-A is larger than the other compounds (MW = 1201) and probably partitions into the SC by a different process. The SC-water partition coefficient for nicorandil was measured with plantar callus which is potentially different from uncallused SC. The PCB

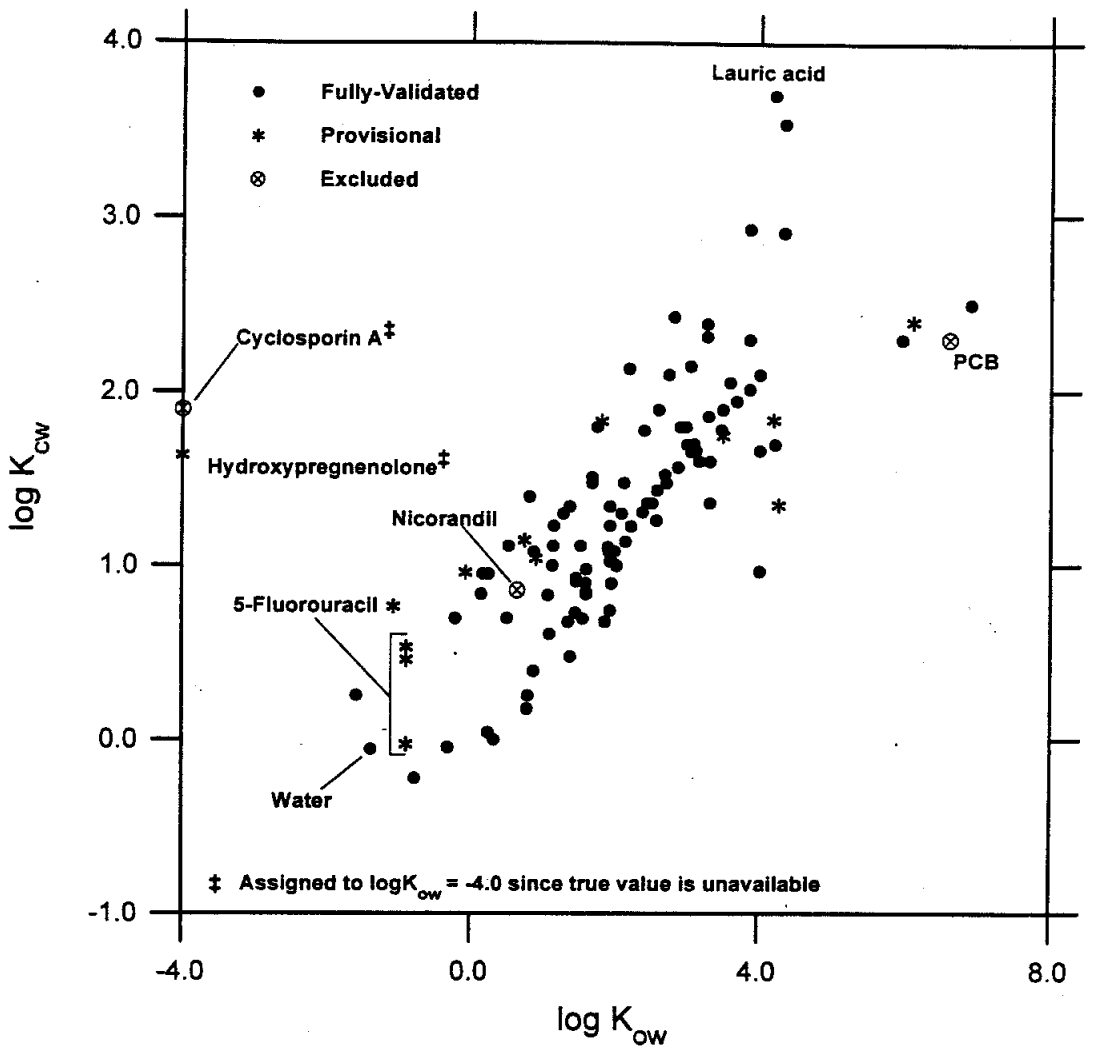

Figure 6.1 Stratum corneum-water partition coefficients of the fully-validated and provisional databases and several values from the excluded database plotted as a function of K_{ow}. Hydroxypregnenolone and cyclosporin-A, which lack appropriate $\log K_{ow}$, are assigned to $\log K_{ow} = -4.0$.

(arochlor 1254, 54% chlorine) partition coefficient was excluded because PCB exists as a polydispersed mixture. The partition coefficients of cyclosporin-A (an excluded measurement) and hydroxypregnenolone (a provisional measurement) are plotted at artificial lipophilicities ($\log K_{ow} = -4$) since accurate $\log K_{ow}$ are not available for these compounds. Unless otherwise stated, hydroxypregnenolone and cyclosporin-A have been omitted from figures because there is no known K_{ow} for interpreting the measurements. Excluded measurements made with powdered human stratum corneum (Hui *et al.*, 1995; Wester *et al.*, 1987) are not shown on this plot but are discussed in more detail later in this analysis. One value for lauric acid (from Smith and Anderson (Smith and Anderson, 1995)) is the largest partition coefficient in the validated database and labeled in Figure 6.1. We will find later that the measurement for lauric acid deviates the most from our conventional regression equation (i.e., Eq. (6.11)).

Figure 6.2 shows the data from Figure 6.1, after adjustment for basis (i.e., all data are reported in terms of the volume of hydrated SC) plotted as a function of $\log K_{ow}$. Also shown in Figure 6.2 is the linear regression of Eq. (6.4) which was developed from the validated SC-water partition coefficients (i.e., the measurements in Table 6A.1). The partition coefficients shown span four orders of magnitude, but are more confined than permeability coefficients (which can vary by up to six orders of magnitude). The regression describes the data reasonably well.

6.4.2. Analysis with the Conventional Model

First we analyze the validated but unadjusted data from Table 6A.1. Using a standard linear regression package, JMP (SAS Institute, 1995)), Eq. (6.4) was found to be:

$$\log K_{cw} = 0.459(0.074) + 0.402(0.029)\log K_{ow}$$
$$(n = 96,\ r^2 = 0.667,\ r^2(\text{adj.}) = 0.663,\ \text{RMSE} = 0.399,\ \text{F-Ratio} = 188.3) \quad (6.10)$$

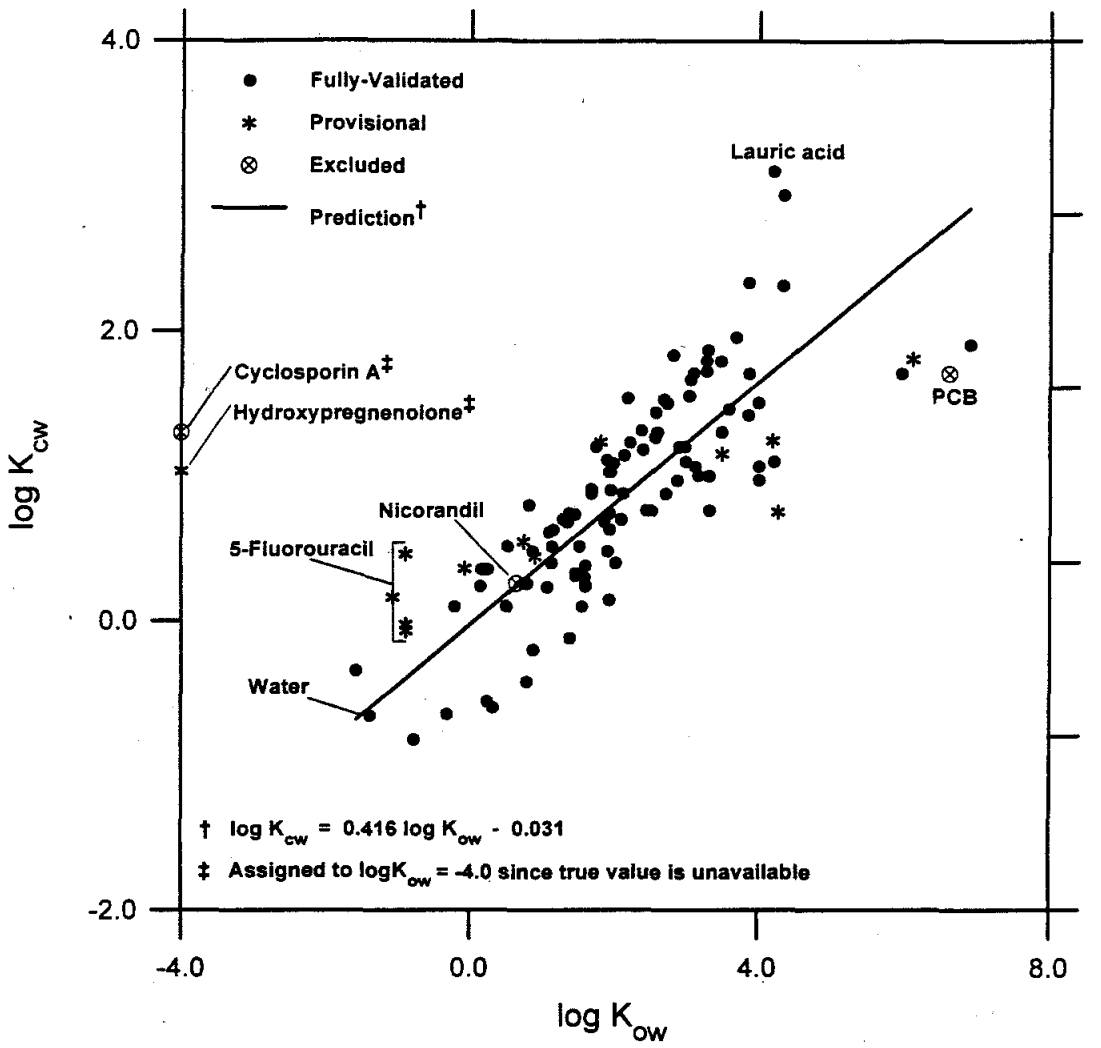

Figure 6.2 Stratum corneum-water partition coefficients of the fully-validated and provisional databases and several values from the excluded database plotted as a function of K_{ow}. Partition coefficients are expressed relative to the hydrated volume of the stratum corneum.

Approximately 67% of the variability in SC-water partition coefficients is explained by variation in $\log K_{ow}$ and a constant according to Eq. (6.10). Standard error of the coefficients are reported within parenthesis. The r^2(adj.) statistic is analogous to r^2 but allows for more relevant comparisons between models with different numbers of fitted parameters (JMP Users Manual, (SAS Institute, 1995)). Specifically, $(1 - r^2)$ = error sum of squares/total sum of squares and $(1 - r^2(\text{adj.}) = (1 - r^2)(n - 1)/(n - p)$ where n = # of data points and p = # of parameters. RMSE is the root mean square error of the model, which is zero when the model perfectly correlates the data. When presented in an equation, F-Ratio is the model F-Ratio (sum of squares for the model divided by the degrees of freedom for the model) / (sum of squares for the error divided by the degrees of freedom for the error), and when presented for a term such as the MW or $\log K_{ow}$, the F-Ratio is the effect F-Ratio (sum of squares for the effect divided by the degrees of freedom for the effect) / (sum of squares for the error divided by the degrees of freedom for the error). The model F-ratio = 1 when there is zero correlation with the parameters and is large for correlations with good predictive power. Because the number of fitted parameters is in the denominator of the F-Ratio, changes in the model F-Ratio with an increase in the number of parameters should reflect the effect on predictive power relative to the number of fitted parameters. Thus, a correlation with a larger number of parameters might give a higher r^2 (or r^2(adj.)) but a lower F-Ratio than a correlation with fewer parameters. This would indicate that the improvement in predictive power (as indicated by a larger r^2) was not as large per parameter as for the equation with fewer parameters.

After adjustment for basis (i.e., all SC-water partition coefficients are reported in terms of the volume of hydrated SC), Eq. (6.4) is found to be:

$$\log K_{cw} = -0.031(0.075) + 0.416(0.029)\log K_{ow}$$
$$(n = 96,\ r^2 = 0.682,\ r^2(\text{adj.}) = 0.678,\ \text{RMSE} = 0.400,\ \text{F-Ratio} = 201.3) \quad (6.11)$$

This correlation, which defines the role of lipophilicity in SC-water partitioning is based on a database that is adequately large and diverse for predictive estimates to be relevant. About 68% of the variability in the 96 values of the validated and adjusted SC-water partition coefficients is explained by variation in $\log K_{ow}$ according to Eq. (6.11). Because a large fraction of the database was adjusted to a basis of hydrated volume of SC, there is only a modest improvement in the regression statistics for Eq. (6.11) over Eq. (6.10). Consistent with this fact, within statistical uncertainty, the constant in Eq. (6.11) is equal to the constant in Eq. (6.10) and log (0.25) = 0.459 – 0.602 = -0.143.

An equation which also allows for differences in MW was developed by regressing Eq. (6.5) to the validated and adjusted data from Table 6A.1:

$$\begin{gathered}\log K_{cw} = -0.146(0.090) + 0.395(0.030)\log K_{ow} - 0.00066(0.00030)\,MW^{*} \\ (n = 96,\ r^2 = 0.698,\ r^2(\text{adj.}) = 0.691,\ \text{RMSE} = 0.392,\ \text{F-Ratio} = 107.3)\end{gathered} \tag{6.12}$$

The asterisk designates that the MW term is not meaningful at the 95% confidence level. Molecular weight effects will no longer be considered in our analysis of SC-water partition coefficients. Notice that SC-water partition coefficients have a weaker dependence upon $\log K_{ow}$ than permeability coefficients.

Importantly, correlations should not be used for estimating partition coefficients for compounds that are very different from those used to develop the database. That is, appropriate bounds on $\log K_{ow}$ and MW need to be set for all permeability correlations. In the fully-validated database, 95% of the chemicals have $-1.1 \leq \log K_{ow} \leq 5.3$ with equal percentages higher and lower than these bounds. Likewise, 80% of the chemicals have $0.3 \leq \log K_{ow} \leq 3.9$. Based on the accuracy of the correlations at describing the existing data, we recommend the less conservative lower bound ($\log K_{ow} = -1.1$) and the more conservative upper bound ($\log K_{ow} = 3.9$) for $\log K_{ow}$. A reasonable lower bound for MW is that of water (i.e., the lowest MW chemical in the database, MW = 18). In the fully-validated database, 10% of chemicals have MW ≥ 465 and 2.5% have MW ≥ 500. The

less conservative bound (i.e., MW ≤ 500) is chosen, since MW was shown to have a small effect on K_{cw}. Generally, the correlations developed from the entire fully-validated database will provide a reasonable estimate of K_{cw} for aqueous organic compounds with lipophilicities in the range ($-1 < \log K_{ow} < 4$) and molecular weights in the range ($18 < MW < 500$).

In developing Eq. (6.11), we have made no assumptions regarding the relative extents of partitioning of ionized and unionized compounds. As such, Eq. (6.11) can be used as a reference correlation for studying the influence of various effects on the ratio of experimental to predicted partition coefficients (K/K_{pred}).

6.4.3. Trends in the Fully-Validated Database

Figure 6.3 shows the effects of temperature and ionization relative to the prediction by Eq. (6.11) for the validated partition coefficients from Table 6A.1 and the provisional measurements from Table 6A.2. Measurements above the upper dashed line are underestimated by Eq. (6.11) by more than an order of magnitude and those below the lower dashed line are overestimated by Eq. (6.11) by more than an order of magnitude. With the exception of three measurements (lauric acid (from Smith and Anderson, (Smith and Anderson, 1995)), HC-21-yl-octanoate (from Raykar *et al.* (Raykar *et al.*, 1988)) and indomethacin (from Surber *et al.* (Surber *et al.*, 1992)), all SC-water partition coefficients can be predicted within a factor of ten (i.e., within one order of magnitude). There appears to be less uncertainty in prediction of SC-water partition coefficients compared to prediction of SC permeability coefficients, even though the database for permeability coefficients is much larger.

Measurements taken at temperatures cooler than 30°C are (on average) overestimated by the predictive equation developed from the entire database, while measurements made at temperatures warmer than 30°C are (on average) overestimated.

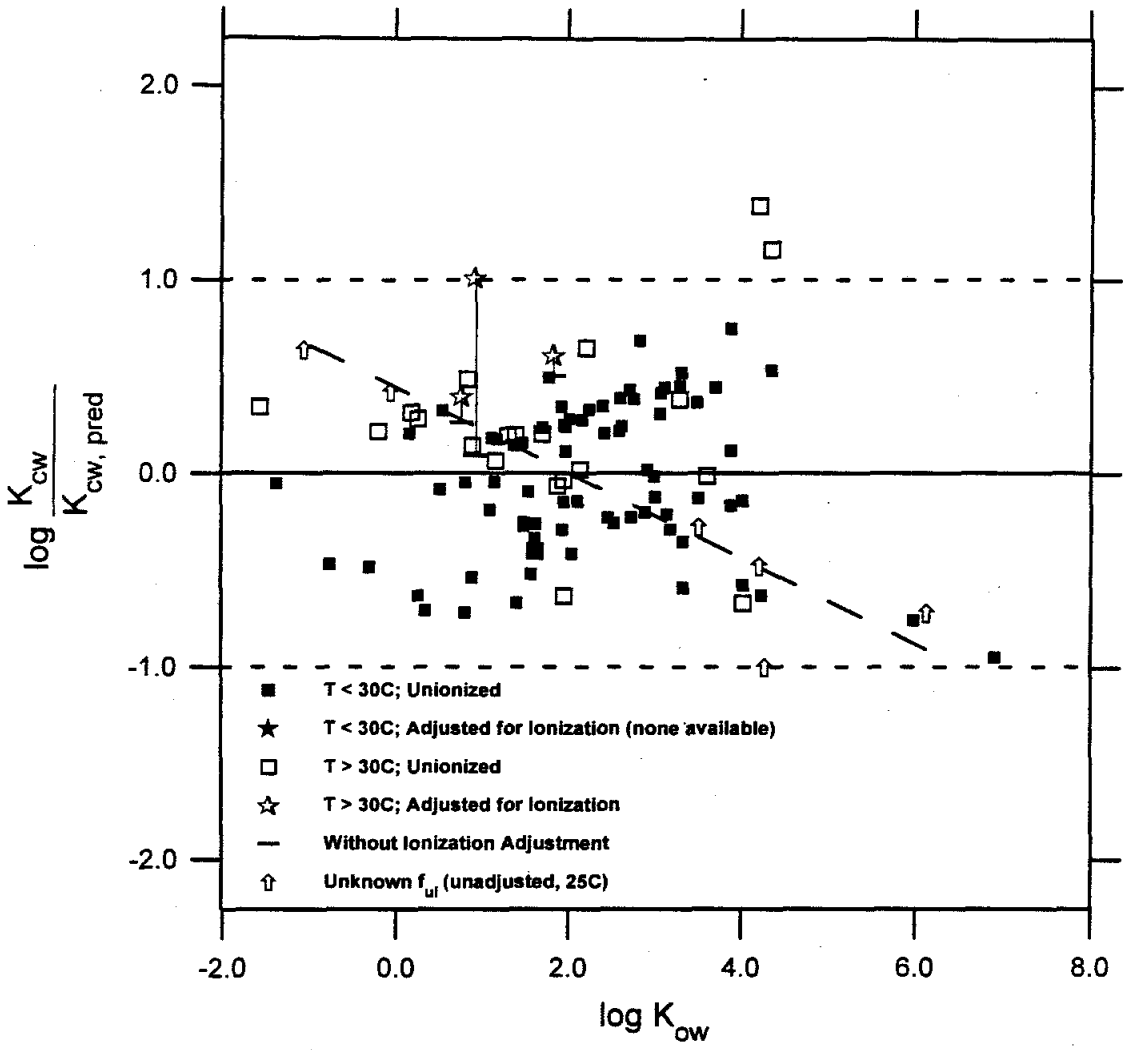

Figure 6.3 A comparison of SC-water partition coefficients in the fully-validated and provisional databases (K_{cw}) to those predicted ($K_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (6.11), with different temperatures and levels of ionization designated.

The residuals (defined as the experimental $\log K_{cw}$ less the predicted $\log K_{cw}$) were calculated. For T < 30°C, the average residual is -0.037 (n = 76, s.d. = 0.39) (overpredicted) and at T ≥ 30°C the average residual is 0.140 (n = 20, s.d. = 0.41) (underpredicted). The standard deviations are large (compared to the mean) because there are other significant sources of variability. As a result effect of temperature on SC-water partitioning is not entirely resolved. The solubility in any given solvent usually (but not always) increases with increasing temperature. The direction of the temperature effect is determined by the solute and the solvent and can change magnitude and sign (i.e., solubility increasing or decreasing with temperature) over a range of temperatures (Lyman *et al.*, 1982). There is no simple means of anticipating the effect of temperature on all compounds, but for many compounds the solubility in octanol and water increase similarly as temperature increases. The results we have gathered indicate that different temperatures introduce a minor contribution to the total variability in K_{cw}.

The line designated by long dashes represents the regression of $\log(K_{cw}/K_{cw,\,pred})$ with $\log K_{ow}$ for the unadjusted (by f_{ui}) measurements of partially ionized chemicals. This regression, $\log(K_{cw}/K_{cw,\,pred}) = 0.44 - 0.22(\log K_{ow})$, provided a reasonably good fit to the data with $r^2 = 0.81$. The measurements after adjustment for ionization (by dividing K_{cw} by f_{ui}) are shown as solid or open stars connected to a horizontal dash at the unadjusted value. For several measurements from Surber *et al.* (Surber *et al.*, 1990a; Surber *et al.*, 1992) it was not possible to calculate f_{ui}, although it is known that $f_{ui} < 1.0$. These chemicals are designated in Figure 6.3 by arrows pointing in the direction that an adjustment for ionization would change $K_{cw}/K_{cw,\,pred}$. In all cases, adjusting for ionization by dividing by the f_{ui} increases $\log(K_{cw}/K_{cw,\,pred})$.

Although the number of data points are quite small (n = 9), the trend seems to be that the ratio $K_{cw}/K_{cw,\,pred}$ is larger than 1 for the more hydrophilic chemicals (i.e., $\log K_{ow}$ < about 2), and $K_{cw}/K_{cw,\,pred}$ is less than 1 for the more lipophilic ionizing chemicals (i.e.,

$\log K_{ow}$ > about 2). Furthermore, adjusting the more lipophilic chemicals by f_{ui} seems to improve the predictability (i.e., $K_{cw}/K_{cw,\,pred}$ moves closer to 1 after adjusting with f_{ui}), while adjusting measurements for the more hydrophilic chemicals degrades predictability (i.e., $K_{cw}/K_{cw,\,pred}$ moves away from 1). Three measurements for 5-fluorouracil were not included because for 5-fluorouracil the $\log K_{ow}$ was measured with the ionized species present, whereas only unionized species were used to measure $\log K_{ow}$ for the other 9 chemicals.

This is consistent with the hypothesis that the relative amount of the unionized and ionized chemical partitioning into the SC is a function of K_{ow}. The partition coefficient of the unionized species ($K_{cw,ui}$) of a lipophilic chemical is likely to be significantly larger than its ionized species ($K_{cw,i}$). In this case, increasing the extent of ionization would decrease the quantity of chemical which absorbs. If the unionized form of a molecule partitions into the SC at least 100 times more than its ionic form, then the partition coefficient of the unionized species ($K_{cw,ui}$) can be estimated from that observed for a mixture of the unionized and ionized species ($K_{cw,obs}$) as: $K_{cw,ui} \approx K_{cw,obs}/f_{ui}$.

Ionized chemicals do absorb limitedly into SC. Since unionized hydrophilic chemicals absorb into the SC to a lesser extent than more lipophilic chemicals, an unionized hydrophilic species may absorb into skin only slightly more readily than its ionized relative. Furthermore, additional chemical interactions may arise between charged molecules, like amino acids, and the protein regions of the SC, which are composed of amino acids. As a consequence, for more hydrophilic ionizing chemicals $K_{cw,\,ui}$ may be of similar magnitude to $K_{cw,\,i}$ which will cause $K_{cw,\,ui} \approx K_{cw,\,obs}$. In this case, $K_{cw,\,obs}/f_{ui}$ will over estimate the magnitude of $K_{cw,\,ui}$.

Figure 6.4 compares the fully-validated and provisional database partition coefficients and predictions according to the year that the measurements were collected. There is no systematic trend over the period of time shown. The uncertainty in measuring

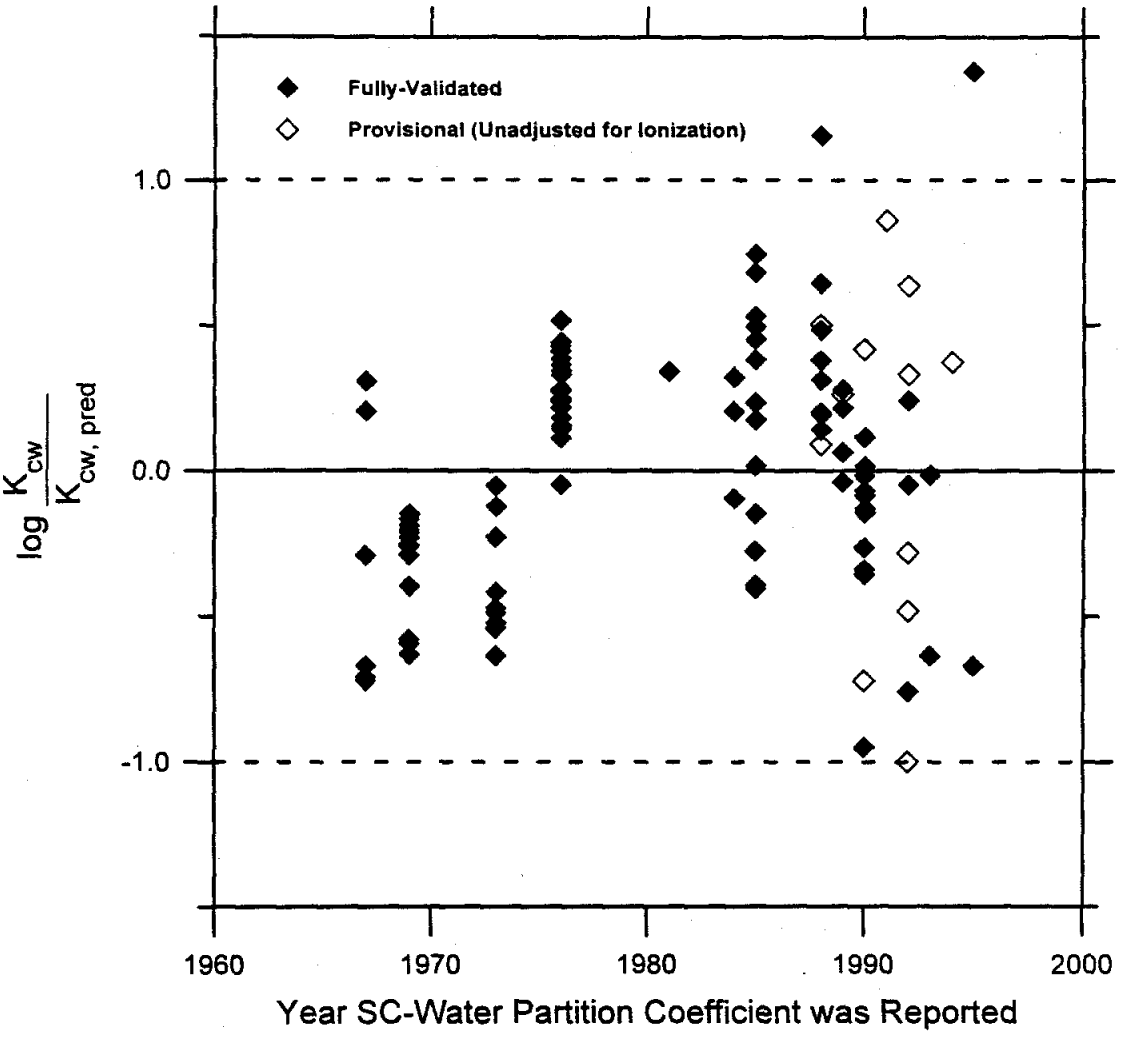

Figure 6.4 A comparison of stratum corneum-water partition coefficients in the fully-validated and provisional databases (K_{cw}) to those predicted ($K_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (6.11), as a function of the year in which the data were published.

partition coefficients is nearly the same (perhaps somewhat larger) in recent measurements as in those measured as early as 1967. Because the chemical complexity of the compounds studied has increased throughout these years, it is difficult to guess the sources of variance.

However, Figure 6.5 shows that partition coefficients measured in particular laboratories can be systematically different from measurements made, for similar compounds, in other laboratories. The fully-validated and provisional (not adjusted for ionization when $f_{ui} < 1$) measurements are compared to Eq. (6.11). Assuming that $\log K_{ow}$ effects were dominant, one would expect that partition coefficients measured in all studies would be randomly scattered about the line $\log K_{cw}/K_{cw,pred} = 0.0$. Some research groups are measuring partition coefficients that are predominantly higher or lower than expected from analysis of the other validated and unionized (since the partition coefficients for partially ionized compounds were not used to develop Eq. (6.11)) measurements. Whether this is due to the skin source, skin preparation, or an unidentified experimental protocol is not known. For example, partition coefficients for the steroids (Scheuplein *et al.*, 1969) and alkanols (Scheuplein and Blank, 1973) are all overestimated by Eq. (6.11). Conversely, all partition coefficients for slightly modified hydrocortisone steroids measured by Raykar and colleagues (Raykar *et al.*, 1988) are all systematically underestimated. Using similar procedures as Raykar and colleagues (Raykar *et al.*, 1988), partition coefficients reported by Anderson *et al.* (Anderson and Raykar, 1989) for miscellaneous cresols are predominantly underestimated. The remaining partition coefficients (i.e., labeled as others) show the pattern that is expected and several independent research groups are nearly correct on average. It may be of some relevance that laboratories reporting larger than predicted partition coefficients also report larger than predicted permeability coefficients; laboratories reporting smaller partition coefficients also report smaller permeability coefficients (see Figure 5.7). This

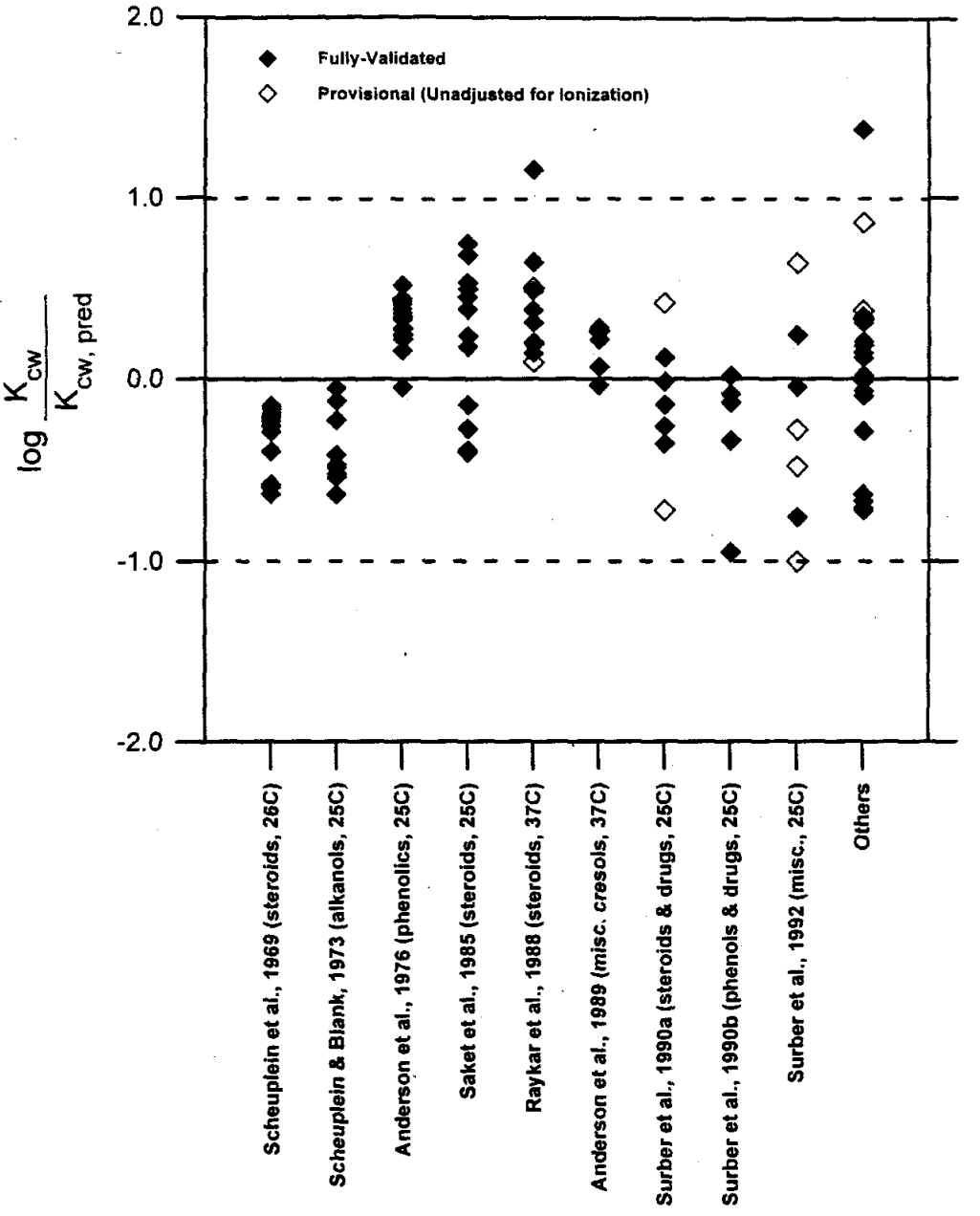

Figure 6.5 A comparison of stratum corneum-water partition coefficients in the fully-validated and provisional databases (K_{cw}) to those predicted ($K_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (6.11), as reported in the prominent investigations.

systematic variation in measurements from different laboratories is responsible for a significant fraction of the total variability in SC-water partition coefficients.

Figure 6.6 compares predictions with replicated SC-water partition coefficients (i.e., multiple SC-water partition coefficients measured for the same compound in different investigations) and unreplicated measurements that appear in the fully-validated and provisional databases. Twenty-six replicate measurements for eleven different compounds are shown. Four of the replicate measurements shown (three for 5-fluorouracil and one for lauric acid) come from the provisional database. These replicates provide the opportunity to observe differences (which should be small) between partition coefficients for the same compound. Except for the replicate measurements on lauric acid, all replicate estimates agree within an order of magnitude (i.e., factor of ten). The measurement for lauric acid (Smith and Anderson, 1995) is quite inconsistent with another measurement for lauric acid (Surber *et al.*, 1992). Additionally, this measurement deviates from its predicted value more than any other measurement in the database and is one of only three measurements which are misestimated by more than an order of magnitude. Differences this large are alarming (considering that these measurements are required to protect human health), but this figure shows that such differences are relatively infrequent. Nevertheless, this figure indicates that measurements differing by a factor of two, or even a factor of five, are not necessarily meaningfully different within the other sources of uncertainty.

Until now we have ignored the set of SC-water partition coefficients from Table 6A.3 which were measured using powdered human stratum corneum (PHSC), prepared by grinding excised foot callus into a powder in the presence of liquid nitrogen and then collecting the fraction that passed through a 50-mesh sieve but was retained by an 80-mesh sieve (Hui *et al.*, 1995). This dataset has been excluded because we wanted to examine it separate from the remainder of the measurements (which, except for

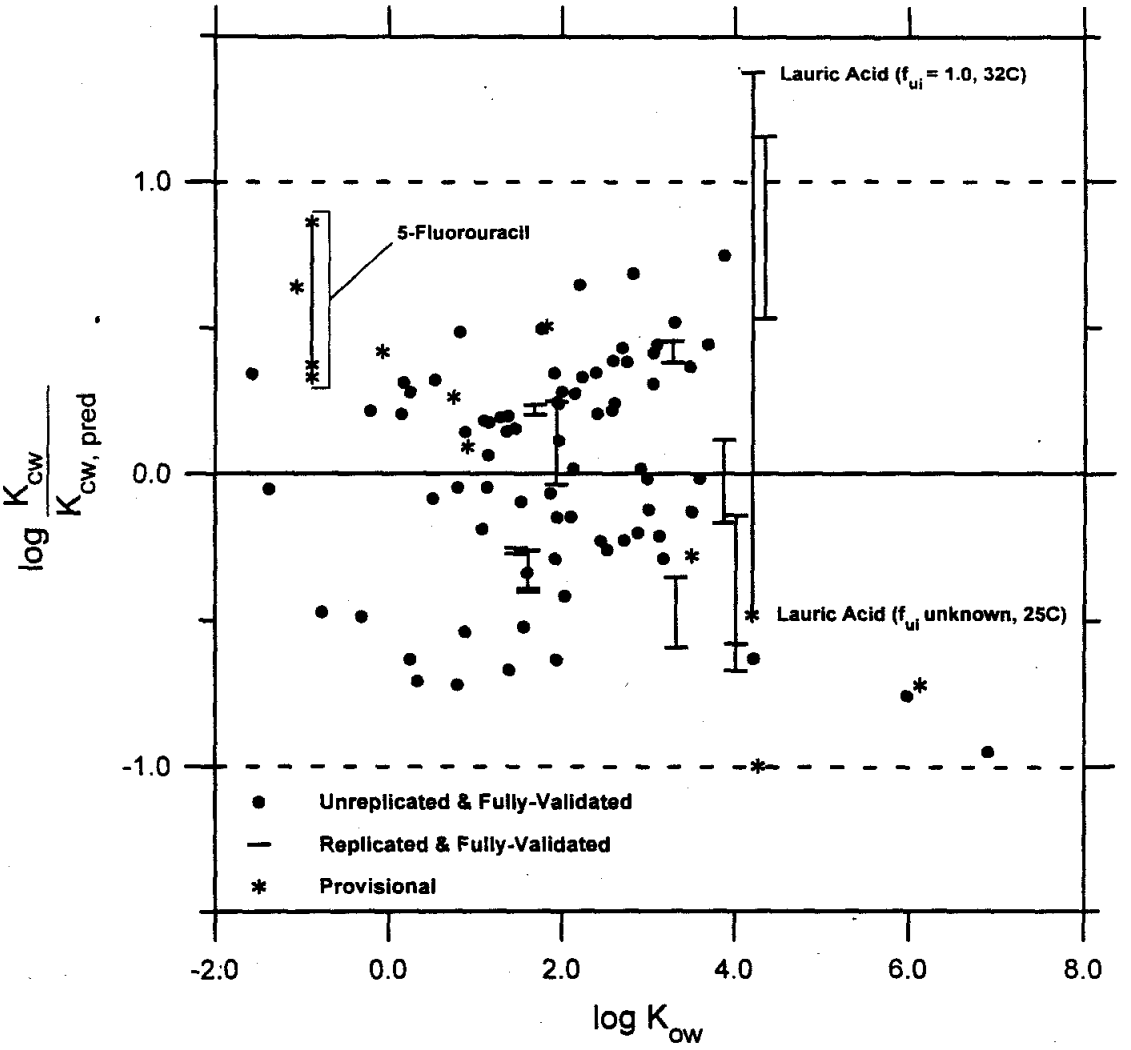

Figure 6.6 A comparison of stratum corneum-water partition coefficients in the fully-validated and provisional databases (K_{cw}) to those predicted ($K_{cw,pred}$) by a correlation developed from the fully-validated database, Eq. (6.11), with replicated (multiple partition coefficients measured for the same chemical) measurements designated.

nicorandil, were measured using excised and uncallused SC (e.g., from abdomen, torso, or breast) to evaluate the relevance of PHSC for measuring K_{cw}. The chemical differences between plantar or palmar callus SC and uncallused SC as well as possible physical alterations introduced by grinding and sieving raise questions regarding the relevance partition coefficient measurements on PHSC.

There are many puzzling results listed in Table 2 from Hui *et al.* (Hui *et al.*, 1995). Some of the more significant inconsistencies are: (1) the natural pH attained by some compounds after addition to distilled water do not make sense (e.g., for urea, which should be very weakly basic they report an unexpectedly low pH of 2.1), (2) the reported pK_a values are very different from those calculated by SPARC (e.g., for atrazine the reported pK_a is 8.15 while SPARC predicts 2.0, and for urea the reported pK_a is 0.18 compared to 1.6 from SPARC), and (3) in several cases the pK_a do not make sense at all (e.g., the unionizing chemicals, hydrocortisone and PCB are erroneously reported to have $pK_a = 7.0$). The characterization of the ionic condition of the chemical is nevertheless mostly in agreement with our calculations. The $\log K_{ow}$ (reported to be from Hansch and Leo (Hansch and Leo, 1979)) are mostly consistent with the $\log K_{ow}$ recommended by Hansch and colleagues (Hansch *et al.*, 1995) except for dopamine (reported as -3.4) which is not listed by Hansch *et al.* (Hansch *et al.*, 1995) and was calculated by Daylight to be -0.05.

Figure 6.7 compares validated SC-water partition coefficients from Table 6A.1 and the predictive correlation developed from these measurements (i.e., Eq.(6.11)) with SC-water partition coefficients measured using PHSC obtained from plantar callus (Hui *et al.*, 1995). Plantar callus is probably more proteinaceous than SC excised from other regions. If this is true, partitioning into the protein domains may dominate, and the predicted K_{cw} would probably be smaller than measured except for highly hydrophilic chemicals which would have more polar regions to partition to than in uncallused SC.

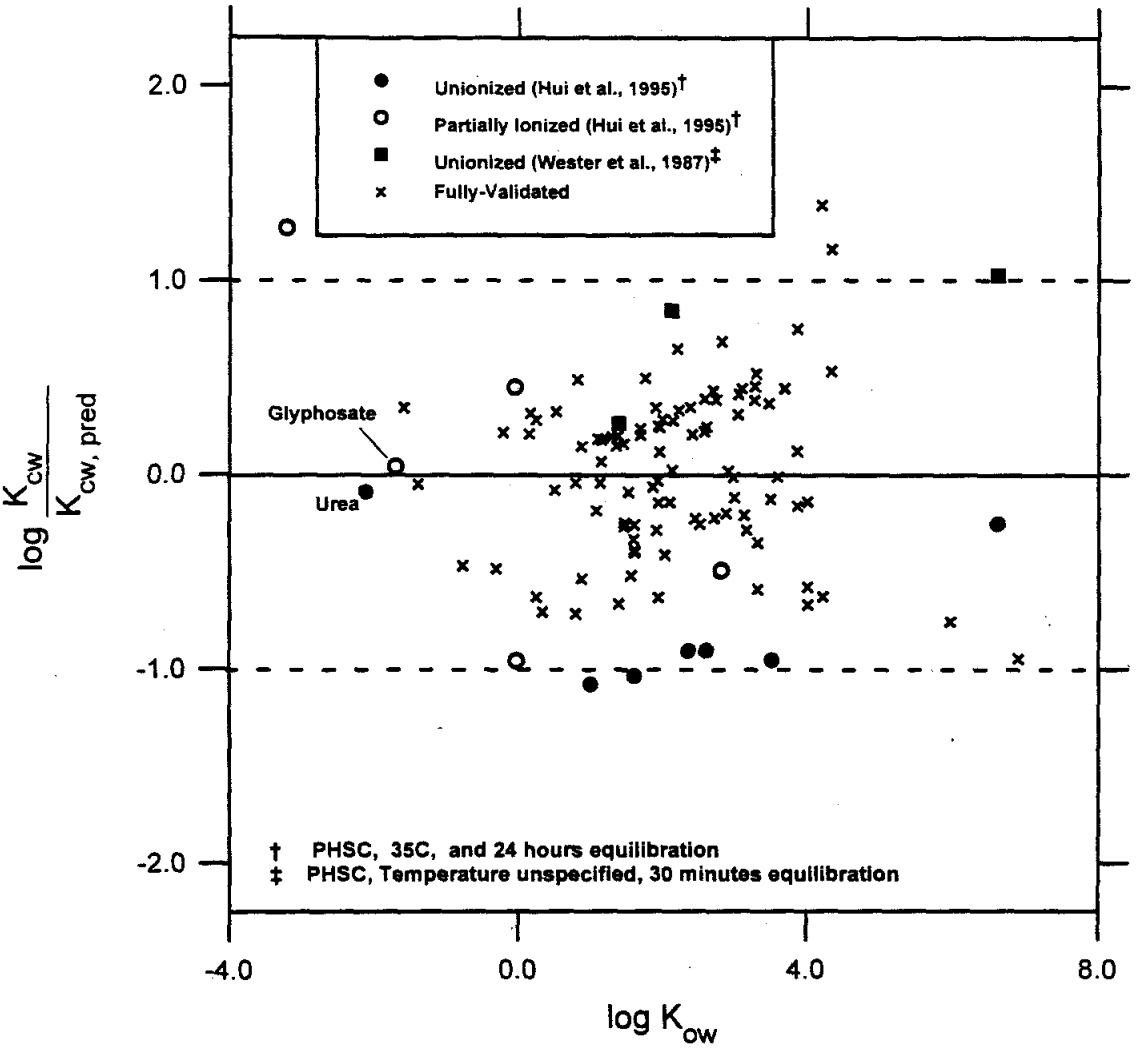

Figure 6.7 A comparison of stratum corneum-water partition coefficients in the fully-validated database and partition coefficients measured using sieved, powdered, human plantar callus stratum corneum (PHSC) from the excluded database (K_{cw}) to those predicted ($K_{cw,prd}$) by a correlation developed from the fully-validated database, Eq. (6.11), with the ionization of the chemical designated.

These expectations generally agree with the data from Hui *et al.* (Hui *et al.*, 1995) which are consistently overpredicted by Eq. (6.11) except for compounds with $\log K_{ow}$ < about zero. The three PHSC measurements reported by Wester *et al.* (Wester *et al.*, 1987) are inconsistent with the data from Hui *et al.* (Hui *et al.*, 1995) and our hypothesis that more polar protein domains dominate. Based on these limited data, it is not possible to say conclusively whether or not measurements of partition coefficients using PHSC represent uncallused SC.

6.4.4. Analysis with the Lipophilic-Hydrophilic Domain Model

Some investigators have theorized that the existence of multiple domains within the stratum corneum should present regions with different polarities. In the simplest theory, the SC consists of polar and nonpolar regions. Hydrophilic chemicals should partition primarily in the polar region, while lipophilic chemicals should partition to the nonpolar regions. Partitioning between the nonpolar regions and water would be expected to increase with a measure of lipophilic character such as $\log K_{ow}$. However, partitioning between the polar region and water should be affected much less by K_{ow} of the partitioning compound.

Here we examine the database of partition coefficients for trends indicating the presence of more than one partitioning mechanism. Figures 6.1 and 6.2 show that partitioning into the SC increases approximately linearly with increases in $\log K_{ow}$. There appears to be more uncertainty in the partition coefficients measured for most lipophilic and most hydrophilic compounds in the database. Because of this uncertainty, it is difficult to decide whether the partition coefficients for hydrophilic compounds deviate from the linear relationship observed for the lipophilic compounds in a statistically meaningful way. One quantitative way to test the hypothesis of polar and nonpolar regions is to regress a mathematical model based on two-phase partitioning (i.e., Eqs.

(6.6) or (6.8)) to the fully-validated unionized database and to compare the regression statistics with those from the conventional correlation (i.e., Eq. (6.11)).

We have regressed the validated data from Table 6A.1 to Eqs. (6.6) and (6.7) to determine goodness of fit statistics (e.g., r^2) for various values of $\log K^{\#}_{ow}$. The goodness-of-fit statistics are nearly constant over the range $-2 < \log K^{\#}_{ow} < 1.5$. There is a local minimum in r^2 at $\log K_{ow} = 0.0$ which is essentially the same as the r^2 obtained from the simple correlation, Eq. (6.11). A local maximum in r^2 occurs at $\log K^{\#}_{ow} = -1.5$. An optimum $\log K^{\#}_{ow}$ may exist in the region $-1.5 < \log K^{\#}_{ow} < 0.0$, but much more data for hydrophilic compounds is required to make decisive conclusions. The r^2 statistic improves as $\log K^{\#}_{ow} > 1$ because the analysis customizes the fit to the $\log K_{ow} < \log K^{\#}_{ow}$ and $\log K_{ow} > \log K^{\#}_{ow}$ regions. This does not indicate a change in uptake mechanism, because the $\log K_{ow}$ dependence is not very different for $K_{ow} < K^{\#}_{ow}$ and $K_{ow} \geq K^{\#}_{ow}$ (i.e., c in Eq. (6.6) is small).

The slightly simplified two-mechanism model in Eqs. (6.7)-(6.8) was also regressed using the fully-validated data from Table 6A.1. In this model, $\log K_{cw}$ is constant for all compounds with $\log K_{ow} < \log K^{\#}_{ow}$. In this case, the r^2 statistic improved monotonically with decreasing $\log K^{\#}_{ow}$ in the range $-1 < \log K^{\#}_{ow} < 1$ indicating that the regressed data are more consistent with the conventional correlation, Eq. (6.11).

Thus, the validated partition coefficients from Table 6A.1 do not indicate that the mechanism of uptake of hydrophilic compounds is different from the mechanism of uptake for lipophilic compounds. This could be genuine or an artifact of the small number of hydrophilic compounds with a rather narrow range of $\log K_{ow}$. The larger (n = 170) database of permeability coefficients analyzed in Chapter 5 suggested a different penetration mechanism for hydrophilic and lipophilic compounds. In the analysis of

permeability coefficients, the optimum $\log K_{ow}^{\#}$ was approximately -0.5. Using $\log K_{ow}^{\#}$ = -0.5 and Eqs. (6.6) and (6.7) to analyze the partition coefficients:

$$\log K_{cw} = -0.104(0.207) + 0.301(0.303)\log K_{ow} + 0.120(0.315)(\log K_{ow} + 0.5)\delta \quad (6.13)$$

$$(r^2 = 0.682,\ r^2(\text{adj.}) = 0.675,\ \text{RMSE} = 0.40,\ \text{F} - \text{Ratio} = 99.8)$$

Consistent with earlier calculations, the coefficient c is not statistically significantly different than zero. Assuming instead the form of Eqs. (6.7)-(6.8) and fixing $\log K_{ow}^{\#}$ = -0.5:

$$\log K_{cw} = -0.290(\pm 0.091) + 0.432(\pm 0.031)\cdot(\log K_{ow} + 0.5)\delta \quad (6.14)$$

$$(r^2 = 0.679,\ r^2(\text{adj.}) = 0.675,\ \text{RMSE} = 0.402,\ \text{F-Ratio} = 198.7)$$

As expected, Eq. (6.14) is quite similar to Eq. (6.11) since only a few data points in the database have $\log K_{ow} < -0.5$.

6.4.5. Analysis with the Linear Solvation-Energy Relationship (LSER) Model

The LSER model, Eq. (6.9), has been used to analyze chemicals from the fully-validated database for which solvatochromic parameters could be found. The solvatochromic parameters, all from Abraham *et al.* (Abraham *et al.*, 1994) were calculated by averaging multiple normalized solvent effects on a variety of chemical properties involving many varied types of indicators. These experimentally determined descriptors are now available for well over 1000 solutes (Abraham *et al.*, 1994).

Abraham and colleagues (Abraham *et al.*, 1995) have previously developed a correlation based on a small database of SC-water partition coefficients:

$$\log K_{cw} = -0.027 - 0.374\,\pi + 0.334\,\alpha - 1.674\,\beta + 1.869\,V_x \quad (6.15)$$

$$(n = 22,\ r^2 = 0.943)$$

We have repeated their analysis on a subset of the validated data from Table 6A.1 for which LSER parameters were available. LSER parameters are available for the

chemicals in Table 6A.1 with names enclosed in brackets (e.g., [benzene]) and Table 6A.4 lists the solvatochromic parameters and K_{cw} for all of these chemicals. Table 6A.4 represents predominantly the lower MW chemicals from the fully-validated database. The result is:

$$\log K_{cw} = -0.16(0.3) - 0.27(0.2)\,\pi^* + 0.42(0.3)\,\alpha^* - 3.15(0.5)\,\beta + 2.22(0.2)\,V_x \quad (6.16)$$
$$(n = 38,\ r^2 = 0.830,\ r^2(\text{adj.}) = 0.810,\ \text{RMSE} = 0.325,\ \text{F-Ratio} = 40.5)$$

where the uncertainty expressed in parenthesis is the standard error of the coefficients. In Eq. (6.16) the terms labeled with asterisk (π^*, α^*) were not meaningful at the 95% confidence level. This correlation indicates that 83% of the variability in $\log K_{cw}$ can be attributed to variation in the LSER parameters (π, α, β, and V_x). After excluding the statistically insignificant terms and regressing the same database:

$$\log K_{cw} = -0.043(0.248) - 2.893(0.463)\,\beta + 1.977(0.181)\,V_x \quad (6.17)$$
$$(n = 38,\ r^2 = 0.816,\ r^2(\text{adj.}) = 0.805,\ \text{RMSE} = 0.330,\ \text{F-Ratio} = 77.4)$$

This correlation indicates that 82% of the variability in $\log K_{cw}$ can be attributed to variations in only β and V_x.

Equation (6.17), which describes the partition coefficient of predominantly low MW compounds is revealing about the mechanism of SC partitioning. The two most important LSER parameters for predicting SC-water partitioning, the hydrogen-bonding basicity and the molecular volume, were also the most important parameters for predicting SC permeability coefficients. There is a particularly high level of variability in the LSER regression coefficients, but, generally the values are as good as can be expected taking into account the rather large experimental errors that can be associated with the difficult determination of partition coefficients in biological tissues. In addition, the chemical diversity of the solutes used in the LSER regression are by no means optimal, which makes precise specification of the coefficients difficult. Prediction of SC permeability coefficients and SC-water partition coefficients would be greatly improved

if measurements would be made on compounds with optimally different combinations of LSER parameters (i.e., different forms of chemical diversity), as determined by statistical model discrimination guidelines.

The partition coefficients for compounds in the LSER database were also analyzed using the conventional correlation (i.e., using lipophilicity alone) to measure the improvement in fit from the more complicated LSER analysis. The result is:

$$\log K_{cw} = -0.255(0.108) + 0.513(0.048) \log K_{ow}$$
$$(n = 38,\ r^2 = 0.758,\ r^2(\text{adj.}) = 0.751,\ \text{RMSE} = 0.373,\ \text{F-Ratio} = 112.5) \quad (6.18)$$

Comparing regression statistics, the predictive power of Eq. (6.17) is modestly greater (r^2 is larger) than Eq. (6.18) although the gain is small relative to the additional parameters (F-Ratio is smaller).

Figure 6.8 compares measured SC-water partition coefficients for compounds in the LSER database with predictions using either the correlation based on LSER parameters, Eq. (6.16), or the correlation with $\log K_{ow}$, Eq. (6.18). To indicate the impredictability of the entire database, we have included the same comparison for the entire database of valid measurements, with predictions from Eq. (6.11). The range of $\log K_{ow}$, represented in the LSER database is similar to the entire valid database. However, the LSER database is composed of functionally simple, low MW, predominantly phenolic compounds. Although the LSER parameters fit the LSER database better than $\log K_{ow}$ alone and provide more chemical information, we believe that K_{ow} is the single most relevant parameter for estimating the SC-water partition coefficient.

6.4.6. Estimation of the Stratum Corneum Diffusion Coefficient

The ratio of SC diffusion coefficient to SC thickness (i.e., D_c/L_c) is another useful combination of parameters that appears in dermal absorption models (Cleek and Bunge,

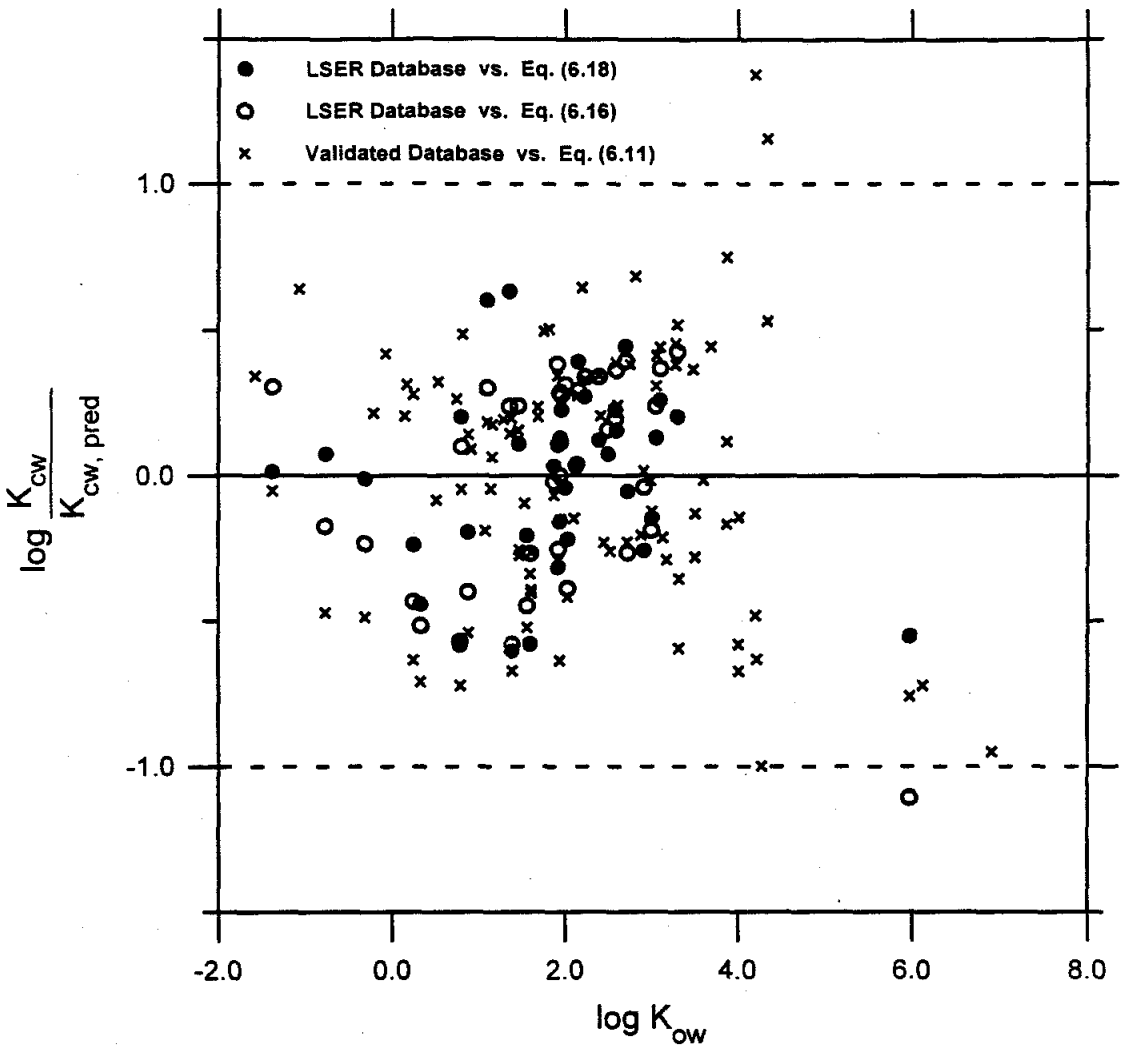

Figure 6.8 A comparison of stratum corneum-water partition coefficients in the LSER database (K_{cw}) to those predicted ($K_{cw,pred}$) by a correlation developed from the LSER database based on LSER, Eq. (6.16), and a correlation developed from the LSER database based on $\log K_{ow}$, Eq. (6.18). Partition coefficients from the fully-validated database are also compared to those predicted by a correlation developed with the entire fully-validated database, Eq. (6.11).

1993). We estimate this ratio using principles that apply to membranes and correlations developed for the SC permeability coefficient and SC-water partition coefficient.

As discussed in Chapter 5, penetration through the SC membrane is frequently modeled as a solution-diffusion process [Cleek, 1993 #38; and many others]. In this case, the steady-state permeability coefficient for crossing the SC from an aqueous vehicle (P_{cw}) into an infinite sink depends upon the diffusivity of the chemical in the SC (D_c), the SC thickness (L_c), and the equilibrium partition coefficient between the SC and the vehicle (K_{cw}) as given below:

$$P_{cw} = \frac{K_{cw}\, D_c}{L_c} \tag{6.19}$$

Logarithmic transformation and rearrangement of Eq. (6.19) gives the relationship; $\log(D_c/L_c) = \log P_{cw} - \log K_{cw}$ which can be used to calculate $\log(D_c/L_c)$ in terms of correlations for $\log P_{cw}$ and $\log K_{cw}$. The correlation which we previously developed to estimate skin penetration from an aqueous vehicle (i.e., Eq. (5.27)),

$$\begin{aligned} &\log P_{cw} = -2.44(0.12) + 0.514(0.04)\log K_{ow} - 0.0050(0.0005)\,MW \\ &(n = 170,\ r^2 = 0.551,\ r^2(\text{adj.}) = 0.546,\ \text{RMSE} = 0.803,\ F-\text{Ratio} = 102.6) \end{aligned} \tag{5.27}$$

is used to express $\log P_{cw}$. Equation (6.11) is used to express $\log K_{cw}$. Using these two correlations we calculate an expression for the ratio D_c/L_c:

$$\log\left(\frac{D_c}{L_c}\right) = -2.408(0.14) + 0.098(0.05)\log K_{ow} - 0.00502(0.0005)\,MW \tag{6.20}$$

Eq. (6.20) is the difference between two regression correlations and was not itself fit to data. Standard errors, contained within parenthesis, were calculated as the square root of the sum of the squared standard errors for the $\log K_{ow}$ or MW terms in Eqs. (5.27) and (6.11). Eq. (6.20) suggests that D_c/L_c would depend weakly on $\log K_{ow}$

An expression for the SC diffusion coefficient can also be developed using LSER correlations for the SC permeability and SC-water partition coefficient. The LSER

correlation which we previously reported in Table 5.2 to estimate skin penetration from an aqueous vehicle,

$$\log P_w = -2.21(0.20) - 3.27(0.41)\,\beta + 1.73(0.17)\,V_x$$
$$(n = 65,\ r^2 = 0.701,\ r^2(\text{adj.}) = 0.691,\ \text{RMSE} = 0.471) \tag{6.21}$$

Assuming $P_{cw} = P_w$ and combining Eqs. (6.21) with (6.17) we obtain an LSER expression for the ratio D_c/L_c:

$$\log\left(\frac{D_c}{L_c}\right) = -2.177(0.38) - 0.377(0.62)\,\beta^* - 2.47(0.25)\,V_x \tag{6.22}$$

In this case, hydrogen bond basicity, β^* does not contribute significantly to $\log(D_c/L_c)$.

Equations (6.20) and (6.22) are two of very few estimates of the SC-diffusion coefficient. Roberts and colleagues (Roberts *et al.*, 1996) have presented a correlation analogous to Eq. (6.20) but using hydrogen bonding group analysis or LSER parameters. These authors also developed correlations of the lag time across the SC (from which D_c/L_c can be calculated) by regressing on the number of hydrogen bonding groups or LSER parameters.

6.4.7. Comparison of the Database with Literature Correlations

Table 6.1 contains an incomplete list of empirical correlations presented in the literature for predicting the SC-water partition coefficient of human skin. Also listed are the chemical classes upon which the correlation was trained, a reference to the training data, and the range of MW and $\log K_{ow}$ of the data on which the correlation was developed (noted as data range). The correlations from Brown and Hattis (Model 1), McKone (Model 7), McKone and Howd (Model 8), Raykar *et al.* (Model 9), and Hui *et al.* (Model 18) are not developed in the conventional form. Except for Brown and Hattis (Brown and Hattis, 1989), these references can be consulted for the theoretical justification of the alternative forms.

Table 6.1 SC-Water Partition Coefficient Correlations for Human Skin

	Equation Reference	Chemical Class	SC-Water Partition Coefficient Correlation	Data Reference	Data Range
1	Brown & Hattis, 1989[a]	N/A	$K_{cw} = 1.277 + 0.1208\ K_{ow}$	N/A	N/A
2	Cleek & Bunge, 1993	Not fit to data[b]	$\log K_{cw} = 0.74\ \log K_{ow}$	Indirectly derived from Flynn Database	$18<MW<765$ $-3<\log K_{ow}<6$
3	Cleek & Bunge, 1993	Alcohols, acids, steroids, phenols (n=42)	$\log K_{cw} = 0.57\ \log K_{ow} - 0.006$ $(r^2 = 0.722)$	Scheuplein, 1967; Scheuplein et al., 1969; Roberts et al., 1977	$46 < MW < 363$ $-0.3 < \log K_{ow} < 4.2$
4	Cleek & Bunge, 1993	Alkanols & acids (n=12)	$\log K_{cw} = 0.72\ \log K_{ow} - 0.26$ $(r^2 = 0.85)$	Scheuplein, 1967	$46 < MW < 144$ $-0.3 < \log K_{ow} < 3.1$
5	Cleek & Bunge, 1993	Alkanols & acids (n=12)	$\log K_{cw} = 0.60\ \log K_{ow}$ $(r^2 = 0.722)$	Scheuplein, 1967	$46 < MW < 144$ $-0.3 < \log K_{ow} < 3.1$
6	El Tayar et al., 1991	Alkanols & steroids (n = 22)	$\log K_{cw} = 0.51\ \log K_{ow} + 0.10$ $(r^2 = 0.943)$	Scheuplein, 1965; Scheuplein et al., 1969	$32 < MW < 363$ $-0.8 < \log K_{ow} < 4.2$

	Equation Reference	Chemical Class	SC-Water Partition Coefficient Correlation	Data Reference	Data Range
7	McKone & Howd, 1992	Alkanols (n=8)	$K_{cw} = 0.64 + 0.25\ K_{ow}^{0.8}$ ($r^2 = 0.90$)	Scheuplein & Blank, 1973	$32 < MW < 130$ $-0.8 < \log K_{ow} < 3.0$
8	McKone, 1990	Not fit to data	$K_{cw} = 0.11\ K_{ow} + 0.5$	Not fit to data	Not fit to data
9	Raykar et al., 1988	Esters of Hydro-cortisone (n = 11)	$\left(K_{cw} = 6.29\ K_{ow}^{0.24} + 0.0225\ K_{ow}^{0.91}\right)^{c}$	Raykar et al., 1988	$418 < MW < 519$ $1.4 < \log K_{ow} < 4.3$
10	Roberts et al., 1996	Misc.[d] (n = 45)	$\log K_{cw} = 0.590\ \log K_{ow} - 0.024$ ($r^2 = 0.839$, $F = 230$)	Scheuplein & Blank, 1971, Lien & Tong, 1973, Anderson et al. 1976, Anderson et al. 1988, Roberts, 1976	$32 < MW < 519$ $-0.8 < \log K_{ow} < 5.5$
11	Roberts et al., 1977	Aromatic alcohols (n=21)	$\log K_{cw} = 0.57\ \log K_{ow} - 0.1$ ($r^2 = 0.984$)	Anderson et al., 1976; Roberts, 1976	$94 < MW < 198$ $0.8 < \log K_{ow} < 3.7$
12	Roberts et al., 1977	Alkanols & acids (n=13)	$\log K_{cw} = 0.66\ \log K_{ow} - 0.1$ ($r^2 = 0.964$)	Scheuplein (1967)	$46 < MW < 144$ $-0.3 < \log K_{ow} < 3.1$

	Equation Reference	Chemical Class	SC-Water Partition Coefficient Correlation	Data Reference	Data Range
13	Roberts et al., 1977	Steroids (n = 14)	$\log K_{cw} = 0.37 \log K_{ow} + 0.6$ ($r^2 = 0.764$)	Scheuplein et al., 1969	$270 < MW < 363$ $1.5 < \log K_{ow} < 4.2$
14	Saket et al., 1985	Hydro-cortisone esters & cortisone esters (n = 11)	$(\log K_{cw} = 0.761 \log K_{ow} - 0.343)^e$ $(\log K_{cw} = 0.739 \log K_{ow} - 0.280)^f$ ($r^2 = 0.968$)	Saket et al., 1985	$194 < MW < 363$ $-0.1 < \log K_{ow} < 6.1$
15	Surber et al., 1990b	Phenols, PCB, DDT, steroids, drugs[g] (n=13)	$\log K_{cw} = 0.26 \log K_{ow} + 0.87$ ($r^2 = 0.81$)	Surber et al., 1990b; Surber et al., 1990b	$151 < MW < 363$ $0.0 < \log K_{ow} < 6.4$
16	Surber et al., 1990b; Surber et al., 1992	Phenols, PCB, DDT (n = 6)	$(\log K_{cw} = 0.28 \log K_{ow} + 0.69)^h$ ($r^2 = 0.903$)	Surber et al., 1990b	$151 < MW < 355$ $0.3 < \log K_{ow} < 6.4$
17	Surber et al., 1990a	steroids, drugs[e] (n=7)	$(\log K_{cw} = 0.344 \log K_{ow} + 0.725)^i$ $(\log K_{cw} = 0.254 \log K_{ow} + 0.987)^j$ ($r^2 = 0.740$)	Surber et al., 1990a	$194 < MW < 363$ $-0.1 < \log K_{ow} < 6.1$

	Equation Reference	Chemical Class	SC-Water Partition Coefficient Correlation	Data Reference	Data Range
18[k]	Hui et al. 1995	Drugs, pesticides, steroids (n=12)	$\log K_{cw} = 0.078\,(\log K_{ow})^2 + 0.868 \log MW - 2.04$ ($r^2 = 0.90$, $S = 0.33$, $F = 42.6$)	Hui et al. 1995	$60 < MW < 363$ $-3.2 < \log K_{ow} < 6.6$

[a] Brown and Hattis (Brown and Hattis, 1989) and several subsequent authors (Shatkin and Brown, 1991; Chinery and Gleason, 1993) incorrectly reference this correlation to Roberts and colleagues (Roberts et al., 1975). The actual source is unknown.
[b] Derived from the permeability coefficient correlation of the Flynn permeability database (Bunge et al., 1994) by assuming that the $\log K_{ow}$ term represented the SC-water partition coefficient.
[c] Based on separate fits of partitioning into the protein and extracted lipids.
[d] Including steroids, alcohols, acids, and various pharmaceuticals
[e] This equation is algebraically reorganized from the equation ($\log K_{ow} = 1.313 \log K_{cw} + 0.45$) which fits the data in this reference. The equation published in this reference ($\log K_{cw} = 1.313 \log K_{ow} + 0.45$) reversed K_{cw} and K_{ow}.
[f] This equation was developed by regression of the data in this reference to an equation of the form, $\log K_{cw} = a + b \log K_{ow}$. This equation is plotted in Figures 6.9 and 6.10 as Model 14.
[g] The drugs are acitretin, diazepam, and caffeine.
[h] A regression of the data provided in this reference indicates that this is the correct equation. The published equation ($\log K_{cw} = 0.69 - 0.28 \log K_{ow}$) does not fit the data.
[i] This equation is algebraically reorganized from the published equation ($\log K_{ow} = -2.11 + 2.91 \log K_{cw}$).
[j] This equation was developed by regression of the data in this reference to an equation of the form, $\log K_{cw} = a + b \log K_{ow}$. This equation is plotted in Figures 6.9 and 6.10 as Model 17.
[k] This correlation was developed with measurements from powdered human stratum corneum (plantar, callus)

The Model 1 equation, from Brown and Hattis (Brown and Hattis, 1989) and also appearing in several subsequent publications (Chinery and Gleason, 1993; Shatkin and Brown, 1991) is incorrectly attributed to a paper by Roberts and colleagues (Roberts *et al.*, 1975). The equation from Brown and Hattis (Brown and Hattis, 1989) does not appear in Roberts *et al.* (Roberts *et al.*, 1975) nor do the data in Roberts *et al.* (Roberts *et al.*, 1975) fit the Model 1 equation. According to Roberts ((Roberts, 1996b)) he does not remember developing such a correlation, so it is unlikely that Model 1 comes from a different investigation of Roberts. So far we have been unable to find the original source of Model 1.

Model 2 was developed by splitting the semi-theoretically derived Potts and Guy permeability correlation (Potts and Guy, 1992) into separate solution and diffusion components. This division is theoretically possible to the extent that Potts and Guy (Potts and Guy, 1992) developed a model with parameters that have assignable physicochemical significance.

Two equations are presented for Model 14. The first model ($\log K_{cw} = 0.761 \log K_{ow} - 0.343$) is an algebraic rearrangement of the equation ($\log K_{ow} = 1.313 \log K_{cw} + 0.45$) which fits the data presented in the Saket *et al.* publication (Saket *et al.*, 1985). The correlation presented by Saket *et al.* ($\log K_{cw} = 1.313 \log K_{ow} + 0.45$) incorrectly reversed the roles of K_{cw} and K_{ow} and did not fit the data. The second model ($\log K_{cw} = 0.739 \log K_{ow} - 0.28$) was developed by regression of the data in this reference to an equation of the form, ($\log K_{cw} = a \log K_{ow} + b$). This equation is plotted in Figures 6.9 and 6.10 as Model 14.

Two equations are also presented for Model 17. The first model ($\log K_{cw} = 0.344 \log K_{ow} + 0.725$) is an algebraic rearrangement of the published equation ($\log K_{ow} = 2.91 \log K_{cw} - 2.11$). The second equation ($\log K_{cw} = 0.254 \log K_{ow} + 0.987$) was developed by

regression of the data in this reference to an equation of the form, $\log K_{cw} = a \log K_{ow} + b$. This equation is plotted in Figures 6.9 and 6.10 as Model 17.

All eighteen of the correlations account for the effect of lipophilicity through $\log K_{ow}$ and only Model 18, developed using PHSC, included a term for the molecular size (i.e., MW). Consistent with our analysis, this indicates that MW has little influence on skin partitioning for the range of MW studied in these databases. Most of the correlations in Table 6.1 are of the conventional form (i.e., $\log K_{cw} = a \log K_{ow} + b$). linearly correlated with $\log K_{ow}$ with or without a constant term (i.e., intercept). Among the models with conventional form, the slope (a) and intercept (b) estimated for each model depends upon the data used in the regression. The slope varies from a low value of 0.25 (for Model 17) to a high value of 0.761 (for Models 2 and 14). Most other models, and the two models developed from the largest databases (i.e., Models 3 and 10) have slopes near 0.6. The intercept varies from a low value of -0.343 (for Model 14) to a high value of 0.99 (for Model 17). Several of the models have forced b = 0. The two models developed from the largest databases (Models 3 and 10) have intercepts that are small and negative (i.e., -0.006 and -0.024, respectively). Intercept and slope values are not independent. Typically, equations with large values for the slope have smaller intercepts and equations (e.g., Model 14), and equations with smaller slopes have larger intercepts (e.g., Model 17).

The 18 correlations from Table 6.1 and Eq. (6.11) are plotted as a function of $\log K_{ow}$ in Figure 6.9. Except for Model 18, the correlations are in relative agreement in the range ($1 < \log K_{ow} < 4$) but begin to differ from one another at the high and low extremes of $\log K_{ow}$. The correlations in Table 6.1 can be divided into 2 groups, those in which $b < 0.4$ and those in which $b > 0.5$. Models 13, 15, 16, and 17 predict a weak dependence on $\log K_{ow}$ (i.e., $b < 0.4$) than do the other correlations. Models 13, 16, and 17 are derived from entirely independent datasets without overlapping compounds and

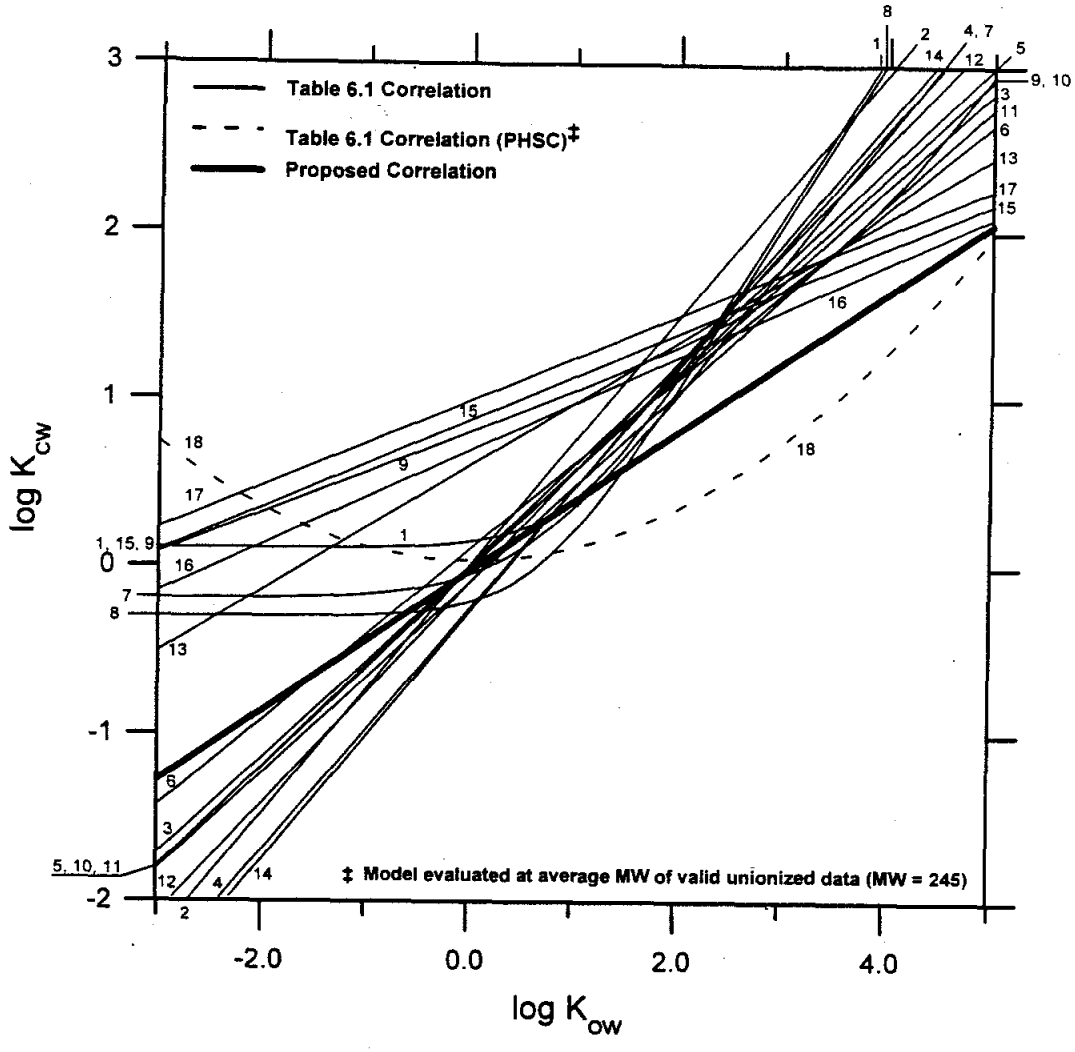

Figure 6.9 Eighteen stratum corneum-water partition coefficient correlations and a proposed correlation (Eq. (6.11)) plotted as a function of $\log K_{ow}$.

Model 15 contains all compounds used in developing Models 16 and 17. Thus, this apparent weak dependence on $\log K_{ow}$ can not be attributed to one influential dataset. Model 9 predicts a weak K_{ow} dependence (slope is 0.24) for $\log K_{ow}$ < about 2.0, but a stronger K_{ow} dependence (slope is 0.91) when $\log K_{ow}$ > about 3. The remaining correlations including the two correlations based on quite large and diverse databases, Model 3 (b = 0.57 from 42 measurements) and 10 (b = 0.59 from 45 measurements), predict a stronger dependence on $\log K_{ow}$. Eq. (6.11) predicts an intermediate dependence upon $\log K_{ow}$ (i.e., b = 0.42). intermediate between the two extreme dependencies. Many of the models intersect near $\log K_{ow} = 2.5$ but this has unknown significance.

Figure 6.10 compares the validated data from Table 6A.1 and the provisional data from 6A.2 (hydroxypregnenolone not shown) to the 18 models in Table 6.1 and Eq. (6.11). The measurements for partially ionized compounds, not adjusted for ionization, are distinguished from the measurements for unionized chemicals. Equation (6.11) provides the best overall fit of the unionized data over the entire range of $\log K_{ow}$. The other correlations tend to overestimate the SC-water partition coefficients because the correlations shown in Figures 6.9 and 6.10 were primarily developed with partition coefficients expressed relative to the dry mass of SC. That is, most of these correlations were developed on parameters which were approximately 4 times larger than the adjusted data shown in Figure 6.10. On average, these correlations would shift down by log(0.25) = -0.60.

More data are needed in the extremes of K_{ow}. Few measurements are available for highly lipophilic compounds and the existing measurements are more variable. It is not surprising then that model predictions vary widely for $\log K_{ow}$ > about 3-4. Similarly, the database is not sufficient to judge whether Models 1, 7, and 8 make appropriate predictions for hydrophilic compounds.

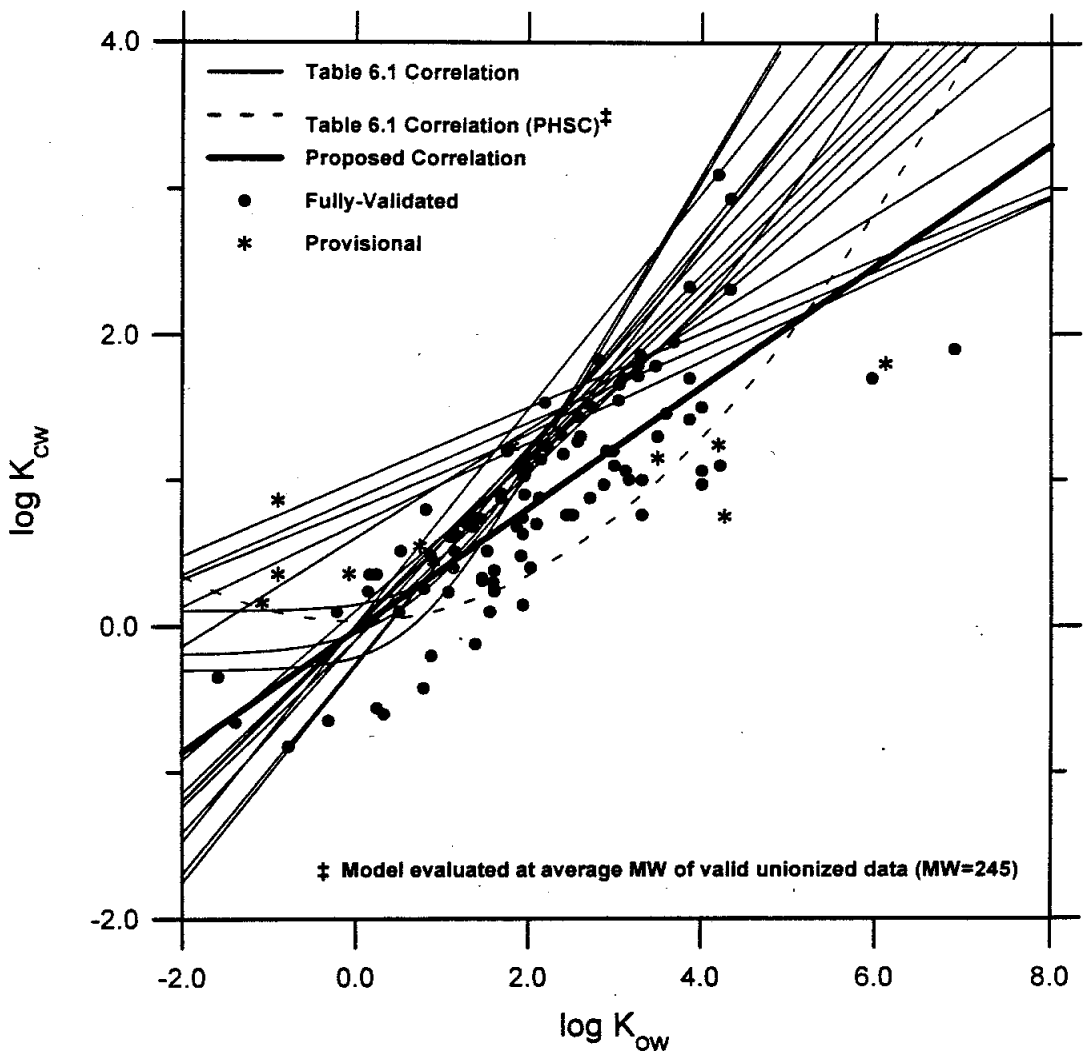

Figure 6.10 Eighteen stratum corneum-water partition coefficient correlations compared to partition coefficients from the fully-validated and provisional databases.

The correlations based on the most limited sets of data (e.g., Models 7 (eight measurements), 16 (six measurements), and 17 (seven measurements)) appear to be consistent with correlations based on a larger set of data, indicating that partition coefficients may not be sensitive to the number of measurements and diversity of the compounds included. The fact that the two correlations, developed from independent databases, predict similar dependencies upon $\log K_{ow}$ also supports this conclusion.

6.4.8. Final Considerations and Recommendations

There are an ample number of SC-water partition coefficients measured to make accurate predictions for moderately lipophilic compounds. However, more measurements are needed at large and small values of K_{ow} (i.e., $\log K_{ow} > 4$ and $\log K_{ow} < 0$, respectively).

The usual procedure in partition coefficient experiments is to measure the mass of absorbed chemical in the SC (M_{sc}). The concentration of chemical in the SC (i.e., the mass of chemical in the SC/ volume of hydrated SC) is then calculated by dividing M_{sc} by the cross sectional area and the hydrated thickness of the SC, L_c, (i.e., $C_{sc} = M_{sc}/(L_cA)$). Unfortunately, the hydrated thickness, L_c, is difficult to measure precisely, introducing uncertainty.

However, mathematical models of dermal absorption never require that L_c and K_{cw} be known independently, and when K_{cw} is reported separately, these models require that L_c be estimated. Experimental determinations of ($K_{cw}L_c$) involve direct measures of the absorbed mass of chemical in the SC, the cross sectional area of the skin sample, and the equilibrium concentration of chemical in the aqueous solution (i.e., $K_{cw}L_c = M_{sc}/(AC_w)$). Thus, in model calculations as well as for improved experimental reliability it is preferred to report the product ($K_{cw}L_c$) rather than K_{cw} alone.

6.5. Conclusions

A set of data validation criteria, based upon physicochemical influences on the SC-water partition coefficient, were used to improve predictive estimates of the SC-water partition coefficient. The goal was to develop the most mechanistically relevant predictive model that is supported by the database assembled. We found that the data are consistent with simple models in terms of $\log K_{ow}$, but do not substantiate more complicated models (based on LSER parameters or based on two mechanisms of partitioning). SC-water partition coefficients for most chemicals can be predicted within an order of magnitude using these simple models involving $\log K_{ow}$.

SC-water partition coefficients measured in different laboratories for the same compound can sometimes differ by more than an order of magnitude. Different ways of expressing the concentration of partitioning chemical in the SC is one source of variation among SC-water partition coefficients. Differences in temperature and different amounts of ionization appear to contribute to this variation, these trends can not be completely understood using the current data. More data for chemicals at the large and small extremes of K_{ow} and for chemicals which ionize is needed to better understand several of these effects. Although the reason is not clearly identified, certain laboratories appear to be measuring partition coefficients which are systematically higher or lower than predicted by a correlation developed using the entire database.

Finally, 18 correlations from the literature are compared to the database and to the correlation which we developed. Many correlations provide adequate estimates. The main difference between correlations, most with the form of the conventional correlation, is the dependency upon K_{ow}. Differences in the K_{ow} dependence for different datasets requires further investigation.

6.6. Notation

A	=	Surface area of chemical exposure
C_{sc}	=	Concentration of absorbing chemical in the SC
C_w	=	Aqueous concentration of the absorbing chemical
D_c	=	Effective diffusivity of the absorbing chemical in the SC
f_{ui}	=	Fraction of the total chemical dose that is unionized in the vehicle
K_{cw}	=	Equilibrium partition coefficient between the SC and water for the absorbing chemical
K'_{cw}	=	SC-water partition coefficient for the absorbing chemical with dry mass of SC as the basis, defined by Eq. (6.2)
$K_{cw,i}$	=	Partition coefficient of the ionized species
$K_{cw,obs}$	=	Partition coefficient observed for a mixture of the unionized and ionized species
$K_{cw,\,pred}$	=	Predicted partition coefficient between the SC and water for the absorbing chemical
$K_{cw,ui}$	=	Partition coefficient of the unionized species
K_{ow}	=	Octanol-water partition coefficient of the penetrating chemical
$K^{\#}_{ow}$	=	Value of K_{ow} at which the piecewise linear regression changes slope and distinguishes the hydrophilic chemicals from the lipophilic chemicals
L_c	=	Effective thickness of the SC
M_{sc}	=	Mass of absorbed chemical in the SC
MW	=	Molecular weight of the absorbing chemical
P_{cw}	=	Steady-state permeability of the SC from water
pH	=	Negative logarithm of the hydrogen ion molarity $-\log_{10}[H^+]$
pK_a	=	Negative logarithm of the acidity equilibrium constant $-\log_{10}[K_a]$
SC	=	Stratum corneum
T	=	Absolute temperature (Kelvin)
V_x	=	Characteristic volume of McGowans (LSER parameter)

Greek

α	=	Hydrogen bond acidity of the absorbing chemical (LSER parameter)
β	=	Hydrogen bond basicity of the absorbing chemical (LSER parameter)
δ	=	Indicator variable defined by Eq. (6.7)
π	=	Dipolarity/polarizability of the absorbing chemical (LSER parameter)
ρ_D	=	Density of aqueous solution
ρ_{sc}	=	Mass dry SC divided by hydrated skin volume

6.7. References

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6.8. Appendix 6A: Tables of Stratum Corneum-Water Partition Coefficients

Table 6A.1 Fully-Validated Stratum Corneum-Water Partition Coefficients

Table 6A.2 Provisional Stratum Corneum-Water Partition Coefficients

Table 6A.3 Excluded Stratum Corneum-Water Partition Coefficients

Table 6A.4 LSER Parameters and Partition Coefficients for Chemicals in the LSER Database

Table 6A.5 Temperature Effects on f_{ui} and Calculation of Unmeasured pH

Table 6A.1 Fully-Validated Stratum Corneum-Water Partition Coefficients

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)[c]	K_{ow}[d]	K_{ow} (adj)[e]	Basis[f]	f_{ul}[g]	pH[h]	t_{equil} (hrs)[i]	Reference
Acetaminophen	0.51	151.2	25	5.0	1.3	Dry Mass	1	< [7.0]	6-24	Surber et al., 1990b
Aldosterone	1.08	360.4	26[j]	6.8	1.7	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
4-Amino-2-nitrophenol	1.53	154.1	25	13.0	2.5	Dry Volume[l]	1	N/A[m]	48	Bronaugh & Congdon, 1984
Atrazine	2.61	215.7	25	79.4	19.9	Dry Mass	1	N/A	24[n]	Surber et al., 1992
[Benzene]	2.13	78.1	31	30.0	7.5	Dry Mass	1	ND	N/A	Blank & McAuliffe, 1985
[Benzo[a]pyrene]	5.97	252.3	25	199.5	49.9	Dry Mass	1	ND	24[n]	Surber et al., 1992
[Benzoic acid]	1.87	122.1	35	4.8	4.8	Wet Volume	1	2.75	24	Parry et al., 1990
[Benzyl alcohol]	1.10	108.1	25	4.1	4.1	Wet Mass[o]	1	ND	48[o]	Roberts, 1976
Betamethasone	1.94	392.5	37[j]	5.6	1.4	Dry Mass	1	4.5	72	Kubota & Maibach, 1993
Betamethasone 17-valerate	3.60	476.0	37[j]	113.9	28.5	Dry Mass	1	4.5	72	Kubota & Maibach, 1993
[p-Bromophenol]	2.59	173.0	25	27.2[p]	27.2	Wet Mass	1	[5.3][q]	48	Anderson et al., 1976
[Butanoic acid]	0.79	88.1	25	1.5	0.4	Dry Mass[k]	1	N/A	N/A	Scheuplein, 1967
[Butanol]	0.88	74.1	25	2.5	0.6	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
Chloramphenicol	1.14	323.1	25	10.0	2.5	Dry Mass	1	ND	24[n]	Surber et al., 1992
[Chlorocresol]	3.10	142.6	25	50.4[p]	50.4	Wet Mass	1	[5.3][q]	48	Anderson et al., 1976
[p-Chlorophenol]	2.39	128.6	25	20.4[p]	20.4	Wet Mass	1	[5.3][q]	48	Anderson et al., 1976
[o-Chlorophenol]	2.15	128.6	25	13.8[p]	13.8	Wet Mass	1	[4.6][q]	48	Anderson et al., 1976
Chloroxylenol	[3.48]	115.5	25	60.8[p]	60.8	Wet Mass	1	[5.3][q]	48	Anderson et al., 1976
Cortexolone	2.52	346.5	26[j]	23.0	5.8	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
Cortexone	2.88	330.5	26[j]	37.0	9.3	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
Corticosterone	1.94	346.5	26[j]	17.0	4.3	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
Cortisone	1.47	360.5	25	8.1	2.0	Dry Mass	1	ND	48	Saket et al., 1985
Cortisone	1.47	360.5	26[j]	8.5	2.1	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
Cortisone acetate	2.10	402.0	25	20.0	5.0	Dry Mass	1	ND	48	Saket et al., 1985
Cortisone butyrate	[1.76]	430.5	25	63.1	15.8	Dry Mass	1	ND	48	Saket et al., 1985
Cortisone hexanoate	[2.82]	458.5	25	269.2	67.3	Dry Mass	1	ND	48	Saket et al., 1985
Cortisone octanoate	[3.87]	486.5	25	851.1	212.8	Dry Mass	1	ND	48	Saket et al., 1985
[m-Cresol]	1.96	108.1	25	10.6[p]	10.6	Wet Mass	1	[5.5][q]	48	Anderson et al., 1976

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)[c]	K_{ow}[d]	K_{ow} (adj)[e]	Basis[f]	f_{ul}[g]	pH[h]	t_{equil} (hrs)[i]	Reference
[o-Cresol]	1.95	108.1	25	10.6[p]	10.6	Wet Mass	1	[5.6][q]	48	Anderson et al., 1976
[p-Cresol]	1.94	108.1	25	10.6[p]	10.6	Wet Mass	1	[5.6][q]	48	Anderson et al., 1976
[p-Cresol]	1.94	108.1	37	22.0	5.5	Dry Mass[s]	1	4	N/A	Anderson et al., 1989
[4-Cyanophenol]	1.60	129.1	25	7.9	2.0	Dry Mass	>0.90	< [6.9]	6-24	Surber et al., 1990b
DDT	6.91	354.5	25	316.2	79.1	Dry Mass	1	ND	6-24	Surber et al., 1990b
Diazepam	2.99	284.8	25	63.1	15.8	Dry Mass	1	N/A	6	Surber et al., 1990a
2,4-Dichlorophenol	3.06	163.0	25	45.4[p]	45.4	Wet Mass	1	[4.4][q]	48	Anderson et al., 1976
β-Estradiol	4.01	272.4	26[j]	46.0	11.5	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
β-Estradiol	4.01	272.4	25	125.9	31.5	Dry Mass	1	ND	6	Surber et al., 1990a
β–Estradiol	4.01	272.4	32[j]	9.3	9.3	Wet Mass	1	ND	48	Megrab et al., 1995
Estriol	2.45	288.4	26[j]	23.0	5.8	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
Estrone	3.13	270.4	26[j]	46.0	11.5	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
[Ethanol]	-0.31	46.0	25	0.9	0.2	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
[p-Ethylphenol]	2.58	122.2	25	18.3[p]	18.3	Wet Mass	1	[5.7][q]	48	Anderson et al., 1976
[Heptanoic acid]	[2.41]	130.2	25	60.3	15.1	Dry Mass[k]	1	N/A	N/A	Scheuplein, 1967
[Heptanol]	2.72	116.0	25	30.0	7.5	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
[Hexanoic acid]	1.92	116.2	25	12.0	3.0	Dry Mass[k]	1	N/A	N/A	Scheuplein, 1967
[Hexanol]	2.03	102.2	25	10.0	2.5	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
Hydrocortisone (HC)	1.61	362.5	25	7.1	1.8	Dry Mass	1	ND	48	Saket et al., 1985
Hydrocortisone (HC)	1.61	362.5	37	6.9	1.7	Dry Mass	1	ND	48	Saket et al., 1985
Hydrocortisone (HC)	1.61	362.5	26[j]	7.0	1.8	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
Hydrocortisone (HC)	1.61	362.5	25	9.5	2.4	Dry Mass	1	ND	6	Surber et al., 1990a
HC-21-yl-acetate	[1.16]	404.0	25	17.0	4.2	Dry Mass	1	ND	48	Saket et al., 1985
HC-21-yl-N,N-dimethylsuccinate	[0.88]	489.6	37	12.0	3.0	Dry Mass	1	ND	48-72	Raykar et al., 1988
HC-21-yl-hexanoate	[3.28]	460.6	37	207.9	52.0	Dry Mass	1	ND	48-72	Raykar et al., 1988
HC-21-yl-hexanoate	[3.28]	460.6	25	245.5	61.4	Dry Mass	1	ND	48	Saket et al., 1985
HC-21-yl-hydroxy hexanoate	[1.29]	476.6	37	20.0	5.0	Dry Mass	1	ND	48-72	Raykar et al., 1988
HC-21-yl-octanoate	[4.34]	488.7	37	3423.3	855.8	Dry Mass	1	ND	48-72	Raykar et al., 1988
HC-21-yl-octanoate	[4.34]	488.7	25	812.8	203.2	Dry Mass	1	ND	48	Saket et al., 1985

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)[c]	K_{cw}[d]	K_{cw} (adj)[e]	Basis[f]	f_{uf}[g]	pH[h]	t_{equil} (hrs)[i]	Reference
HC-21-yl-pentanoate	[2.75]	446.0	25	125.9	31.5	Dry Mass	1	ND	48	Saket et al., 1985
HC-21-yl-pimelamate	[0.82]	503.6	37	25.0	6.3	Dry Mass	1	ND	48-72	Raykar et al., 1988
HC-21-yl-propionate	[1.69]	418.5	37	30.0	7.5	Dry Mass	1	ND	48-72	Raykar et al., 1988
HC-21-yl-propionate	[1.69]	418.5	25	32.4	8.1	Dry Mass	1	ND	48	Saket et al., 1985
HC-21-yl-succinamate	[0.17]	461.6	37	9.0	2.3	Dry Mass	1	ND	48-72	Raykar et al., 1988
4-Hydroxybenzyl alcohol	0.25	124.1	37	9.0	2.3	Dry Mass[s]	1	4	N/A	Anderson et al., 1989
α-(4-Hydroxyphenyl)acetamide	[-0.21]	151.2	37	5.0	1.3	Dry Mass[s]	1	4	N/A	Anderson et al., 1989
17α-Hydroxyprogesterone	3.17	330.5	26[j]	40.0	10.0	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
[4-Iodophenol]	2.91	220.0	25	63.1	15.8	Dry Mass	1	< [7.0]	6-24	Surber et al., 1990b
Lauric acid	4.20	200.3	32	5060.0	1265.0	Dry Mass	1	3.2	24-48	Smith & Anderson, 1995
[Methanol]	-0.77	32.0	25	0.6	0.2	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
Methyl 4-hydroxyphenyl acetate	[1.15]	166.0	37	13.0	3.3	Dry Mass[s]	1	4	N/A	Anderson et al., 1989
Methyl HC-21-yl-pimelate	[2.20]	518.6	37	136.0	34.0	Dry Mass	1	ND	48-72	Raykar et al., 1988
Methyl HC-21-yl-succinate	[1.38]	476.6	37	22.0	5.5	Dry Mass	1	ND	48-72	Raykar et al., 1988
Methyl hydroxybenzoate	1.96	152.1	25	7.9	7.9	Wet Mass[o]	1	ND	48[o]	Roberts, 1976
[β-Naphthol]	2.70	144.2	25	33.4[p]	33.4	Wet Mass	1	[5.2][q]	48	Anderson et al., 1976
2-Nitro-p-phenylenediamine	0.53	153.1	25	13	2.5	Dry Volume[l]	1	N/A[m]	48	Bronaugh & Congdon, 1984
[m-Nitrophenol]	2.00	139.1	25	12.1[p]	12.1	Wet Mass	1	[4.8][q]	48	Anderson et al., 1976
[p-Nitrophenol]	1.91	139.1	25	12.8[p]	12.8	Wet Mass	1	[3.9][q]	48	Anderson et al., 1976
N-Nitrosodiethanolamine	[-1.58]	134.1	32	1.8	0.5	Dry Mass	1	ND	96	Bronaugh et al., 1981
[Octanoic acid]	3.05	144.2	25	141.3	35.3	Dry Mass[k]	1	N/A	N/A	Scheuplein, 1967
[Octanol]	3.00	130.2	25	50.0	12.5	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
[Pentanoic acid]	1.39	102.1	25	3.0	0.8	Dry Mass[k]	1	N/A	N/A	Scheuplein, 1967
[Pentanol]	1.56	88.0	25	5.0	1.3	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
4-Pentyloxyphenol	3.50	180.2	25	79.4	19.9	Dry Mass	1	< [6.2]	6-24	Surber et al., 1990b
[Phenethyl alcohol]	1.36	122.2	25	4.8	4.8	Wet Mass[o]	1	ND	48[o]	Roberts, 1976
[Phenol]	1.46	94.1	25	5.4[p]	5.4	Wet Mass	1	[5.4][q]	48	Anderson et al., 1976
o-Phenylenediamine	0.15	108.1	25	6.9	1.3	Dry Volume[l]	1	[7.6]	48	Bronaugh & Congdon, 1984
Pregnenolone	4.22	316.5	26[j]	50.0	12.5	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)[c]	K_{ow}[d]	K_{ow} (adj)[e]	Basis[f]	f_{ui}[g]	pH[h]	t_{equil} (hrs)[i]	Reference
Progesterone	3.87	314.5	26[j]	104.0	26.0	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
Progesterone	3.87	314.5	25	199.5	49.9	Dry Mass	1	ND	6	Surber et al., 1990a
[Propanoic acid]	0.33	74.1	25	1.0	0.3	Dry Mass[k]	1	N/A	N/A	Scheuplein, 1967
[Propanol]	0.25	60.0	25	1.1	0.3	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
[Resorcinol]	0.80	110.1	25	1.8[p]	1.8	Wet Mass	1	[5.4][q]	48	Anderson et al., 1976
Testosterone	3.32	288.4	26[j]	23.0	5.8	Dry Mass[k]	1	ND	N/A	Scheuplein et al., 1969
Testosterone	3.32	288.4	25	39.8	10.0	Dry Mass	1	ND	6	Surber et al., 1990a
[Thymol]	3.30	150.2	25	72.7[p]	72.7	Wet Mass	1	[6.0][q]	48	Anderson et al., 1976
2,4,6-Trichlorophenol	3.69	197.5	25	89.0[p]	89.0	Wet Mass	1	[3.6][q]	48	Anderson et al., 1976
[Water]	-1.38	18.0	25	0.9	0.2	Dry Mass[r]	1	ND	N/A	Scheuplein & Blank, 1973
[3,4-Xylenol]	2.23	122.2	25	16.9[p]	16.9	Wet Mass	1	[5.8][q]	48	Anderson et al., 1976

a. The compound investigated. Compounds contained within brackets (e.g. [Benzene]) were included in the LSER analysis.
b. Reported $\log K_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for chloroxylenol [3.48]), in which case they were calculated (Daylight, 1995).
c. Temperature used to measure the partition coefficient.
d. Reported stratum corneum-water partition coefficients prior to any adjustment.
e. Stratum corneum-water partition coefficients adjusted for basis and reported on the basis of hydrated-SC volume.
f. Basis on which the unadjusted stratum corneum-water partition coefficient was reported. SC concentration is expressed relative to a gram of dry SC (Dry Mass), a gram of hydrated SC (Wet Mass), or a unit volume of dry SC (Dry Volume), or a unit volume of hydrated SC (Wet Volume).
g. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 6A.3.
h. Reported solution pH unless contained within brackets (e.g. for p-bromophenol [5.3]) in which case the pH was calculated from the reported chemical concentration and calculated pKa values (see Table 6A.3). Chemicals that are undissociated are indicated by ND when no pH was reported.
i. Time allowed for the absorbing chemical to equilibrate with the stratum corneum.
j. The temperature for measuring the SC-water partition coefficients is assumed to equal that used by these authors in experiments to determine permeability coefficients.
k. Scheuplein and coworkers used the same experimental procedure as Scheuplein (1965), which used a dry-mass SC basis.
l. Bronaugh & Congdon adjusted their measured values by a factor of 1.32 to place it on a dry-SC volume basis. We first convert their data to a dry mass basis by multiplying by 1.32 g dry SC/mL dry SC and adjusting by the 0.25 conversion factor.
m. For 4-amino-2-nitrophenol and 2-nitro-p-phenylenediamine the pH attained can not be calculated because the concentration was not provided. Although, if the starting pH was near 7 the pH will change in a direction that f_{ui} is always 1.
n. The times varied with chemical, but most of the equilibration times reported were 24 hours (Surber et al., 1992).
o. There is reasonable evidence to assume that values are consistent with previous measurements (e.g., Anderson et al., 1976).
p. The Anderson et al., 1976 values for phenolics were determined by a desorption technique in which the solution and SC concentration were

measured after solute-laden SC was allowed to equilibrate with a solute-free aqueous solution.

q. The concentrations were consistently dilute (circa 1% (w/v)), but were not reported. We have used a concentration of 1% (w/v) to estimate the pH and the fraction unionized. In all cases, $f_{ui} = 1$.

r. The Scheuplein n-alcohols and water have a dry mass basis as explained in the 1964 Army Report.

s. These authors reference Raykar and colleagues (Raykar et al., 1988) for experimental protocol. We assume that the basis is the same as in Raykar et al. (1988).

Table 6A.2 Provisional Stratum Corneum-Water Partition Coefficients

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	K_{cw}[c]	K_{cw} (adj)[d]	Basis[e]	f_u[f]	pH[g]	t_{equil} (hrs)[h]	Reference
Acitretin	[6.12]	326.4	25	251.2	62.8	Dry Mass	N/A	N/A[i]	24	Surber et al., 1990a
Caffeine	-0.07	194.2	25	9.1	2.3	Dry Mass	N/A	N/A[i]	6	Surber et al., 1990a
5-Fluorouracil (+ - + -)	-0.89	130.1	32	0.9	0.9	Wet Mass	<0.1	4.75[j]	24	Cornwell & Barry, 1994
5-Fluorouracil (+ - + -)	-0.89	130.1	25	3.4[k]	0.9	Dry Mass[k]	<0.1	N/A[i]	24[l]	Surber et al., 1992
5-Fluorouracil (+ - + -)	-0.89	130.1	20	2.9	2.9	Wet Mass	<0.1	4.75[j]	4	Williams & Barry, 1991
HC-21-yl-hemipimelate (-)	[1.82]	504.6	37	68.0	17.0	Dry Mass	0.8	4	48-72	Raykar et al., 1988
HC-21-yl-hemisuccinate (-)	[0.91]	462.5	37	11.0	2.8	Dry Mass	0.1	5.5	48-72	Raykar et al., 1988
4-Hydroxyphenyl acetic acid	0.75	152.1	37	14.0	3.5	Dry Mass[m]	0.7	4	N/A	Anderson et al., 1989
Hydroxypregnenolone[n]	N/A	N/A	26[o]	43.0	10.8	Dry Mass[p]	1	ND	N/A	Scheuplein et al., 1969
Ibuprofen	3.50	206.3	25	56.2[k]	14.1	Dry Mass[k]	N/A	N/A[i]	24[l]	Surber et al., 1992
Indomethacin	4.27	357.8	25	22.4[k]	5.6	Dry Mass[k]	N/A	N/A[i]	24[l]	Surber et al., 1992
Lauric acid	4.20	200.3	25	69.2[k]	17.3	Dry Mass[k]	N/A	N/A[i]	24[l]	Surber et al., 1992
Uracil	-1.07	112.1	25	5.8[k]	1.5	Dry Mass[k]	N/A	N/A[i]	24[l]	Surber et al., 1992

a. The compound investigated. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, 5-fluorouracil with two positive and two negative charges is indicated by (+ - + -).
b. Reported $\log K_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless bracketed, in which case they were calculated (Daylight, 1995).
c. Reported stratum corneum-water partition coefficients prior to any adjustment.
d. Stratum corneum-water partition coefficients adjusted for basis and reported on the basis of hydrated-SC volume.
e. Basis on which the unadjusted stratum corneum-water partition coefficient was reported. SC concentration is expressed relative to a gram of dry SC (Dry Mass), a gram of hydrated SC (Wet Mass), or a unit volume of dry SC (Dry Volume), or a unit volume of hydrated SC (Wet Volume).
f. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 6A.3.
g. Reported solution pH unless contained within brackets in which case the pH was calculated from the reported chemical concentration and calculated pKa values (see Table 6A.3).
h. Time allowed for the absorbing chemical to equilibrate with the stratum corneum.
i. The natural pH depends on the chemical concentration which was not reported.
j. This pH was attained by a saturated 5-fluorouracil solution (personal communication, Barry, 1996).
k. This information was provided by personal communication with the first author (Surber, 1996).
l. The times varied with chemical, but most of the equilibration times reported were 24 hours (Surber et al., 1992).
m. These authors reference Raykar and colleagues (1988) for experimental protocol. We assume that the basis is the same as in Raykar et al. (1988).
n. Hydroxypregnenolone is provisional because we could not unambiguously identify the molecular formula for this steroid.
o. The temperature for measuring the SC-water partition coefficients is assumed to equal that used by these authors in experiments to determine P_{cw}.
p. Scheuplein and coworkers used the same experimental procedure as Scheuplein (1965), which used a dry-mass SC basis.

Table 6A.3 Excluded Stratum Corneum-Water Partition Coefficients

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	K_{cw}[c]	K_{cw} (adj)[d]	Basis[e]	f_{ui}[f]	pH[g]	t_{equil} (hrs)[h]	Reference
[Alachlor]	3.52	270.0	35	[12.0]	3.0	Dry Mass	1	6.6	24	Hui et al., 1995
[Aminopyrine]	1.00	231.3	35	[0.68]	0.2	Dry Mass	0.91	4.8	24	Hui et al., 1995
[Atrazine]	2.61	215.7	35	[5.5]	1.4	Dry Mass	1	6.2	24	Hui et al., 1995
[Benzene]	2.13	78.1	N/A	199.0	49.8	Dry Mass	1	ND	0.5	Wester et al., 1987
Cyclosporin-A[i]	N/A	1201.0	25	79.4	19.9	Dry Mass	<0.1	N/A	24[j]	Surber et al., 1992
[Dopamine hydrochloride] (+)	[-0.05][k]	153.2	35	[10.0]	2.5	Dry Mass	<0.1	4	24	Hui et al., 1995
[2,4-D] (-)	2.81	221.0	35	[17.8]	4.4	Dry Mass	<0.1	5.9	24	Hui et al., 1995
[Glycine] (+ -)	-3.21	75.0	35	[3.1]	0.8	Dry Mass	<0.1	4.5	24	Hui et al., 1995
[Glyphosate] (- - - +)	[-1.7][l]	169.0	35	[0.9]	0.2	Dry Mass	<0.1	7.3	24	Hui et al., 1995
[Hydrocortisone]	1.61	362.5	35	[1.5]	0.4	Dry Mass	1	8.1	24	Hui et al., 1995
[Malathion]	2.36	330.0	35	[4.7]	1.1	Dry Mass	1	5.2	24	Hui et al., 1995
Nicorandil[m]	[0.65]	211.2	37	7.25	1.8	Dry Mass	1	[7.2]	24	Sato et al., 1989
[p-Nitroaniline]	1.39	138.1	N/A	25.6	6.4	Dry Mass	1	[7.0]	0.5	Wester et al., 1987
[PCB] (54% chlorine)[n]	6.62[o]	325.1	35	1175.0	293.7	Dry Mass	1	6.5	24	Hui et al., 1995
PCB (54% chlorine)[n]	6.62[o]	325.1	25	199.5	49.9	Dry Mass	1	ND	6-24	Surber et al., 1990b
[PCB] (54% chlorine)[n]	6.62[o]	325.1	N/A	22256.0	5563.9	Dry Mass	1	ND	0.50	Wester et al., 1987
[Theophylline]	-0.02	180.2	35	[0.4]	0.1	Dry Mass	0.33	7.6	24	Hui et al., 1995
[Urea]	-2.11	60.1	35	[0.5]	0.1	Dry Mass	1	2.1[p]	24	Hui et al., 1995

a. The compound investigated. Compounds contained within brackets (e.g. [Alachlor]) were measured using sieved, powdered human stratum corneum (PHSC) using plantar calluses. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, glyphosate with three negative charges and one positive charge is indicated by (- - - +).

b. Reported $\log K_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for dopamine hydrochloride [-0.05]), in which case they were calculated (Daylight, 1995).

c. Reported stratum corneum-water partition coefficients prior to any adjustment. Values within brackets (e.g., for alachlor [12.0]) were digitized.

d. Stratum corneum-water partition coefficients adjusted for basis and reported on the basis of hydrated-SC volume.

e. Basis on which the unadjusted stratum corneum-water partition coefficient was reported. SC concentration is expressed relative to a gram of dry SC (Dry Mass), a gram of hydrated SC (Wet Mass), or a unit volume of dry SC (Dry Volume), or a unit volume of hydrated SC (Wet Volume).

f. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 6A.3.

g. Reported solution pH unless contained within brackets (e.g. for nicorandil [7.2]) in which case the pH was calculated from the reported chemical concentration and calculated pKa values (see Table 6A.3). Chemicals that are undissociated are indicated by ND when no pH was reported.

h. Time allowed for the absorbing chemical to equilibrate with the stratum corneum.

i. *Cyclosporin*-A is excluded because it is much larger than the other compounds in the database and may partition by different mechanisms than smaller compounds.
j. The individual times varied. Most equilibration times reported were 24 hours (Surber et al., 1992).
k. The $\log K_{ow}$ was calculated for dopamine not the hydrochloride salt.
l. This $\log K_{ow}$ was reported by the authors to be recommended by Hansch and Leo (1979). However, this value was not recommended by Hansch et al. (1995).
m. Excluded because the stratum corneum used was plantar callus (Sato et al., 1989).
n. PCB was excluded because it was a polydispersed mixture and not a single compound.
o. The $\log K_{ow}$ for PCB was calculated as the average of 14 Starlist values for variously substituted pentachlorobiphenyls (Hansch et al., 1995).
p. The authors state that urea was added to distilled water (presumably near pH 7). If true, the reported pH of 2.1 can not be attained unless a pH adjustment was made (suggesting that pH = 2.1 may be in error). At any pH value higher than 2.1, urea is essentially unionized. Since it is likely that the actual pH > 2.1, we have assumed $f_{ui} = 1$.

Table 6A.4 LSER Parameters[a] and Partition Coefficients for Chemicals in the LSER Database

COMPOUND	π	α	β	V_X [cm^3/mol/100][b]	T (C)	K_{cw} (adj)[c]	Reference
Benzene	0.52	0.00	0.14	0.716	31	7.5	Blank & McAuliffe, 1985
Benzo[a]pyrene	1.98	0.00	0.44	1.954	25	49.9	Surber et al., 1992
Benzoic Acid	0.90	0.59	0.40	0.932	35	4.8	Parry et al., 1990
Benzyl alcohol	0.87	0.33	0.56	0.916	25	4.1	Roberts, 1976
p-Bromophenol	1.17	0.67	0.20	0.950	25	27.2	Anderson et al., 1976
Butanoic acid	0.62	0.60	0.45	0.747	25	0.4	Scheuplein, 1967
Butanol	0.42	0.37	0.48	0.731	25	0.6	Scheuplein & Blank, 1973
Chlorocresol	1.02	0.65	0.22	1.038	25	50.4	Anderson et al., 1976
o-Chlorophenol	0.88	0.32	0.31	0.898	25	13.8	Anderson et al., 1976
p-Chlorophenol	1.08	0.67	0.20	0.898	25	20.4	Anderson et al., 1976
m-Cresol	0.88	0.57	0.34	0.916	25	10.6	Anderson et al., 1976
o-Cresol	0.86	0.52	0.30	0.916	25	10.6	Anderson et al., 1976
p-Cresol	0.87	0.57	0.31	0.916	25	10.6	Anderson et al., 1976
p-Cresol	0.87	0.57	0.31	0.916	37	5.5	Anderson et al., 1989
4-Cyanophenol	1.63	0.79	0.29	0.930	25	2.0	Surber et al., 1990b
Ethanol	0.42	0.37	0.48	0.449	25	0.2	Scheuplein & Blank, 1973
p-Ethylphenol	0.90	0.55	0.36	1.057	25	18.3	Anderson et al., 1976
Heptanoic acid	0.60	0.60	0.45	1.169	25	15.1	Scheuplein, 1967
Heptanol	0.42	0.37	0.48	1.154	25	7.5	Scheuplein & Blank, 1973
Hexanoic acid	0.60	0.60	0.45	1.028	25	3.0	Scheuplein, 1967
Hexanol	0.42	0.37	0.48	1.013	25	2.5	Scheuplein & Blank, 1973
4-Iodophenol	1.22	0.68	0.20	1.033	25	15.8	Surber et al., 1990b
Methanol	0.44	0.43	0.47	0.308	25	0.2	Scheuplein & Blank, 1973
β-Naphthol	1.08	0.61	0.40	1.144	25	33.4	Anderson et al., 1976

COMPOUND	π	α	β	V_X [cm^3/mol/100][b]	T (C)	K_{ow} (adj)[c]	Reference
m-Nitrophenol	1.57	0.79	0.23	0.949	25	12.1	Anderson et al., 1976
p-Nitrophenol	1.72	0.82	0.26	0.949	25	12.8	Anderson et al., 1976
Octanoic acid	0.60	0.60	0.45	1.310	25	35.3	Scheuplein, 1967
Octanol	0.42	0.37	0.48	1.295	25	12.5	Scheuplein & Blank, 1973
Pentanoic acid	0.60	0.60	0.45	0.888	25	0.8	Scheuplein, 1967
Pentanol	0.42	0.37	0.48	0.872	25	1.3	Scheuplein & Blank, 1973
Phenol	0.89	0.60	0.30	0.775	25	5.4	Anderson et al., 1976
2-Phenylethanol	0.91	0.30	0.64	1.057	25	4.8	Roberts, 1976
Propanoic Acid	0.65	0.60	0.45	0.606	25	0.3	Scheuplein, 1967
Propanol	0.42	0.37	0.48	0.590	25	0.3	Scheuplein & Blank, 1973
Resorcinol	1.00	1.10	0.58	0.834	25	1.8	Anderson et al., 1976
Thymol	0.60	0.27	0.35	1.340	25	72.7	Anderson et al., 1976
Water	0.45	0.82	0.35	0.167	25	0.2	Scheuplein & Blank, 1973
3,4-Xylenol	0.86	0.56	0.39	1.057	25	16.9	Anderson et al., 1976

a. LSER parameters reported by Abraham and colleagues (Abraham *et al*., 1994b)

b. The reported V_X have been normalized by a factor of 100 to make the values comparable in magnitude to the other LSER parameters.

c. Partition coefficients are from Table 6A.1

Table 6A.5 Temperature Effects on f_{ui} and Calculation of Unmeasured pH

COMPOUND	$pK_a(25)$[a]	T (°C)	ΔH (kcal/mol)[b]	$pK_a(T)$[c]	C_w (mol/L)[d]	pH[e]	f_{ui}[f]	Reference
Acetaminophen	9.8	25	5	9.8	> 3.3E-7	< [7.0][g]	1	Surber et al., 1990b
Aminopyrine	4.06	35	10	3.82		4.8	0.91	Hui et al., 1995
p-Bromophenol	9.4	25	5	9.4	0.058[h]	[5.3]	1	Anderson et al., 1976
Chlorocresol	9.55	25	5	9.55	0.070[h]	[5.3]	1	Anderson et al., 1976
o-Chlorophenol	8.25	25	5	8.25	0.078[h]	[4.6]	1	Anderson et al., 1976
p-Chlorophenol	9.43	25	5	9.43	0.078[h]	[5.3]	1	Anderson et al., 1976
Chloroxylenol	9.68	25	5	9.68	0.086[h]	[5.3]	1	Anderson et al., 1976
m-Cresol	10.13	25	5	10.13	0.092[h]	[5.5]	1	Anderson et al., 1976
o-Cresol	10.33	25	5	10.33	0.092[h]	[5.6]	1	Anderson et al., 1976
p-Cresol	10.31	25	5	10.31	0.092[h]	[5.6]	1	Anderson et al., 1976
4-Cyanophenol	7.77	25	5	7.77	> 3.9E-7	< [6.9][g]	> 0.88	Surber et al., 1990b
2,4-Dichlorophenol	7.67	25	5	7.67	0.061[h]	[4.4]	1	Anderson et al., 1976
p-Ethylphenol	10.29	25	5	10.29	0.082[h]	[5.7]	1	Anderson et al., 1976
4-Iodophenol	9.33	25	5	9.33	> 3.2E-7	< [7.0][g]	1	Surber et al., 1990b
Lauric Acid	4.73	32	0	4.73		3.2	1	Smith & Anderson, 1995
β-Naphthol	9.34	25	5	9.34	0.069[h]	[5.2]	1	Anderson et al., 1976
Nicorandil	2.87	37	5	2.72	2.37E-03	[7.2]	1	Sato et al., 1989
p-Nitroaniline	1.35	N/A	7.5	1.35[i]	3.55E-05	[7.0]	1	Wester et al., 1987
m-Nitrophenol	8.39	25	5	8.39	0.072[h]	[4.8]	1	Anderson et al., 1976
p-Nitrophenol	6.83	25	5	6.83	0.072[h]	[3.9]	1	Anderson et al., 1976
4-Pentyloxyphenol	10.19	25	5	10.19	> 6.1E-7	< [6.2][g]	1	Surber et al., 1990b
Phenol	10	25	5	10	0.106[h]	[5.4]	1	Anderson et al., 1976
o-Phenylenediamine	4.24	25	7.5	4.24	7.72E-04	[7.6]	1	Bronaugh & Congdon, 1984
Resorcinol	9.86	25	5	9.86	0.091[h]	[5.4]	1	Anderson et al., 1976
Theophiline	8.14	35	10	7.9		7.6	0.33	Hui et al., 1995
Thymol	10.82	25	5	10.82	0.066[h]	[6.0]	1	Anderson et al., 1976

COMPOUND	$pK_a(25)$[a]	T (°C)	ΔH (kcal/mol)[b]	$pK_a(T)$[c]	C_w (mol/L)[d]	pH[e]	f_{ul}[f]	Reference
2,4,6-Trichlorophenol	5.94	25	5	5.94	0.051[h]	[3.6]	1	Anderson et al., 1976
3,4-Xylenol	10.44	25	5	10.44	0.082[h]	[5.8]	1	Anderson et al., 1976

a. pK_a values calculated in SPARC at 25C using methods described in Section 5.2.1.
b. These heats of ionization are approximate values obtained from the literature (Sober, 1968). See also Section 5.2.1.
c. Calculated using an integrated form of the van't Hoff equation, Eq. (5.2).
d. Solution concentration provided only when it was needed to calculate the pH.
e. The pH was reported in the original paper, unless contained within brackets, in which case it was calculated from $pK_a(T)$ and the solution concentration assuming that pH was 7.0 prior to chemical addition.
f. The fraction unionized was calculated using Eq. (5.1) when one pK_a is dominant. Otherwise it was determined using a more rigorous solution of simultaneous equilibrium (as discussed in Section 5.2.1).
g. The pH was calculated for the lowest solute concentration used to measure partition coefficients.
h. The concentrations were consistently dilute (circa 1% (w/v)), but not reported. We have used a concentration of 1% (w/v) to calculate the pH and the fraction unionized.
i. We use the pK_a at 25C to calculate the pH since the temperature was not reported.

6.9. Appendix 6B: Documentation on Partition Coefficients

This appendix contains specific information about the stratum corneum-water partition coefficients included in the database. Details are arranged alphabetically by the last name of the leading author of the investigation.

Anderson et al., 1989

Stratum corneum-water partition coefficients for 5 chemicals (α-(4-hydroxyphenyl) acetamide, 4-hydroxybenzyl alcohol, 4-hydroxyphenylacetic acid, methyl 4-hydroxyphenylacetate, p-cresol) were reported in this paper. The compound 4-hydroxyphenylacetic acid was more than 10% unionized in the vehicle (pH = 4.0), so this measurement is listed in the provisional database. The other compounds were unionized. The time for equilibration was not specified. We assumed that partition coefficients were reported on a mass-of-dry-SC basis, since the paper they referenced for experimental procedure (Raykar *et al.*, 1988) used a dry SC basis.

Anderson et al., 1976

Stratum corneum-water partition coefficients for various phenolics were reported in this paper. Mean partition coefficients, based on measurements in up to 4 SC samples, were selected from Table 3. Partition coefficients at other temperatures (12.6 - 34.5°C) were reported but are not incorporated into this analysis since temperature (over this range) appeared to have little influence on SC-water partitioning. The concentration used was low (frequently about 1% w/v or 1g/100mL) but was not reported. We have used a concentration of 1% w/v to calculate the natural pH attained and to calculate the fraction of the various phenols unionized. This is consistent with the analysis of Chapter 5. We chose these values over those reported by Roberts and colleagues (Roberts *et al.*, 1975) because those values are likely averages of less measurements.

Barry et al., 1985

Partition coefficients for three chemicals (mannitol, hydrocortisone, progesterone) were reported. The partition coefficients were taken directly from Table 1. Coefficients are reported relative to the mass of dry stratum corneum. The equilibration time was 14-days, and the skin was likely altered by this excessive amount of contact with aqueous solution. The compounds were not ionized in the vehicle.

Blank and McAuliffe, 1985

The partition coefficient of benzene was reported. The value we included was taken from Table II (water vehicle). The partition coefficient is based on the dry weight of the stratum corneum. It was suggested that enough time was allowed for equilibrium to establish (but exact time was not provided). Benzene was unionized in the vehicle.

Bronaugh and Congdon, 1984

SC-water partition coefficients for o-phenylenediamine, 2-nitro-p-phenylenediamine, and 4-amino-2-nitrophenol were taken from Table I. The partition coefficients reported by the authors (given on the basis of hydrated SC volume) are the measured partition coefficients (given on the basis of dry SC) multiplied by a density, 1.32 grams of dry SC per mL of dry SC. Adjusted permeability coefficients which we report reflect a removal of the 1.32g/mL correction applied by Bronaugh and Congdon and adjustment with the 0.25 conversion factor (going from a mass-dry to volume-hydrated basis) that was discussed in the body of this chapter. Unlike the permeation experiments by these authors, the pH was allowed to go to natural levels. The concentrations necessary for determining the natural pH and fraction unionized were not reported for 2-nitro-p-phenylenediamine nor 4-amino-2-nitrophenol, however if the starting pH was near 7, ionization will change the pH so that $f_{ui} = 1$. The concentration used in the measurement for o-phenylenediamine was reported in Table II. In Table 6A.5 we list a calculated pH of 7.6 for this measurement. The equilibration time for these measurements was 48 hours.

Bronaugh et al., 1981

The SC-water partition coefficient of N-nitrosodiethanolamine was taken from Table I. The partition coefficient is reported on a basis of dry mass of SC. The time allowed for equilibration was four days. N-nitrosodiethanolamine was unionized in the vehicle.

Cornwell and Barry, 1994

SC-water partition coefficients were taken from Table 4 as the average of five control (i.e., no penetration enhancer added) measurements. These coefficients are on a basis of (moles/hydrated mass)/(moles/volume of solution) and required adjustment. The equilibration time was 24 hours. The pH was calculated based on pK_a values from

SPARC and using a concentration of 10mg/mL. This measurement is listed in the provisional database.

Hui et al., 1995

The SC-water partition coefficients for sieved powdered human stratum corneum trimmed from plantar calluses were digitized from Figure 4. The partition coefficients were reported relative to the dry mass of the stratum corneum (personal communication, Wester, 1995). Several of these compounds (dopamine, glycine, urea, glyphosate, theophylline, aminopyrine, 2,4-D, and alachlor) are partially ionized at the natural pH which were reported in Table 2. Equilibration was attained over 24 hours. These measurements are contained in the excluded database

Kubota and Maibach, 1993

The SC-water partition coefficients of betamethasone (BMS) and BMS-17-valerate were calculated as the linear regression slope (zero intercept forced) of the equilibrium SC (y-axis) and aqueous solution concentrations (x-axis) from Figures 3a and 3b. The individual data were digitized. Both compounds were unionized in the vehicle (pH = 4.5). Equilibrium was attained over 72 hours. The partition coefficients were reported on a basis of dry SC mass.

Megrab et al., 1995

The β-estradiol SC-water partition coefficient was digitized from Figure 2. The pH came to natural levels and estradiol did not dissociate. The equilibration time was 48 hours. Partition coefficients were reported on the basis of hydrated SC mass.

Parry et al., 1990

The SC-water partition coefficient for benzoic acid, reported the Results and Discussion section, is already based on the unionized fraction of benzoic acid. The partition coefficient was reported on the basis of hydrated SC volume (using the ratio mass of dry stratum corneum to hydrated stratum corneum volume), and does not require further adjustment. The time allowed for equilibration was 24 hours.

Raykar et al., 1988

The partition coefficients were taken directly from Table IV (labeled there as intrinsic partition coefficients); average partition coefficients (calculated as the sample size-weighted average for measurements made with skin with different lipid contents) were reported for compounds 1i-1k (methyl-hydrocortisone-21-yl-pimelate, hydrocortisone-21-yl-hexanoate, hydrocortisone-21-yl-octanoate). The partition coefficients are reported on the basis of dry mass of SC. Hydrocortisone-21-yl-hemisuccinate (1d) [measurement at pH = 5.5 used], and hydrocortisone-21-yl-hemipimelate (1e) [measurement at pH = 4.0 used] were partially ionized (i.e., $f_{ui} < 0.9$), so these measurements are included in the provisional database. The remaining nine chemicals were unionized. The time allowed for equilibration was 48-72 hours.

Roberts, 1976

Stratum corneum-water partition coefficients for three compounds (benzyl alcohol, phenethyl alcohol, and methyl paraben) were reported in Roberts thesis but were not reported in the paper by Anderson and colleagues (Anderson *et al.*, 1976). Although we do not have the thesis, personal communication with Roberts (Roberts, 1996a) has revealed that these measurements were made by using the procedure reported by Anderson and colleagues (Anderson *et al.*, 1976). Table 4.10 of Roberts thesis contains the benzyl alcohol, phenethyl alcohol, and methyl hydroxybenzoate partition coefficients in addition to the 18 partition coefficients published by Anderson and colleagues (Anderson *et al.*, 1976). We have assumed that, like the data reported by Anderson *et al.* (Anderson *et al.*, 1976), these 3 data points are on the basis of hydrated mass of SC. We also assume that equilibration times were comparable if not identical to those in Anderson *et al.* (Anderson *et al.*, 1976).

Roberts et al., 1975

Stratum corneum-water partition coefficients for seven chemicals (benzyl alcohol, phenethyl alcohol, phenol, p-cresol, m-cresol, o-cresol, p-bromophenol) were reported. None of these values were included in the database because the measurements are probably included in the results by Anderson and colleagues (Anderson *et al.*, 1976).

Saket et al., 1985

Partition coefficients for hydrocortisone and five esters (hydrocortisone, hydrocortisone acetate, hydrocortisone propionate, hydrocortisone valerate, hydrocortisone hexanoate, hydrocortisone octanoate), and cortisone and four esters

(cortisone acetate, cortisone butyrate, cortisone hexanoate, cortisone octanoate) were reported. The partition coefficient of hydrocortisone at 37°C was taken from Table 1 (abdominal skin). All other partition coefficients (at 25°C for abdominal skin) were taken from Table 2. According to one of the authors, I.W. Kellaway, the partition coefficients are based on the dry weight of stratum corneum, and equilibrium was established over 48-hours (Kellaway, 1996). All chemicals were unionized in the vehicle.

Sato et al., 1991

A partition coefficient for nicorandil into human plantar callus is shown in Figure 3. The digitized partition coefficient from this figure is not meaningfully different from the previously determined partition coefficient (Sato *et al.*, 1989) and may in fact be the same measurement reported again. They do not give evidence in the publication that supports the idea that new partition coefficient measurements were made (the skin source and experimental procedure are identical to the previous (1989) study). We have only included the measurement of the previous investigation (Sato *et al.*, 1989) for that reason.

Sato et al., 1989

The partition coefficient for nicorandil is reported in Table 4. The skin was plantar callus, which probably has more proteinaceous material than abdominal, thigh or dorsal stratum corneum, and consequently may have more hydrophilic partitioning properties. The partition coefficient was reported on the basis of dry SC mass. Twenty-four hours were allowed for the partitioning process to equilibrate. Nicorandil was unionized at the basic pH attained by the 0.05 (w/v)% solution used in these studies. The partition coefficient is listed in the excluded database since plantar callus was used.

Scheuplein et al., 1969

SC-water partition coefficients for several steroids were taken directly from Table I. These compounds do not ionized. Equilibration time was not provided. The basis was dry SC mass. The measurement for hydroxypregnenolone is included in the provisional database because we have not determined the structure and properties of this compound.

Scheuplein and Blank, 1973

SC-water partition coefficients for water and the normal alcohols (methanol through octanol) were taken directly from Table I. These compounds do not ionize. The time of equilibration was not provided. The basis was dry SC mass.

Smith and Anderson, 1995

The SC-water partition coefficient for the unionized form of lauric acid was reported in the text as 5060 ± 880. This result is based on measurements which were made at pH = 3.2, 5.5, and 7.9. The equilibration time is between 24 and 48 hours. The measurements is based on the dry mass of SC. The authors adjusted the measurements by a factor of 1g/mL to put them on a volume basis. In the database we report the partition coefficients on a dry mass SC basis and adjust it with the standard conversion factor. We will interpret the partition coefficient as having a dry-mass SC basis and adjust it with a more realistic conversion factor. They report an estimated SC-water partition coefficient for ionized lauric acid, 72.2, which is approximately 70 times smaller than for the unionized form.

Surber et al., 1992

Twenty-two stratum corneum-water partition coefficients are analyzed in this paper. Six of these chemicals (acetamidophenol, cyanophenol, DTT, iodophenol, PCB, and pentyloxyphenol) appeared first in Surber *et al.* (Surber *et al.*, 1990b), seven others (acitretin, caffeine, diazepam, estradiol, hydrocortisone, progesterone, and testosterone) appeared first in Surber *et al.* (Surber *et al.*, 1990a). Partition coefficients for nine additional chemicals (atrazine, benz[a]pyrene, chloramphenicol, cyclosporin-A, 5-fluorouracil, ibuprofen, indomethazine, lauric acid, and uracil) are presented for the first time in this paper. Through personal communication with Surber (1996): (1) we obtained exact values for the data plotted in Figure 3, (2) we learned that the partition coefficients are all based on the dry mass of stratum corneum, (3) we were informed that no buffer was used to control the solution pH, and (4) that compound #18 in Figure 3 is PCB. According to the authors, enough time was allowed for equilibrium to be established although the exact time was not reported (Surber *et al.*, 1992). The mean partition coefficients obtained from Surber and those shown in Figure 3 are averages of measurements made at different concentrations. 5-Fluorouracil and cyclosporin-A, were ionized in the vehicle solution regardless of their concentrations (which were not specified). For several other compounds attaining a natural pH in solution (i.e., ibuprofen, indomethazine, lauric acid, and uracil), the fraction unionized (f_{ui}), depends

upon the unreported concentration in the vehicle. Through personal communication with Surber (1996), we learned that the compounds studied in this investigation were measured using radiolabeled drug to give final concentrations about 5 million cpm/mL water or isopropyl myristate since some compounds would destroy the SC at higher concentrations (personal communication, Surber, 1996). These measurements (except for cyclosporin-A which was excluded for other reasons) are placed in the provisional database. Surber (1996), additionally confirmed that the partition coefficients for PCB and DDT shown in Figure 3 (and the actual values sent to us by Surber) were interchanged from the correct values presented in the earlier paper (Surber *et al.*, 1990b).

Surber et al., 1990a

Partition coefficients were reported for seven chemicals (acitretin, progesterone, testosterone, diazepam, estradiol, hydrocortisone, and caffeine). Mean partition coefficients for the unionized compounds (progesterone, testosterone, diazepam, estradiol, and hydrocortisone) were taken from Table IV. SC-water partition coefficients for acitretin and caffeine, also from Table IV, were placed in the provisional database, since the concentrations of these compounds (attaining a natural pH) were not reported and f_{ui} could not be determined. All partition coefficients are reported on a dry-mass SC basis. Equilibrium was demonstrated by unchanging vehicle concentrations. The time required ranged from 6-hours (for progesterone, testosterone, estradiol, caffeine, hydrocortisone, diazepam) to 24-hours (for acitretin).

Surber et al., 1990b

Partition coefficients were determined for six chemicals (4-acetamidophenol, 4-cyanophenol, 4-iodophenol, 4-pentyloxyphenol, PCB, DDT). Since all compounds were essentially unionized even at the most dilute concentration, measurements made at different concentrations could legitimately be combined, and, partition coefficients for all species were taken directly from Table 6. The partition coefficients are based on the mass of dry SC. Six to twenty-four hours were allowed for the chemical to partition between stratum corneum and the vehicle. The chemicals were all essentially unionized in the vehicle. The measurement for PCB is listed in the excluded database because it was measured with a polydispersed mixture rather than a single compound.

Wester et al., 1987

The SC-water partition coefficients for sieved powdered human stratum corneum, from plantar callus, were reported in Table 2. The partition coefficients are reported relative to the dry mass of the stratum corneum (personal communication, Wester, 1995).

Benzene and PCB (54% chlorine) were unionized but 4-nitroaniline, at a concentration of 4.9μg/mL, was partially ionized at its natural pH. The solution-SC contact time in these experiments was only 30 min. In this powdered form, the SC may equilibrate more rapidly than in its membrane form. However, the authors did not experimentally establish that 30 min was adequate. These measurements are contained in the excluded database.

Williams and Barry, 1991

The SC-water partition coefficient of saturated 5-fluorouracil was taken from the control value in Table II. The equilibration time was four hours. At the natural pH for saturated 5-fluorouracil at 20°C, 5-fluorouracil exists as a zwitterion. The partition coefficient is based on the mass of hydrated SC. The measurement is listed in the provisional database.

7. PERMEABILITY COMPARISONS WITH ANIMAL DATA

7.1. Introduction

Excised animal skin has been used extensively to study percutaneous absorption of drugs and other solutes because of its availability. Percutaneous absorption of toxic compounds can be measured satisfactorily with excised skin (*in vitro*), but adequate supplies of human skin are often unavailable, or when available have often been stored for lengthy periods, which can alter its barrier function. Except for a few animals (such as primates), it is usually possible to obtain animal skin which is excised shortly before use. Although we consider only *in vitro* results here, it is worth mentioning that *in vivo* experiments are usually restricted to animals. The toxicity of many compounds limits their testing in humans *in vivo* as does the cost and time required for approvals and experiments.

The relevance of permeability coefficients measured in animals to dermal absorption in humans can be examined by histologic or with empirical comparisons. For example, when certain physiological (e.g., skin morphology, thickness, capillary perfusion) and chemical (e.g., lipid composition, enzymes, water content) factors of a certain animals skin are similar to human skin, that animal is considered more relevant. For instance, the skin of rodents lacks sweat glands and abounds in hair and hair follicles (not true in the hairless and nude strains), while human skin does not, making rodents potentially less relevant than other animals possessing sweat glands and less hair. Usually the higher terrestrial mammals (e.g., the rhesus monkey) have skin that is physiologically and chemically more similar to human skin than non-mammals (e.g., the

black rat snake). However, some researchers have found that snake skin, though chemically and structurally quite different from terrestrial mammalian skin (Rigg and Barry, 1990), reproduces human skin measured permeability coefficients quite closely (Itoh *et al.*, 1990a; Itoh *et al.*, 1990b; Takahashi *et al.*, 1993). This demonstrates that assessing the relevancy of an animal skin purely from the histological approach can be misleading.

Alternatively, an animal skin can be judged as more relevant when permeability coefficients measured in animal skins compare meaningfully with those measured in human skin. By this standard, the skin of a relevant substitute animal must behave at least qualitatively, if not quantitatively, like human skin (Durrheim *et al.*, 1980). In practice both measures of relevance should be used in combination to infer human absorption with animal skin measurements. The empirical method is most useful for establishing quantitative relationships for permeability coefficients from animal and humans skins, while histologic comparisons are useful for anticipating potential departures from these relationships.

The relationships between animal and human permeability coefficients has been studied from the histological (Bronaugh *et al.*, 1982) and empirical (Bronaugh *et al.*, 1982; Marzulli *et al.*, 1969; Wester and Noonan, 1980) perspectives. Most investigations report that the skin of animals is different, usually more permeable, than human skin. In Appendix C, we review nine prior investigations or reviews of the relationship of permeability coefficients for animal and human skins. With one exception (Sato *et al.*, 1989), none of these studies recommends a quantitative approach for adjusting permeability coefficients from animal skins to represent human skin. Several investigations present relative rankings of permeability coefficients observed for several different animal species (among others, (Marzulli *et al.*, 1969; Wester and Noonan, 1980)). These rankings agree qualitatively (that many other species are more permeable

than human skin) but the relative order of species and the quantitative results are often inconsistent. Many rankings are derived from permeability coefficients measured *in vivo*, from different vehicles, at different temperatures, with compounds at different extents of ionization, using skin from different locations. That is, there are a number of reasons for differences in these permeability coefficients that are external to the fact that they were measured in different species. Significantly, these investigations are based on permeability coefficients for only a few chemicals. As shown in Chapter 5, permeability coefficients measured in human skin by different laboratories for the same chemical can differ by an order of magnitude or more. Thus, the experimental uncertainties in the measurement are so large that conclusions drawn from only a few chemicals may be meaningless.

The nine studies reviewed in Appendix C (and others like them) utilize only a small fraction of the permeability coefficient data available for animal skins. It should be possible to improve our understanding of the relationships between permeability coefficients measured in animal and human skins by utilizing many more of the available measurements and by restricting comparisons of skins from different species to data from a standardized experiment and analysis. In Chapter 5, we listed a large set of permeability coefficient data all measured in human skin using a single type of experiment and meeting certain quality criteria. Specifically, all data in the fully-validated database of Chapter 5 are *in vitro* measurements on human skin from aqueous solutions for which (1) the temperature was specified and between 20 - 40°C, (2) the penetrating chemical was at least 10% unionized with a known validated or calculable K_{ow}, and (3) the calculated permeability coefficients probably represent the steady-state value. Here, we describe several new databases of measurements from animal skins which meet these same criteria. The goal is to use these databases of permeability coefficients for human and animal skins from comparable experiments to identify the

similarities and differences in the penetration of chemicals in human, mammalian, and other (primarily shed snake) skins.

7.2. Theory

The steady-state permeability across the stratum corneum (SC) from an aqueous vehicle (P_{cw}) into an infinite sink depends on the diffusivity of the chemical in the SC (D_c), the SC thickness (L_c), and the equilibrium partition coefficient between the SC and the water vehicle (K_{cw}) as given below:

$$P_{cw} = \frac{K_{cw} D_c}{L_c} \tag{7.1}$$

where K_{cw} is defined as the concentration of chemical in the SC (mass/volume of SC at absorbing conditions) divided by the equilibrium concentration in the vehicle (mass/volume) (Parry *et al.*, 1990). As defined here, D_c is an effective diffusivity based on the SC thickness rather than the true diffusivity based on the actual molecular diffusion path length, which is not known.

Equation (7.1) is the basis for several common semi-theoretical models of SC permeability coefficients (Potts and Guy, 1992). In many of these, the SC-water partition coefficient is assumed to be related to the octanol-water partition coefficient through a power function:

$$K_{cw} = K_{ow}^{g} \tag{7.2}$$

where the exponent (g) accounts for differences in lipophilic character of the SC lipids (Potts and Guy, 1992). However, for many organic solvents, the relationship is offset by a constant, and an equation of the form,

$$K_{cw} = a \cdot K_{ow}{}^{f} \tag{7.3}$$

or

$$\log K_{cw} = \log(a) + f \cdot \log K_{ow} \tag{7.4}$$

is more appropriate (Lyman *et al.*, 1982). The diffusion of small molecules in membranes like skin is generally considered to be an activated process which varies exponentially with the size of the penetrant:

$$D_c = D_o \exp(-\beta \cdot MV) \tag{7.5}$$

where D_o is the diffusion coefficient of a hypothetical molecule having zero molecular volume (MV), and β is a constant. The MV is often approximated with molecular weight (MW). Substituting Eqs. (7.3) and (7.5) into Eq. (7.1) followed by logarithmic transformation and rearrangement, yields:

$$\log P_{cw} = \left[\log(a) + \log(D_o / L_c)\right] + f \cdot \log K_{ow} + \beta^{\#} \cdot MW \tag{7.6}$$

where $\beta^{\#} = \beta \log(e)$. Thus, linear regressions of logarithmically transformed skin permeability measurements with Eq. (7.6) will provide values for $[\log(a) + \log(D_o / L_c)]$, f, and $\beta^{\#}$ which have attributable physicochemical meaning. Relationships between permeability coefficients measured in human and animal skins can be deduced by comparing regressions of Eq. (7.6) from different animal skins.

Occasionally, permeability coefficients have been measured in the skin of different species for the same compound. In this case, the ratio of the animal and human skin permeability coefficients can be modeled with an equation of the form:

$$\log\left(\frac{P_{cw,animal}}{P_{cw,human}}\right) = \Delta\left[\log(a) + \log(D_o / L_c)\right] + \Delta f \cdot \log K_{ow} + \Delta\beta^{\#} \cdot MW \tag{7.7}$$

Linear regression of logarithmically transformed skin permeability coefficient ratios with Eq. (7.7) will provide values for $\Delta[\log(a) + \log(D_o / L_c)]$, Δf, and $\Delta\beta^{\#}$ which have attributable physicochemical meaning and will allow for relationships with human permeability coefficients to be developed.

Permeability coefficients may be affected by chemical properties other than $\log K_{ow}$ and MW. For example, steric interactions, limitations of MW as a representation

of molecular size, and unidentified factors that cause some compounds to act as penetration enhancers may affect permeability coefficients but would not be conveyed entirely by $\log K_{ow}$ and MW. Equation (7.6) will not account for these additional influences on permeability coefficients. Equation (7.7) will also not account for these additional influences, but, by directly ratioing animal to human permeability coefficients, the error may be reduced. Equation (7.6) implicitly assumes that permeability coefficients are only effected by $\log K_{ow}$ and MW, which, is a more restrictive assumption than the assumption built into Eq. (7.7) that all unexplored factors have quantitatively the same effect in all species. That is, with the same amount of data, analysis with Eq. (7.7) is preferable to analysis with Eq. (7.6) as long as these additional factors influence permeability coefficients the same way in both human and animal skins.

7.3. Database Collection and Validation

All permeability coefficients collected in this chapter were measured *in vitro* through animal skin from aqueous vehicles. Prior to developing correlations, a critical review process was used to remove data which were unable to meet a set of quality criteria. Permeability coefficients reported without pertinent details are reserved for future analysis, allowing time for contact with the authors, but are excluded for purposes of data regressions described later. Two collectively exhaustive (taken together they contain all permeability measurements we have considered) and mutually exclusive (measurements appearing in one database do not appear in others) databases are presented: (1) the fully-validated database (containing the measurements used to develop correlations), and (2) an excluded database (permeability coefficients do not meet the validation criteria). Details about how the permeability coefficients were extracted from the original references are described in Appendix 7B.

The fully-validated database contains only meaningful permeability coefficients. Five criteria were used to validate this database: (1) the temperature must be expected to

be in the range 20-40°C, (2) more than 10% of the penetrating compound must be in an unionized form, (3) a valid $\log K_{ow}$ must be available for the penetrating compound (obtained from the Starlist of Hansch and colleagues (Hansch *et al.*, 1995) or else calculated consistently with this database using Daylight software (PCModels, 1995)), (4) the measurement must have been determined with data that reasonably approximates steady state, and (5) the donor and receptor fluids do not compromise (more than water does) the barrier of the skin.

Permeability coefficients were adjusted for ionization using the procedure developed in Chapter 5. We assume that penetration is attributed to the unionized species alone and divide the observed permeability coefficients ($P_{cw,obs}$, based on the total concentration) by the fraction unionized (f_{ui}). That is, $P_{cw} = P_{cw,obs}/f_{ui}$ where f_{ui} is calculated from the pH and the acid dissociation constant (pK_a) as given by:

$$f_{ui} = \frac{1}{\left(1+10^{g}\right)} \tag{7.8}$$

where the exponent, $g = (pH - pK_a)$ for acids and $(pK_a - pH)$ for bases. SPARC [SPARC Performs Automated Reasoning in Chemistry] (SPARC, 1995) was used to calculate pK_a values at 25°C. We adjusted all pK_a values for temperature by using an integrated form of the van't Hoff equation (Smith and Van Ness, 1987) with enthalpies of ionization (defined for the direction of the pK_a) from (Sober, 1968): 0.0 for carboxylic acids, 5.0 for phenolic compounds, 10 for amines (primary, secondary, or tertiary), 7.5 for aniline (or amines attached directly to an aromatic ring system), 5 for aromatic nitrogen (pyridine derivatives and isoquinoline). Multiple pK_a values for the same molecule were adjusted independently for effects of temperature. When more than one pK_a was important the fraction unionized was determined by writing dissociation reactions for all relevant species.

When buffer solutions were not used and the pH was not reported, we calculated the pH using the solution concentration, pK_a values for all dissociation reactions, and assuming that the pH was 7.0 prior to solute addition. A general treatment of simultaneous equilibrium, involving equations for all linearly-independent reactions, the water dissociation reaction ($K_a = 1.0x10^{-14}$), a molecular balance on the active species, and an equation requiring solution electroneutrality, is required to calculate the natural pH (Brescia *et al.*, 1975). A more detailed discussion of the adjustment for ionization and associated calculations is provided in Chapter 5.

7.4. Results and Discussion

7.4.1. Inspection of the Data

The originally reported permeability coefficients, P_{cw} (rep.), the ionization-adjusted coefficients, P_{cw} (adj.), $logK_{ow}$, MW, and other experimental details (temperature, pH, skin type, and animal strain) are collected in tables in Appendix 7A. Tables 7A.1-7A.4 list data from hairless mouse, hairless rat, rat, and shed snake skin, respectively. Table 7A.5 contains permeability coefficients measured in assorted animal skins (dog, guinea pig, marmoset, mouse, nude mouse, nude rat, pig, rabbit). The excluded measurements are labeled with an E (for excluded) in the left hand margin, identifying them as failing one or more of the validation criteria. Several excluded measurements have the potential for validation provided that additional relevant information can be obtained from the investigators. Ionized species are identified by the different formal charges using (-) to represent each formal negative charge and (+) to represent each formal positive charge. For example, alanine is represented as (+-) to designate that it has one formal positive charge and one formal negative charge. The identifications are consistent with pK_a values calculated by SPARC and may not be consistent with the reported formal charges on the chemical. Adjustment of the pK_a

values for temperature, calculation of the naturally attained pH (when a pH was not specified), and calculation of the fraction of the compound that was unionized are summarized in Table 7A.7. Table 7A.6 contains stratum corneum-water partition coefficients measured in animal skins. While useful, the data listed in Table 7A.6 were not one of the main objectives of this thesis and this table will not be discussed further.

Figures 7.1-7.6 show how skin permeability coefficients measured in hairless mouse, hairless rat, rat, shed snake, and the lesser-studied animals (guinea pig, marmoset, rabbit, pig, dog, mouse, nude rat) relate with $\log K_{ow}$. In all figures, compounds which are more than 90% ionized are identified by the form of the dominant ionic species (i.e., cation, anion, or zwitterion). All data identified by its ionic species are excluded. Measurements excluded for reasons other than ionization are identified separately. Permeability coefficients for hairless mouse, hairless rat, rat, and shed snake skin are shown in Figures 7.1-7.4, respectively. Figures 7.5 and 7.6 show permeability coefficients measured in several animals which have been studied less (dog, guinea pig, marmoset, mouse, nude mouse, nude rat, pig, and rabbit). Figure 7.5 shows permeability coefficients identified by animal species and including validated and excluded measurements. Figure 7.6 shows the data from Figure 7.5 with the validated and excluded data points identified. Ionized species with undetermined $\log K_{ow}$ are plotted to the left of the vertical line at $\log K_{ow} = -6.0$ (cations at $\log K_{ow} = -6.5$, anions at -7.0, and zwitterions at -7.5). Permeability coefficients that are referenced in the text or excluded for reasons other than ionization are identified on these plots.

Figure 7.1 shows 144 permeability coefficients for 82 compounds (84 fully-validated and 60 excluded data points) measured in hairless mouse skin (Table 7A.1). All of the measurements excluded from this database were more than 90% ionized. Etorphine is distinguished on this figure because, in Chapter 5, we used the fact that the human permeability coefficient is larger than the hairless mouse permeability coefficient

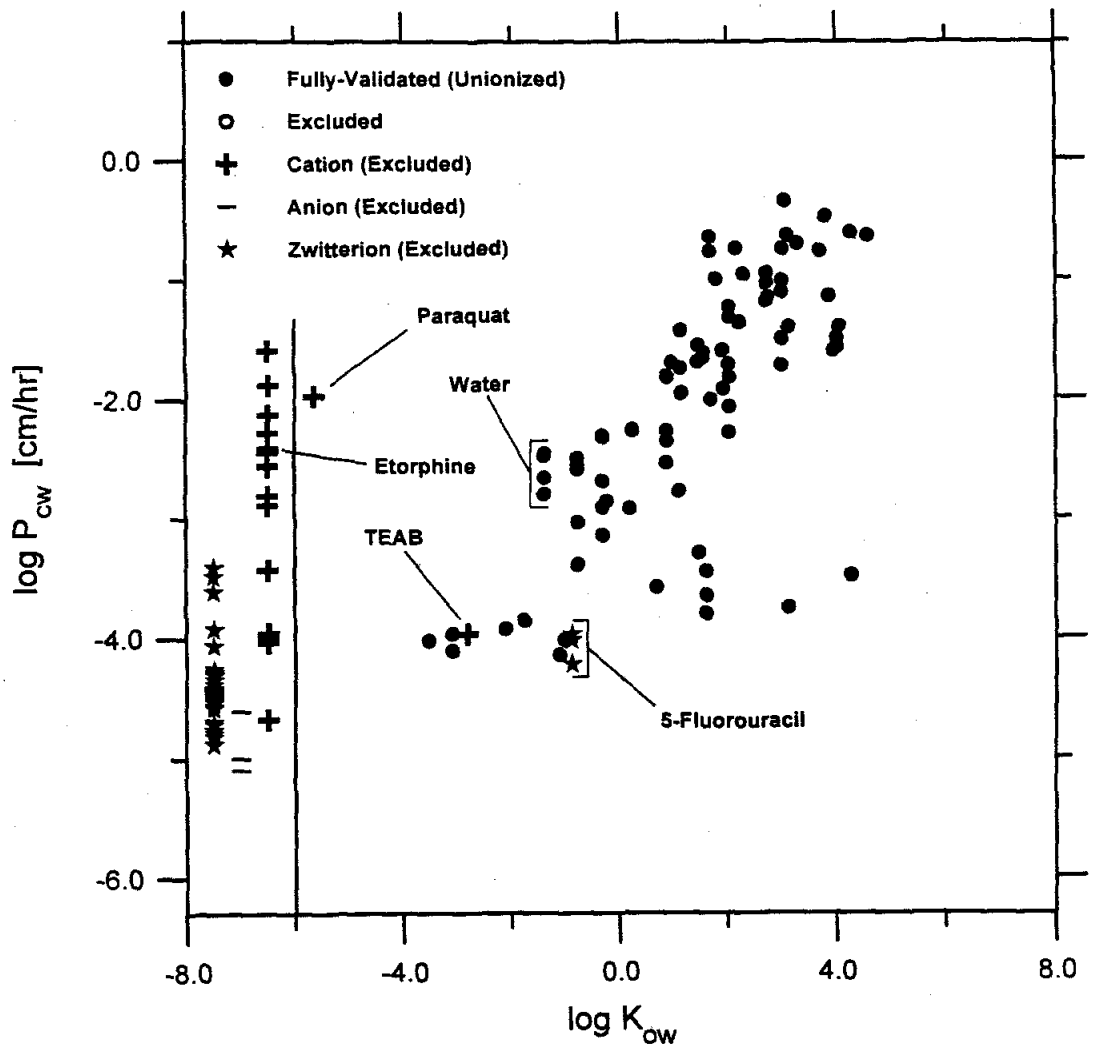

Figure 7.1 Permeability coefficients from hairless mouse as a function of K_{ow}. Ionic species without an appropriate $\log K_{ow}$ are plotted to the left of all other measurements at an assigned $\log K_{ow}$ (cations at -6.5, anions at -7.0, and zwitterions at -7.5).

to support our exclusion of that measurement from the human skin database. Notice that the hairless mouse permeability coefficient of etorphine is consistent with other measurements for cations, which was not the case with the human permeability coefficient for etorphine.

The permeability coefficients shown in this plot span approximately four orders of magnitude. Notice that $\log P_{cw}$ varies almost linearly with $\log K_{ow}$ and, as we will soon show, a portion of the variance can be explained by differences in MW. The permeability coefficients for anionic and zwitterionic species are generally lower than for the unionized species. Permeability coefficients for cationic species are highly variable. Based on pK_a values calculated in SPARC, all but four of the forty permeability coefficients from the Ruland and Kreuter (Ruland and Kreuter, 1991) investigation of amino acids are shown as zwitterions (aspartic acid and glutamic acid (both at pH = 7.4) are shown as anions, and lysine and arginine (both at pH = 7.4) are shown as cations). Ruland and Kreuter concluded that charged and net neutral species permeate skin at the same rates (i.e., penetration rates were not statistically significantly different). However, with only four charged species to compare with 36 zwitterionic species to test their claim, this conclusion may not be general. Figures 7.1 and 5.2 are quite similar, suggesting that the underlying mechanism of dermal absorption is similar in both species. Several specific comparisons with the human permeability coefficients are noteworthy: (1) there are less extremely low (i.e., $\log P_{cw} < -4.0$) permeability coefficients in hairless mouse skin than in human skin, (2) both species have similar linear dependence on $\log K_{ow}$, (3) the largest permeability coefficient values are similar in both species ($-0.7 < \log P_{cw} < -0.9$), (4) the permeability coefficients of zwitterions are similar in magnitude in both species, (5) in both species, permeability coefficients for anions and zwitterions are lower than permeability coefficients for cations, (6) cations have highly variable rates of penetration, and (7) hairless mouse skin is more permeable to water than human skin.

Figure 7.2 shows 41 permeability coefficients for 33 different compounds (18 fully-validated and 23 excluded data points) measured in hairless rat skin (Table 7A.2). The database contains permeability coefficients for structurally diverse compounds, predominantly pharmaceutically active compounds, with varied chemical properties. Vinpocetine was excluded (1) because the fraction unionized can not be determined because the concentration was not reported, and (2) because K_{ow} could not be adequately calculated (vinpocetine has structural fragments that are not adequately represented in Daylight software). Vinpocetine is plotted at the calculated $\log K_{ow}$. Three additional permeability coefficients for macromolecular dextrans (fluorescein isothiocyanate-labeled dextrans) FD-4 (average MW = 4,400), FD-10 (average MW = 9,600), and FD-70 (average MW = 69,000) were also excluded (Ogiso *et al.*, 1994). These macromolecules probably penetrate through a pathway (e.g., appendageal structures like hair folicles and sweat glands) that is not built into the solution-diffusion model of permeability which we apply. Because K_{ow} were not obtained for these compounds they are plotted on the left hand axis (i.e., plotted at an arbitrarily assigned value of $\log K_{ow} = -8.0$). Permeability coefficients for these dextrans are lower but not significantly lower than those of highly ionized species. This may suggest that large molecules and highly polar molecules penetrate skin by the same pathway. Permeability coefficients for these three macromolecules decrease less with MW than the other much smaller chemicals in the database. This is consistent with penetration through a path which has a large enough free volume that these very large molecules are not size segregated. The permeability coefficients of water and 5-fluorouracil measured in hairless rat are similar but slightly larger than those measured in human skin. However, the permeability coefficient of paraquat measured in hairless rat is much larger than that measured in human skin.

Figure 7.3 shows 17 permeability coefficients for eleven compounds (14 fully-validated and three excluded data points) measured in rat skin (Table 7A.3). This

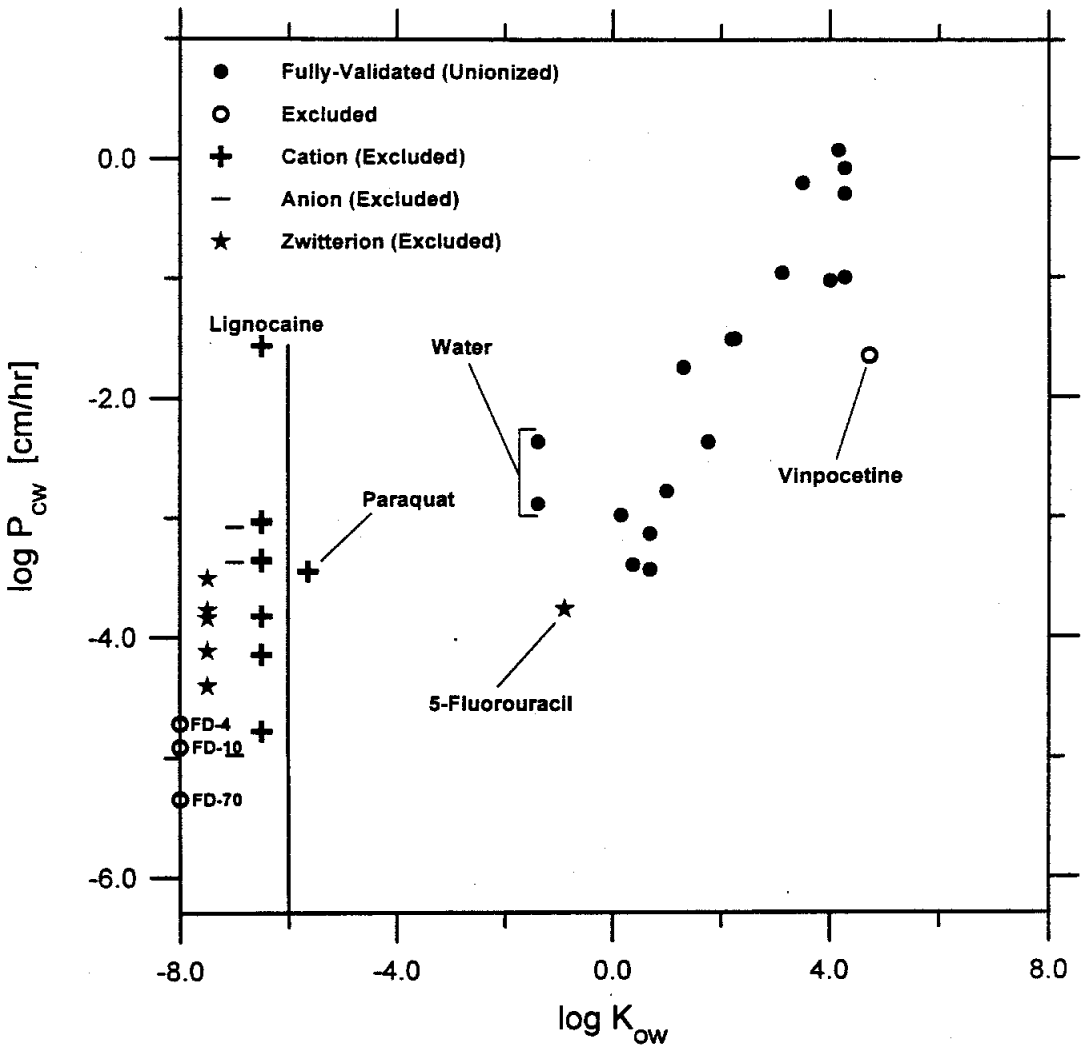

Figure 7.2 Permeability coefficients from hairless rat as a function of K_{ow}. Ionic species without an appropriate $\log K_{ow}$ are plotted to the left of all other measurements at an assigned $\log K_{ow}$ (cations at -6.5, anions at -7.0, and zwitterions at -7.5).

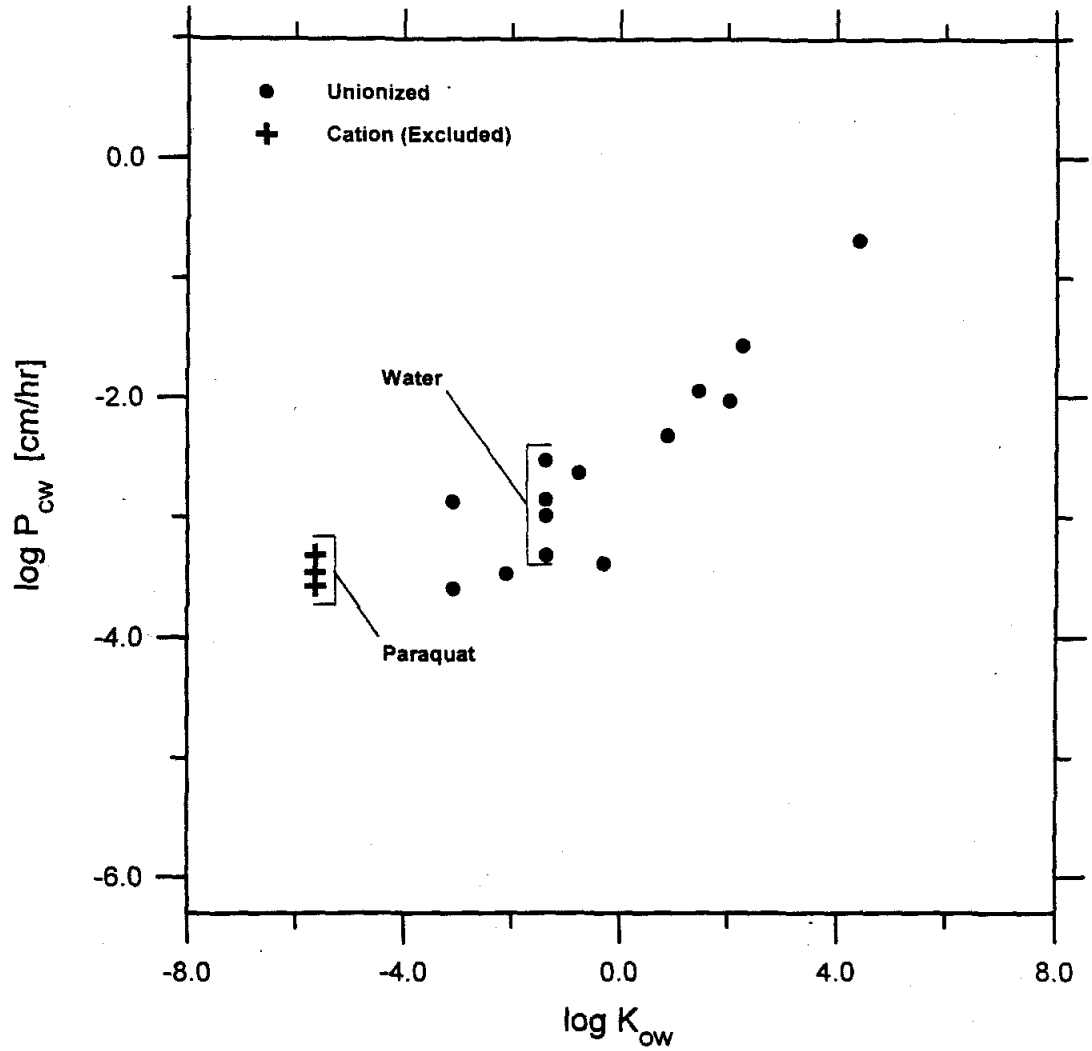

Figure 7.3 Permeability coefficients from rat as a function of K_{ow}.

database is small and consists mainly of phenols, alcohols and water. Because all chemicals in this database are of relatively low MW, $\log P_{cw}$ is more clearly linear with $\log K_{ow}$. Water permeability coefficients are very similar but slightly lower in rat skin than in human skin. However, permeability coefficients for paraquat are significantly higher in rats than in humans.

Figure 7.4 shows 37 permeability coefficients for 28 compounds (33 fully-validated and four excluded data points) measured in shed snake skin (Table 7A.4). Although the database is small, it is diverse and consists of compounds, predominantly pharmaceutically active compounds, spanning a wide range of molecular structures and properties. Three species of snake, elaphe obsoleta (black rat snake), python molurus, and python reticlatus, were used to make these permeability measurements. Elaphe obseleta was used most commonly. As shown, differences between permeability coefficients measured in the shed skin of different snake species appears to be minor. For example, the water permeability coefficients measured in elaphe obsoleta and python molurus skin are nearly identical. The permeability coefficients for 11α-hydroxyprogesterone have been excluded because the $\log K_{ow}$ calculated for this compound using Daylight (PCModels, 1995) are not valid. The data are plotted at the calculated $\log K_{ow} = 6.86$. It is possible that these permeability coefficients would not be significant outliers if a valid $\log K_{ow}$ were available. Permeability coefficients for water are very close but slightly higher in snake than in human and 5-fluorouracil is more permeable in snake than human.

Figures 7.5 and 7.6 show 38 permeability coefficients (24 fully-validated and 14 excluded datapoints) measured in seven other animal species (guinea pig, marmoset, rabbit, pig, dog, mouse, or nude rat) from Table 7A.5. Among the permeability coefficient measurements with pig skin, those made by Bhatti and colleagues (Bhatti *et al.*, 1988) were made on pig ear skin and those made by Sato et al. (Sato *et al.*, 1989)

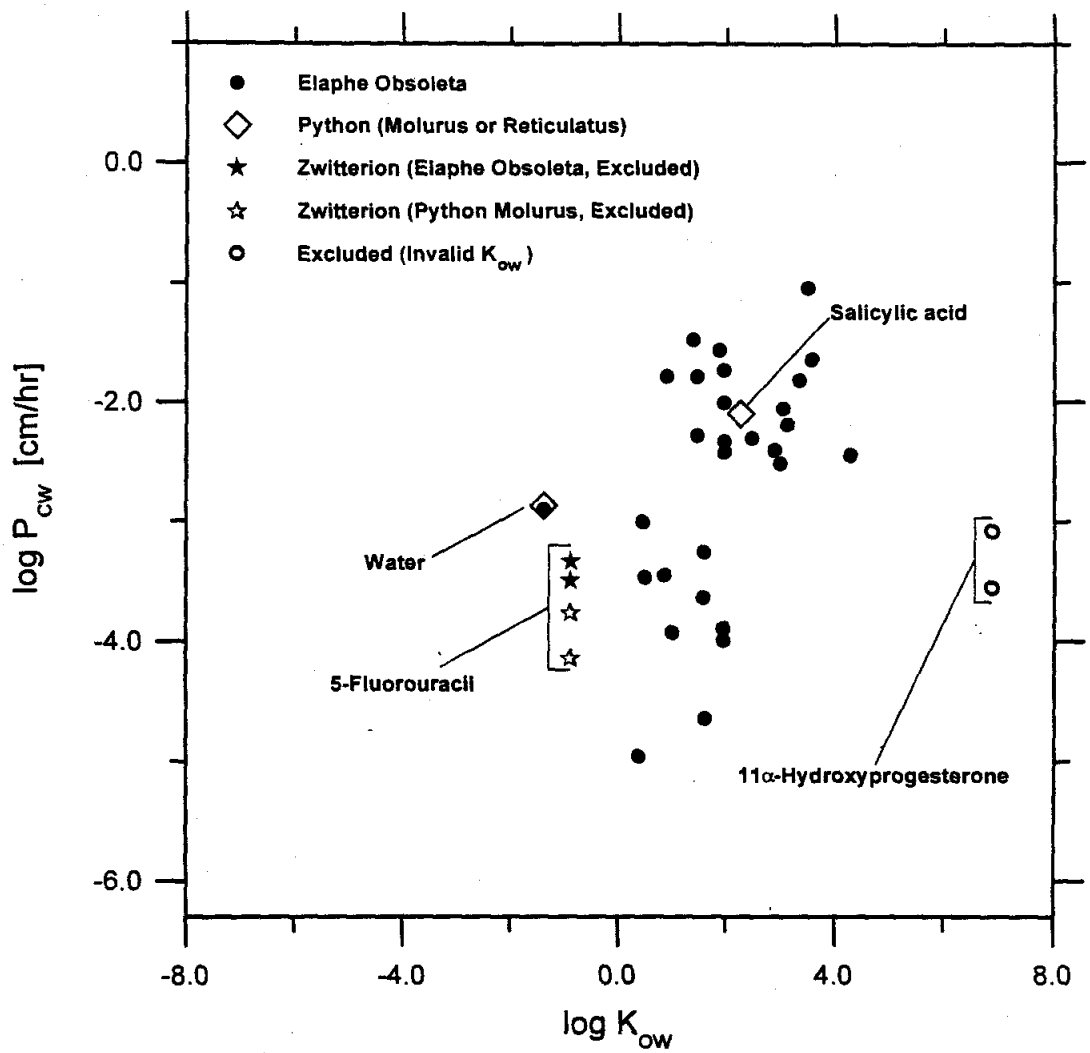

Figure 7.4 Permeability coefficients from shed snake skin as a function of K_{ow}.

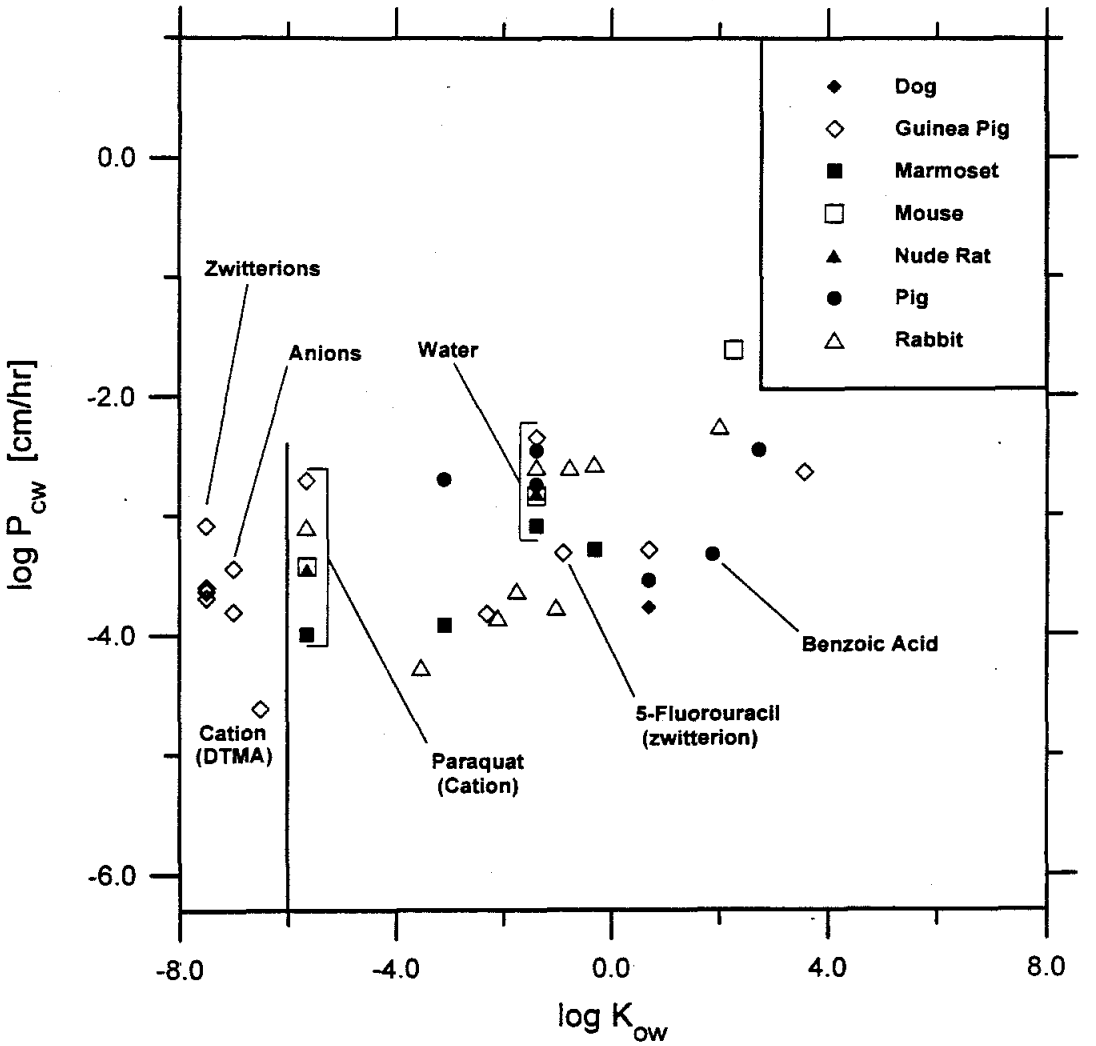

Figure 7.5 Permeability coefficients from various animal skins as a function of K_{ow} identified by animal species and including valid and excluded measurements. Ionic species without an appropriate $\log K_{ow}$ are plotted to the left of all other measurements at an assigned $\log K_{ow}$ (cations at -6.5, anions at -7.0, and zwitterions at -7.5).

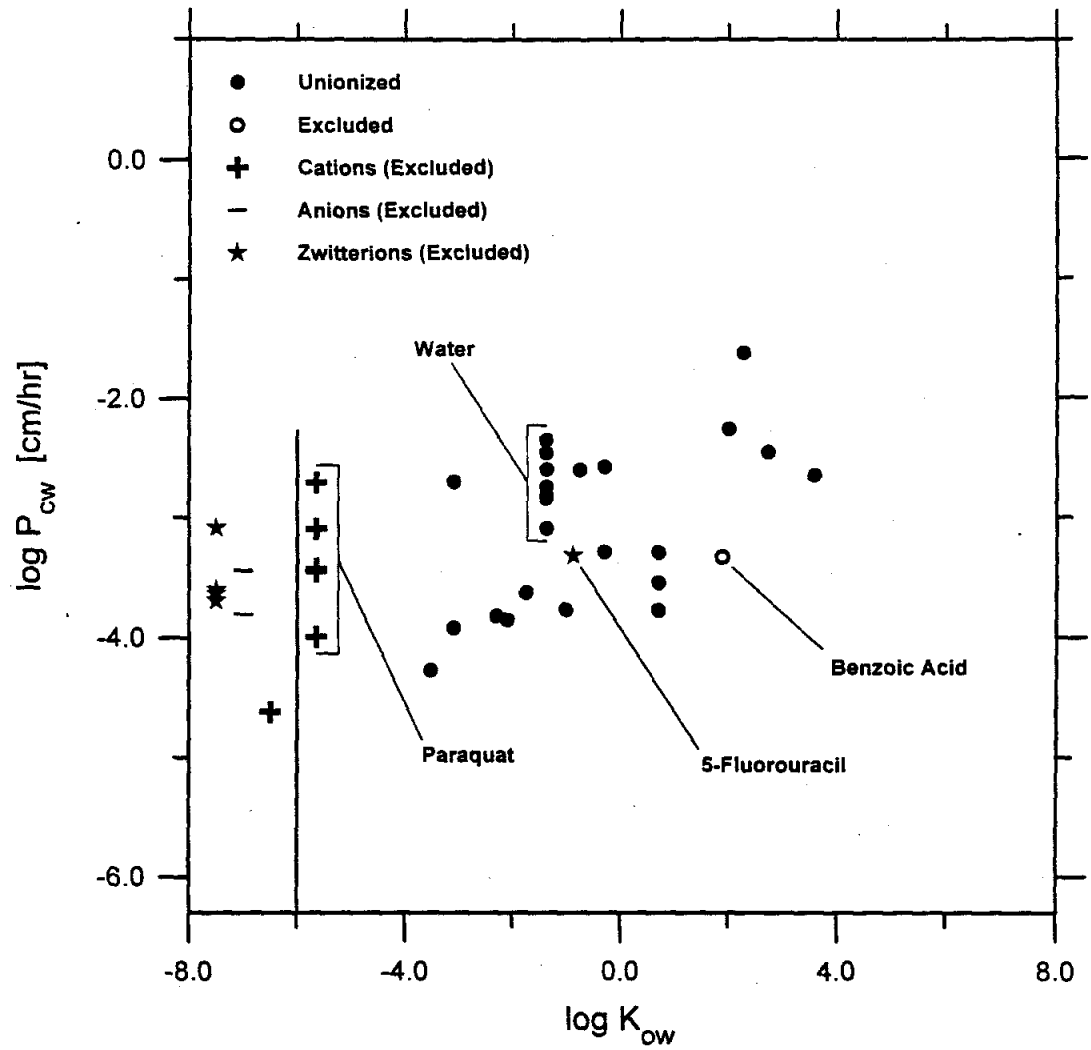

Figure 7.6 Permeability coefficients for various animal skins from Figure 7.5 as a function of K_{ow} with validated and excluded data points identified. Ionic species without an appropriate $\log K_{ow}$ are plotted to the left of all other measurements at an assigned $\log K_{ow}$ (cations at -6.5, anions at -7.0, and zwitterions at -7.5).

were made with skin from the backs of immature pigs. The measurement for benzoic acid was determined at an unknown level of ionization and was therefore excluded.

There is not enough data on any individual species in Figures 7.5 and 7.6 to develop empirical relationships with human permeability coefficients. However, relational analysis can be informative until more data is assembled. Among the compounds investigated in more than one species, some relationships in Figure 7.5 stand out: (1) for ethanol marmoset is less permeable than rabbit; (2) for mannitol marmoset is less permeable than pig; (3) for nicorandil the order of increasing permeability is dog, pig, guinea pig; (4) for paraquat the order of increasing permeability is marmoset, nude rat, mouse, rabbit, guinea pig; (5) for water the order of increasing permeability coefficients is marmoset, mouse, nude rat, rabbit, guinea pig with pig being somewhere between guinea pig and nude rat. Of probable interest is the close consistency between the orders of penetration observed for water and paraquat.

This database is not conducive to definitive analysis, since relationships may be strongly influenced by a single investigation (e.g., most of the replicate measurements were made by Walker *et al.* (Walker *et al.*, 1983) and all marmoset permeability coefficients were measured by Scott *et al.*, (Scott *et al.*, 1991)). More data are required before accurate conclusions can be made regarding relative rates of penetration in these animals and humans.

Figures 7.1-7.6 bear many similarities to Figure 5.2 which contains the human permeability coefficients. It is not surprising that skin from different terrestrial species have similar characteristics of dermal penetration. Several mechanistic trends are consistently observed and also make good chemical sense: (1) the permeability coefficient of lipophilic compounds clearly increases linearly with increasing $\log K_{ow}$ of the penetrant (2) hydrophilic compounds and ions (at least anions and zwitterions) penetrate through the SC with rates that are lower and perhaps less dependent upon

$\log K_{ow}$ than rates of lipophilic compounds, (3) permeability coefficients for water have remarkably similar magnitude in all of the species studied, (4) anions and zwitterions appear to penetrate more slowly than cations, and (5) the penetration of cations appears to be significantly more variable than the penetration of other ionized compounds (i.e., anions or zwitterions).

7.4.2. Correlation of the Entire Validated Database

The valid permeability coefficients for each species were examined in terms of K_{ow} and MW, Eq. (7.6), and then compared with a similar correlation developed in Chapter 5 for human skin. Uncertainties in the regression coefficients are quantified by the standard error of the coefficients and are contained within parenthesis in the regression equations. In addition to the squared correlation coefficient (r^2) an adjusted squared correlation coefficient (r^2(adj.)) is provided to allow for more relevant comparisons between models with different numbers of fitted parameters (JMP User's Guide, (SAS Institute, 1995)). Specifically, $(1 - r^2)$ = error sum of squares/total sum of squares and $(1 - r^2(\text{adj.}) = (1 - r^2)(n - 1)/(n - p)$ where n = # of data points and p = # of parameters. RMSE is the root mean square error of the model, which is zero when the model perfectly correlates the data. When presented with an equation, F-Ratio is the model F-Ratio (the sum of squares for the model divided by the degrees of freedom for the model) / (the sum of squares for the error divided by the degrees of freedom for the error). When presented for a single parameter, such as the MW or $\log K_{ow}$, the F-Ratio is the effect F-Ratio (the sum of squares for the effect divided by the degrees of freedom for the effect) / (the sum of squares for the error divided by the degrees of freedom for the error). The model F-ratio = 1 when there is zero correlation with the parameters and is large for correlations with good predictive power. Because the number of fitted parameters is in the denominator of the F-Ratio, changes in the model F-Ratio with an

increase in the number of parameters should reflect the effect on predictive power relative to the number of fitted parameters. Thus, a correlation with a larger number of parameters might give a higher r^2 (or r^2(adj.)) but a lower F-Ratio than a correlation with fewer parameters. This would indicate that the improvement in predictive power (as indicated by a larger r^2) was not as large per parameter as for the equation with fewer parameters.

The validated hairless mouse permeability coefficients ($P_{cw,HLMouse}$) from Table 7A.1 were linearly regressed with Eq. (7.6) to obtain:

$$\log P_{cw,HLMouse}\,[\text{cm / hr}] = -2.27(0.13) + 0.47(0.05)\log K_{ow} - 0.0024(0.0007)\,\text{MW} \qquad (7.9)$$

$$(n = 84,\ r^2 = 0.580,\ r^2(\text{adj.}) = 0.570,\ \text{RMSE} = 0.681,\ \text{F} - \text{Ratio} = 55.9)$$

Both $\log K_{ow}$ (F-Ratio = 110.1, p = 0.0000) and MW (F-Ratio = 13.3, p = 0.0005) contribute significantly to $P_{cw,HLMouse}$ at the 95% confidence level. Equation (7.9) shows that approximately 58% of the variability in the 84 $\log P_{cw,HLMouse}$ measurements can be explained by variations in $\log K_{ow}$ and MW. Equation (7.9) is a very good correlation for the skin permeability coefficient in hairless mouse skin for organic chemicals with a wide range of MW and K_{ow}. The logarithmically-transformed ratio of hairless mouse to human skin permeability coefficients (i.e., $\log(P_{cw,HLMouse}/P_{cw,Human})$) can be determined by subtracting the correlation developed for the logarithmically transformed permeability coefficient in human skin, Eq. (5.27), from Eq. (7.9):

$$\log\left(\frac{P_{cw,HLMouse}}{P_{cw,Human}}\right) = 0.165(0.18) - 0.044(0.06)\log K_{ow} + 0.0026(0.0008)\,\text{MW} \qquad (7.10)$$

The standard error for a certain coefficient was calculated as the square root of the sum of the squared standard errors for the corresponding coefficients in the hairless mouse and human regression equations.

The validated hairless rat permeability coefficients ($P_{cw,HLRat}$) from Table 7A.2 were linearly regressed using Eq. (7.6) to obtain:

$$\log P_{cw,HLRat}\,[cm/hr] = -2.07(0.37) + 0.742(0.108)\log K_{ow} - 0.0050(0.0021)\,MW \qquad (7.11)$$

$$(n = 18,\ r^2 = 0.815,\ r^2(adj.) = 0.791,\ RMSE = 0.550,\ F-Ratio = 33.1)$$

Again, both $\log K_{ow}$ (F-Ratio = 46.9, p = 0.0000) and MW (F-Ratio = 5.7, p = 0.0304) terms contribute significantly at the 95% confidence level. However, the MW term is almost insignificant at this level of confidence (it would be insignificant at $p \geq 0.05$). Equation (7.11) shows that approximately 81.5% of the variability in the 18 $\log P_{cw,HLRat}$ measurements can be explained by variation in $\log K_{ow}$ and MW. Because the MW term is only borderline significant, we can develop a simpler and equally relevant (or perhaps more relevant) model by neglecting the MW contribution:

$$\log P_{cw,HLRat}\,[cm/hr] = -2.83(0.21) + 0.545(0.080)\log K_{ow} \qquad (7.12)$$

$$(n = 18,\ r^2 = 0.745,\ r^2(adj.) = 0.729,\ RMSE = 0.625,\ F-Ratio = 46.7)$$

Equation (7.12) predicts a significantly smaller dependence on $\log K_{ow}$ than Eq. (7.11). The 95% level of confidence was adopted to discriminate between the significant and the insignificant parameters so Eq. (7.11) will be used in further analysis. Both Eqs. (7.11) and (7.12) are limited by the size of the hairless rat skin permeability coefficient database and provide only an approximate relationship.

The logarithmically-transformed ratio of hairless rat to human skin permeability coefficients (i.e., $\log(P_{cw,HLRat}/P_{cw,Human})$) can be determined by subtracting the correlation developed for the logarithmically transformed permeability coefficient in human skin, Eq. (5.27), from Eq. (7.11):

$$\log\left(\frac{P_{cw,HLRat}}{P_{cw,Human}}\right) = 0.37(0.39) + 0.23(0.12)\log K_{ow} + 6.0\times10^{-5}(0.002)\,MW \qquad (7.13)$$

The standard error for a certain coefficient was calculated as the square root of the sum of the squared standard errors for the corresponding coefficients in the hairless rat and human regression equations.

The valid rat permeability coefficients ($P_{cw,Rat}$) from Table 7A.3 were linearly regressed using Eq. (7.6) to obtain:

$$\log P_{cw,Rat}\,[\text{cm / hr}] = -2.70(0.16) + 0.31(0.05)\log K_{ow} - 0.002(0.001)\,MW^{*} \quad (7.14)$$
$$(n = 14,\ r^2 = 0.846,\ r^2(\text{adj.}) = 0.818,\ \text{RMSE} = 0.350,\ \text{F} - \text{Ratio} = 30.1)$$

The effect of $\log K_{ow}$ is significant (F-Ratio = 41.3, p = 0.0000), but the effect of MW is not significant (F-Ratio = 2.6, p = 0.134) at the 95% confidence level. The asterisk attached to the MW term (i.e., MW^{*}) signifies this insignificance. More than 84% of the variation in $\log P_{cw,Rat}$ can be explained in terms of variation in $\log K_{ow}$ and MW by Eq. (7.14), but, the database is small and non-diverse. Neglecting the effect of MW gave the correlation:

$$\log P_{cw,Rat} = -2.49(0.10) + 0.336(0.047)\log K_{ow} \quad (7.15)$$
$$(n = 14,\ r^2 = 0.809,\ r^2(\text{adj.}) = 0.793,\ \text{RMSE} = 0.373,\ \text{F} - \text{Ratio} = 50.8)$$

Like Eq. (7.14), Eq. (7.15) is limited by the size of the rat skin permeability coefficient database and provides only an approximate relationship. The logarithmically-transformed ratio of rat to human skin permeability coefficients (i.e., $\log(P_{cw,Rat}/P_{cw,Human})$) can be determined by subtracting the correlation developed for the logarithmically transformed permeability coefficient in human skin, Eq. (5.27), from Eq. (7.15):

$$\log\left(\frac{P_{cw,Rat}}{P_{cw,Human}}\right) = -0.05(0.16) - 0.178(0.06)\log K_{ow} + 0.0050(0.0005)\,MW \quad (7.16)$$

The standard errors, enclosed within parenthesis are calculated as described previously.

The validated database of permeability coefficients through shed snake skin from Table 7A.4 were linearly regressed using Eq. (7.6) to obtain:

$$\log P_{cw,snake}\,[cm/hr] = -2.20(0.2) + 0.76(0.08)\log K_{ow} - 0.0098(0.001)\,MW$$
$$(n = 31,\ r^2 = 0.776,\ r^2(adj.) = 0.760,\ RMSE = 0.475,\ F-Ratio = 48.5) \qquad (7.17)$$

The $\log K_{ow}$ (F-Ratio = 80.6, p = 0.0000) and MW (F-Ratio = 74.8, p = 0.0000) terms are both significant at the 95% confidence level. Equation (7.17) shows that approximately 77.6% of the variability in the 31 $\log P_{cw,Snake}$ measurements can be explained by variation in $\log K_{ow}$ and MW alone. Equation (7.17) is a reasonably good correlation for the skin permeability coefficient in shed snake skin for a wide range of organic compounds. The logarithmically-transformed ratio of shed snake to human skin permeability coefficients (i.e., $\log(P_{cw,Snake}/P_{cw,Human})$) can be determined by subtracting the correlation developed for the logarithmically transformed permeability coefficient in human skin, Eq. (5.27), from Eq. (7.17):

$$\log\left(\frac{P_{cw,Snake}}{P_{cw,Human}}\right) = 0.244(0.23) + 0.263(0.09)\log K_{ow} - 0.0048(0.001)\,MW \qquad (7.18)$$

The standard errors, enclosed within parenthesis, are calculated as described previously. Comparing Eqs. (7.10), (7.13), (7.16) and (7.18), we see that only shed snake skin depends more strongly on MW than human skin.

Figures 7.7 and 7.8 show the permeability coefficient correlations for human (Eq. (5.27), hairless mouse (Eq. (7.9)), hairless rat (Eq. (7.12)), rat (Eq. (7.15)), and shed snake skin (Eq. (7.17)) plotted as a function of $\log K_{ow}$ for relatively small molecules (MW = 100) and larger molecules (MW = 300). The correlations for human, hairless mouse, and shed snake skin are most relevant because these databases are the largest and most diverse. The permeability coefficient correlations for the hairless rat and rat skins have been developed from more limited databases and are shown as dashed lines. Permeability coefficients in all species increase linearly with $\log K_{ow}$.

As illustrated in Figures 7.7 and 7.8, differences between species in the K_{ow} dependence of permeability coefficients can cause the relative order of penetration rates

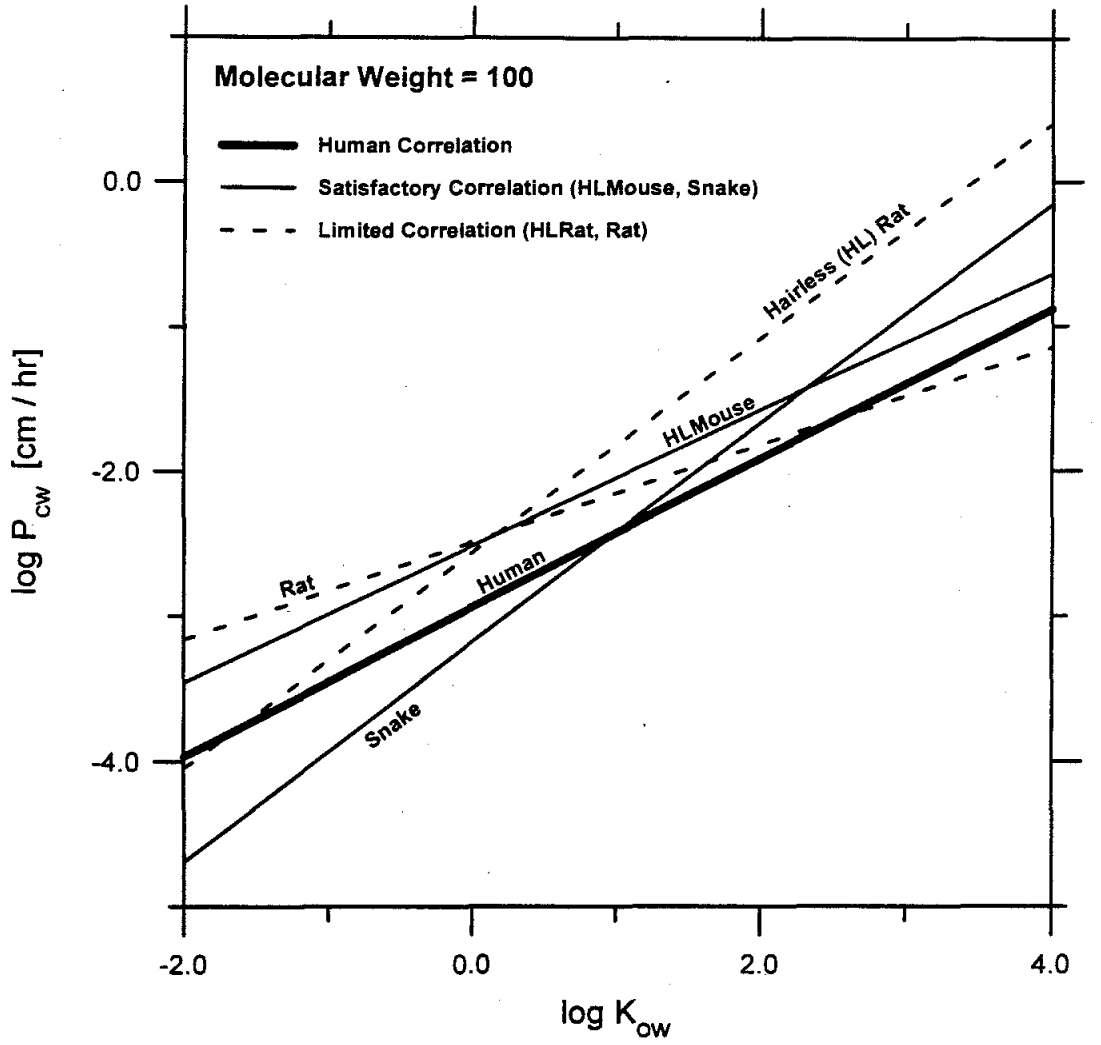

Figure 7.7 Permeability coefficient correlations for human, hairless mouse, hairless rat, rat and shed snake skin as a function of K_{ow} at MW = 100.

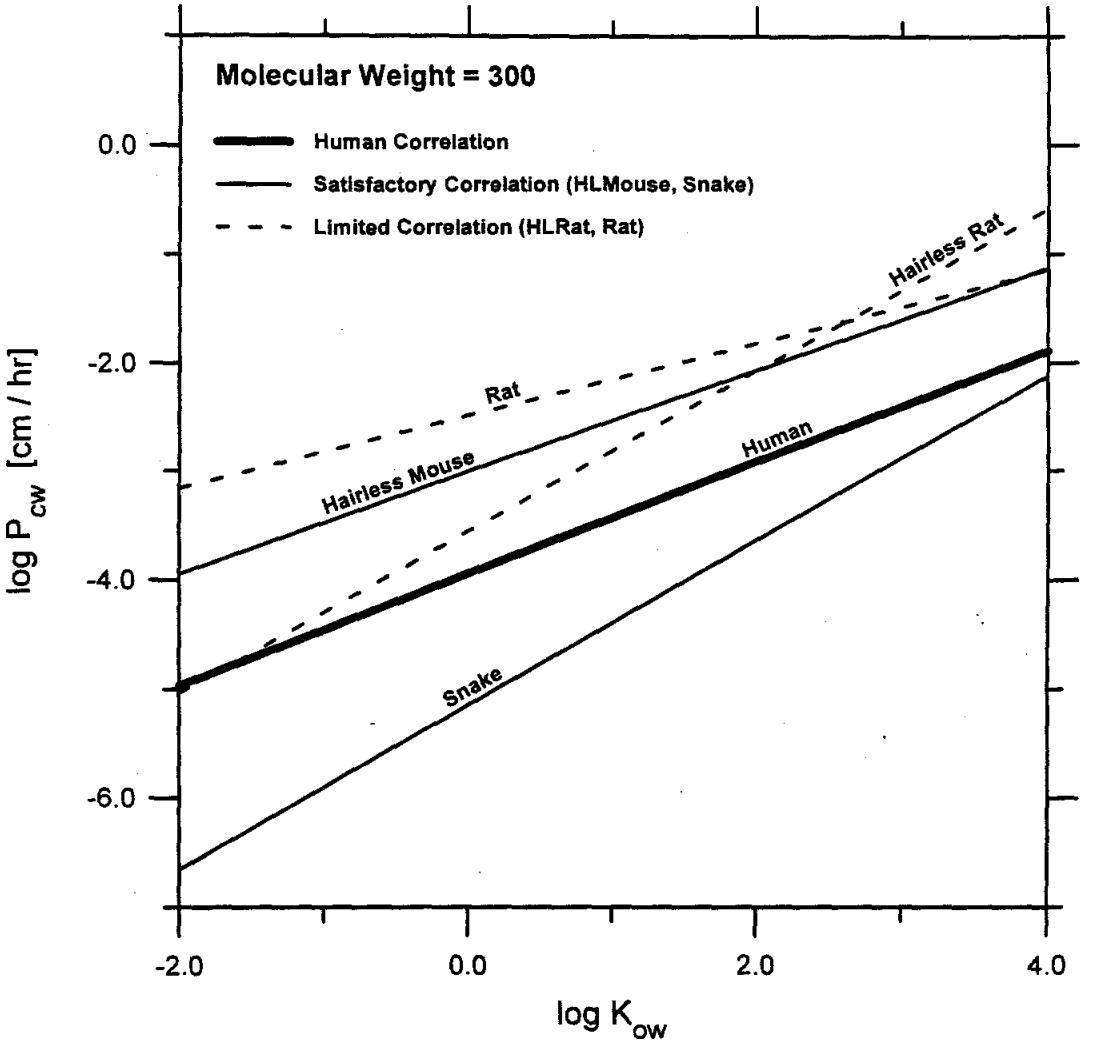

Figure 7.8 Permeability coefficient correlations for human, hairless mouse, hairless rat, rat, and shed snake skin as a function of K_{ow} at MW = 300.

to change with K_{ow}. For example, for a chemical with MW = 100 and $\log K_{ow} = 4$, the predicted order for permeability coefficients is snake > hairless mouse > human, while for a chemical with MW = 100 and $\log K_{ow} = -2.0$ the predicted order is hairless mouse > human > snake. However, when MW = 300, the relative order among these three species is predicted to be independent of $\log K_{ow}$. These plots show clearly that relative rankings of permeability coefficients in different species may depend on chemical properties of the penetrant.

Figure 7.9 shows the permeability coefficient correlations for human, hairless mouse, snake, hairless rat, and rat skin plotted as a function of MW when $\log K_{ow} = 2.0$. Like Figure 7.7, Figure 7.9 illustrates dramatically that the relative order of permeability coefficients between species depends on chemical properties of the penetrating compound. A chemical with $\log K_{ow} = 2.0$ and MW = 75 would penetrate slightly more rapidly through snake skin than through either hairless mouse or human skin, while a chemical with $\log K_{ow} = 2.0$ and MW = 300 would penetrate much more rapidly through hairless mouse skin than through either human or snake skin.

7.4.3. Correlation of the Common Compounds

New databases were created consisting of chemicals for which fully-validated data appear in the animal and human skin databases. In each species, multiple permeability coefficients for a single compound were independently adjusted for ionization and then averaged. For each chemical a ratio of average animal and average human permeability coefficients was then calculated. The ratios of animal to human permeability coefficients for many different chemicals were then linearly regressed as a function of MW and $\log K_{ow}$. When both MW and $\log K_{ow}$ effects were insignificant at the 95% confidence level, the average value of permeability ratios was determined.

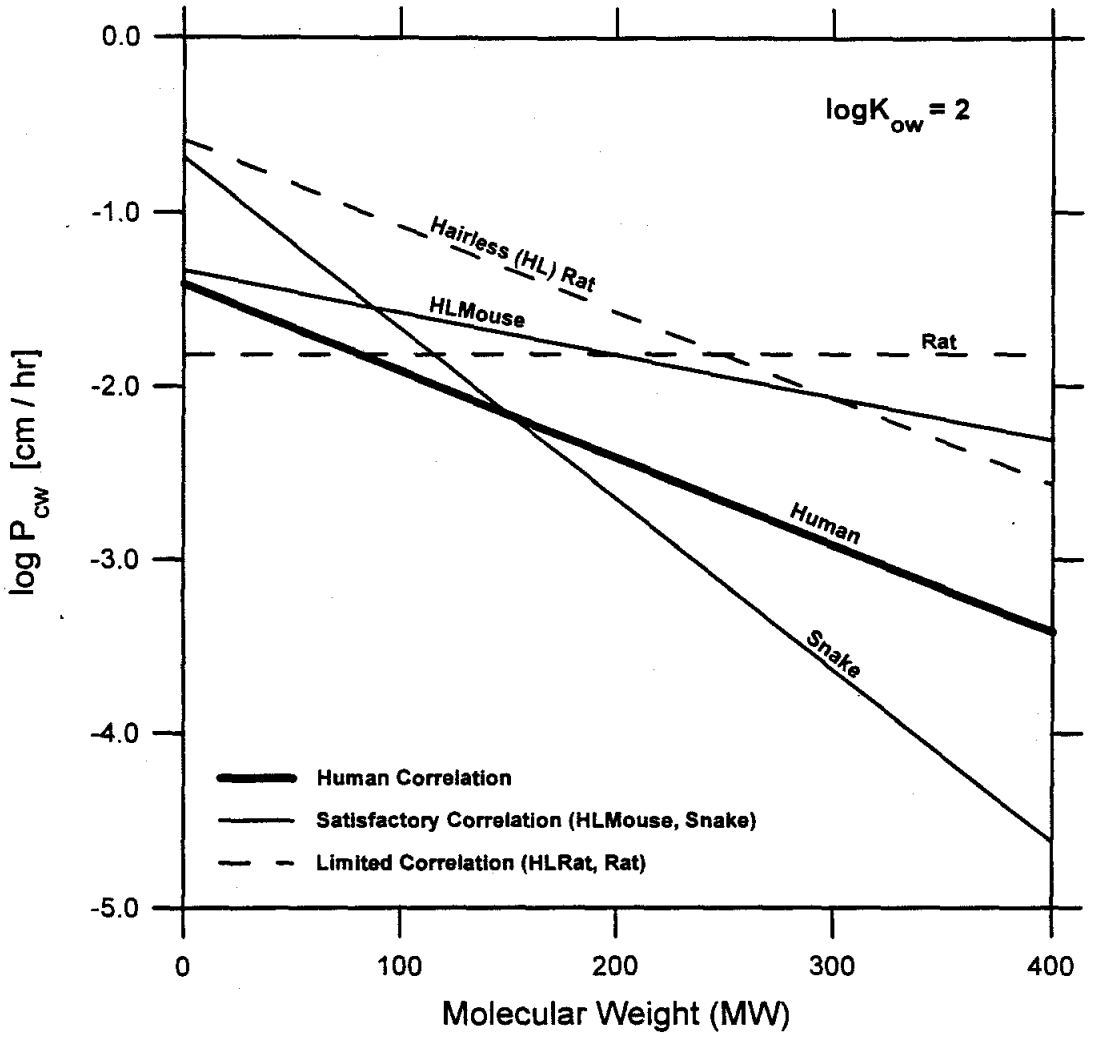

Figure 7.9 Permeability coefficient correlations for human, hairless mouse, hairless rat, rat and shed snake skin as a function of MW at $\log K_{ow} = 2$.

Permeability coefficient ratios for 31 compounds common to both the human and hairless mouse databases (31 compounds from 64 human permeability coefficient measurements and 67 hairless mouse permeability coefficient measurements) were linearly regressed using Eq. (7.7). There was no significant effect of MW or $\log K_{ow}$ in the database. The average ratio of hairless mouse to human permeability coefficients was found to be 3.1 (mean $\log(P_{cw,HLMouse}/P_{cw,Human}) = 0.491$), standard error of mean = 0.134, upper 95% mean = 0.765, lower 95% mean = 0.216).

Figure 7.10 shows the ratio of permeability coefficients for 31 compounds common to the hairless mouse and human databases compared with the prediction plotted as a function of $\log K_{ow}$. Several of the labeled data are clear outliers (e.g., cortisone, hydrocortisone, indomethacin, and progesterone) for reasons that have not been fully identified. However, it is usually true that these measurements were also outliers in either human or hairless mouse skins (e.g., the permeability coefficient for indomethacin (Morimoto *et al.*, 1992) was an outlier in human skin). The solid line is the average value of the permeability coefficient ratios which is consistent with the finding that MW or $\log K_{ow}$ did not contribute significantly to the permeability ratio. Equation (7.10) is also shown, by the dashed lines, for MW = 100 and MW = 300. Predictions based on the entire valid databases for hairless mouse and human skins and the subset of measurements for compounds common to both databases are in relative agreement that the ratio of permeability coefficients in hairless mouse and human skin is essentially insensitive to K_{ow} of the penetrants.

Figure 7.11 shows the ratio of permeability coefficients for 31 compounds common to the hairless mouse and human databases compared with predictions plotted as a function of MW. The solid line is the average of the permeability coefficient ratios. Equation (7.10) is also shown by the dashed lines for $\log K_{ow} = 2$ and $\log K_{ow} = 4$. Prediction based on the entire valid hairless mouse database and the fully-validated

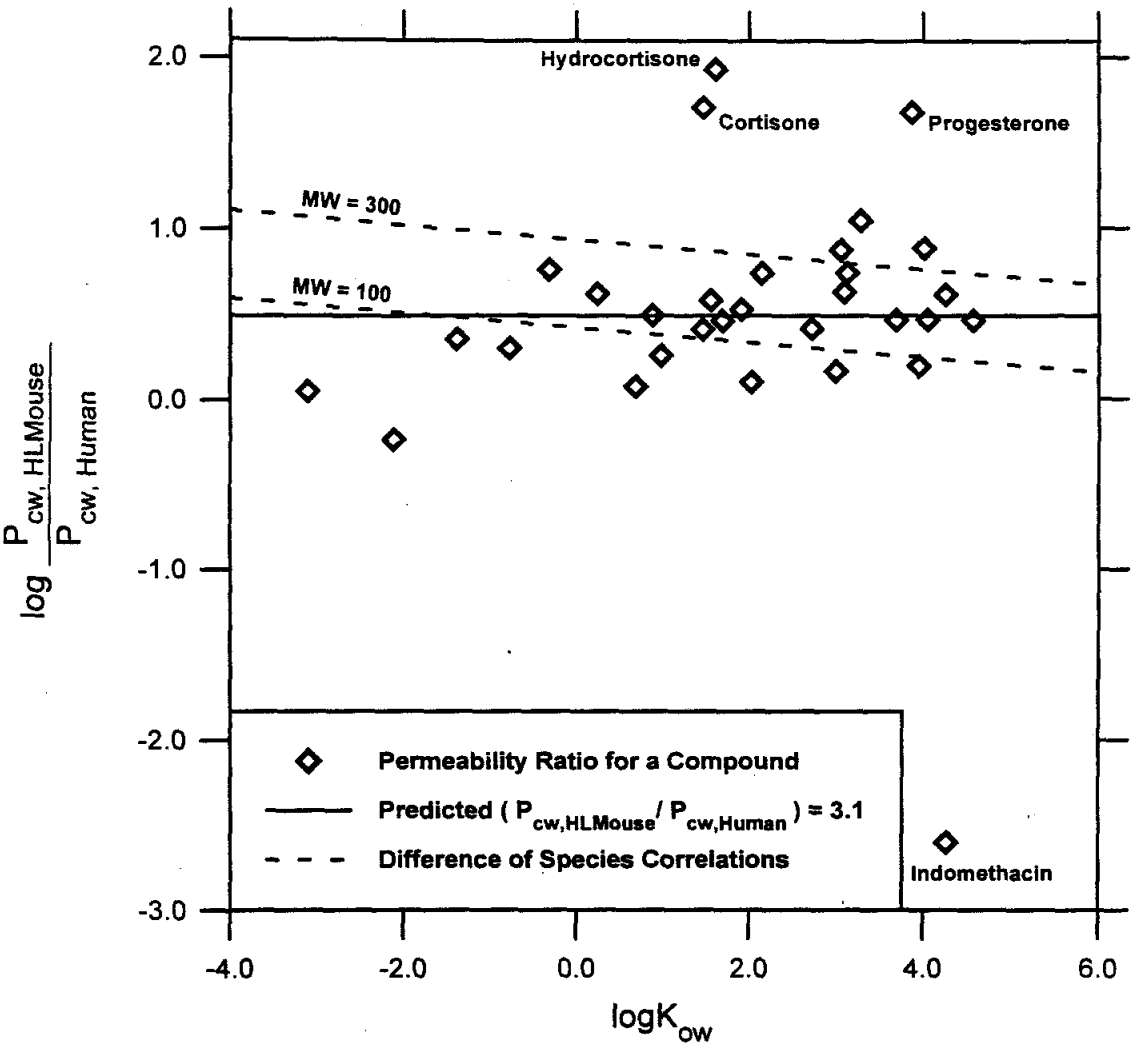

Figure 7.10 Ratios of average permeability coefficients for 31 compounds common to the validated hairless mouse and human databases plotted as a function of K_{ow} compared to the average ratio (solid horizontal line) and to the difference between the correlations developed from the hairless mouse database and the fully-validated human database (dashed lines) evaluated at MW = 100 and 300.

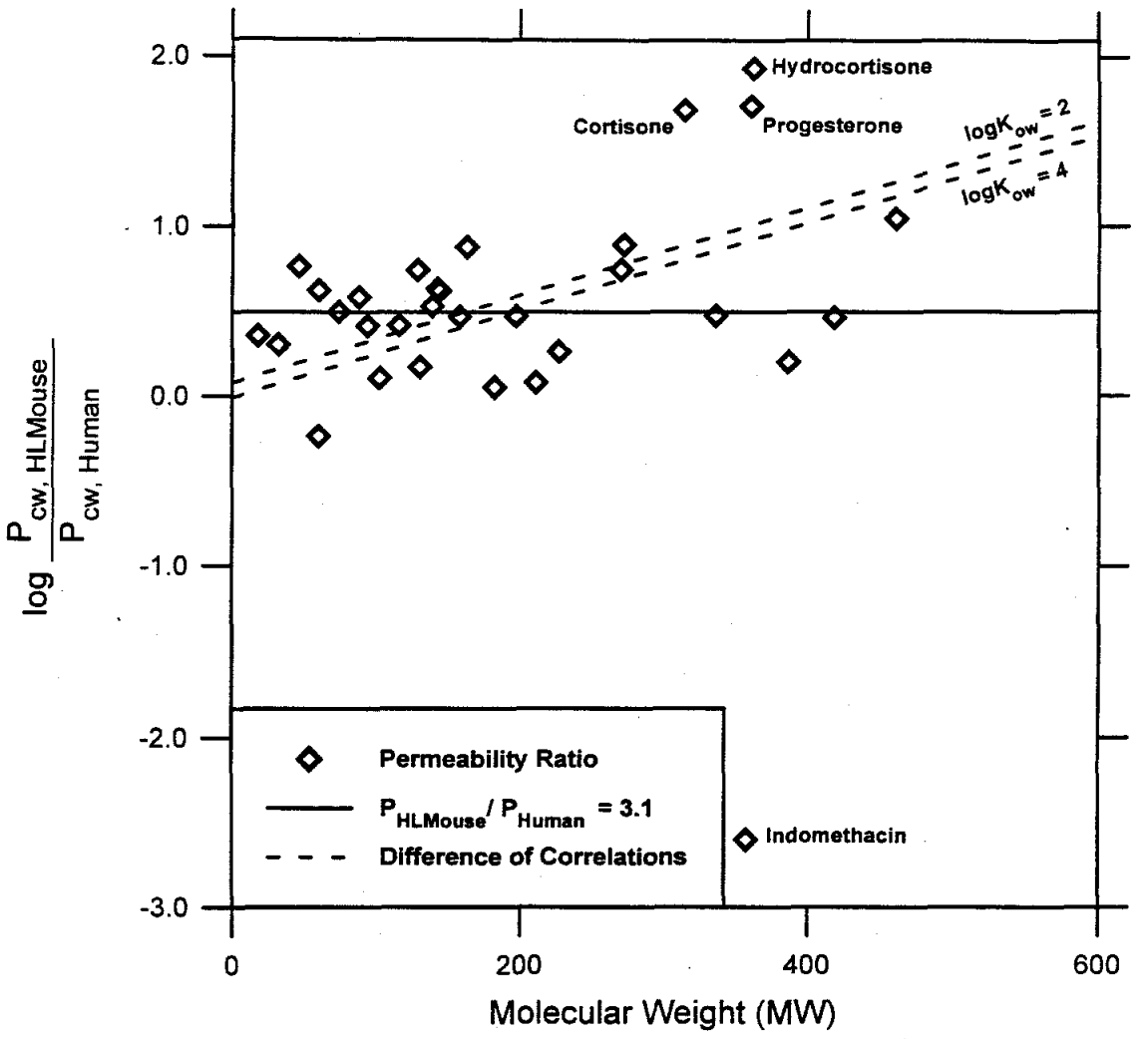

Figure 7.11 Ratios of average permeability coefficients for 31 compounds common to the validated hairless mouse and human databases plotted as a function of MW compared to the average ratio (solid horizontal line) and to the difference between the correlations developed from the hairless mouse database and the fully-validated human database (dashed lines) evaluated at $\log K_{ow} = 2$ and 4.

human database (i.e., the dashed lines) predict an increase in the permeability ratios as MW increases. This effect is not evident in the data shown, although only a few chemicals with MW > 300 are common to the two databases. It may be that the dashed lines, which were developed from data with a larger range of MW, may accurately represent the effect of MW.

Permeability coefficient ratios for 14 validated compounds common to both the human and shed snake skin databases (calculated from 31 human permeability coefficients and 18 snake permeability coefficients) were linearly regressed using Eq. (7.7). The resulting correlation showed no significant effect of MW or $\log K_{ow}$ in the database. The average ratio of shed snake skin to human skin permeability coefficients was found to be 0.43 (mean $\log(P_{cw,Snake}/P_{cw,Human}) = -0.371$), standard error of mean = 0.196, upper 95% mean = 0.052, lower 95% mean = -0.795).

Figure 7.12 shows the ratio of permeability coefficients for the 14 compounds common to the snake and human databases compared with predictions plotted as a function of $\log K_{ow}$. The two labeled ratios for the steroids deoxycorticosterone (DC) (called deoxycorticosterone in the animal investigation and cortexone in the human investigation) and hydrocortisone are outliers. The solid line is the average value of the permeability coefficient ratios that are shown, which is consistent with the finding that both MW and $\log K_{ow}$ do not significantly influence the permeability coefficient ratio for compounds common to the human skin and shed snake skin databases. Equation (7.18) is also shown as dashed lines for MW = 100 and MW = 300. Predictions based on the entire valid shed snake skin and human skin databases show a different effect of $\log K_{ow}$ than observed for compounds common to both databases. It is important to remember that the full databases contain measurements for many more compounds with a broader range of properties than in the data for compounds common to both databases. Thus, it may be that the dashed lines accurately represent the effect of K_{ow}.

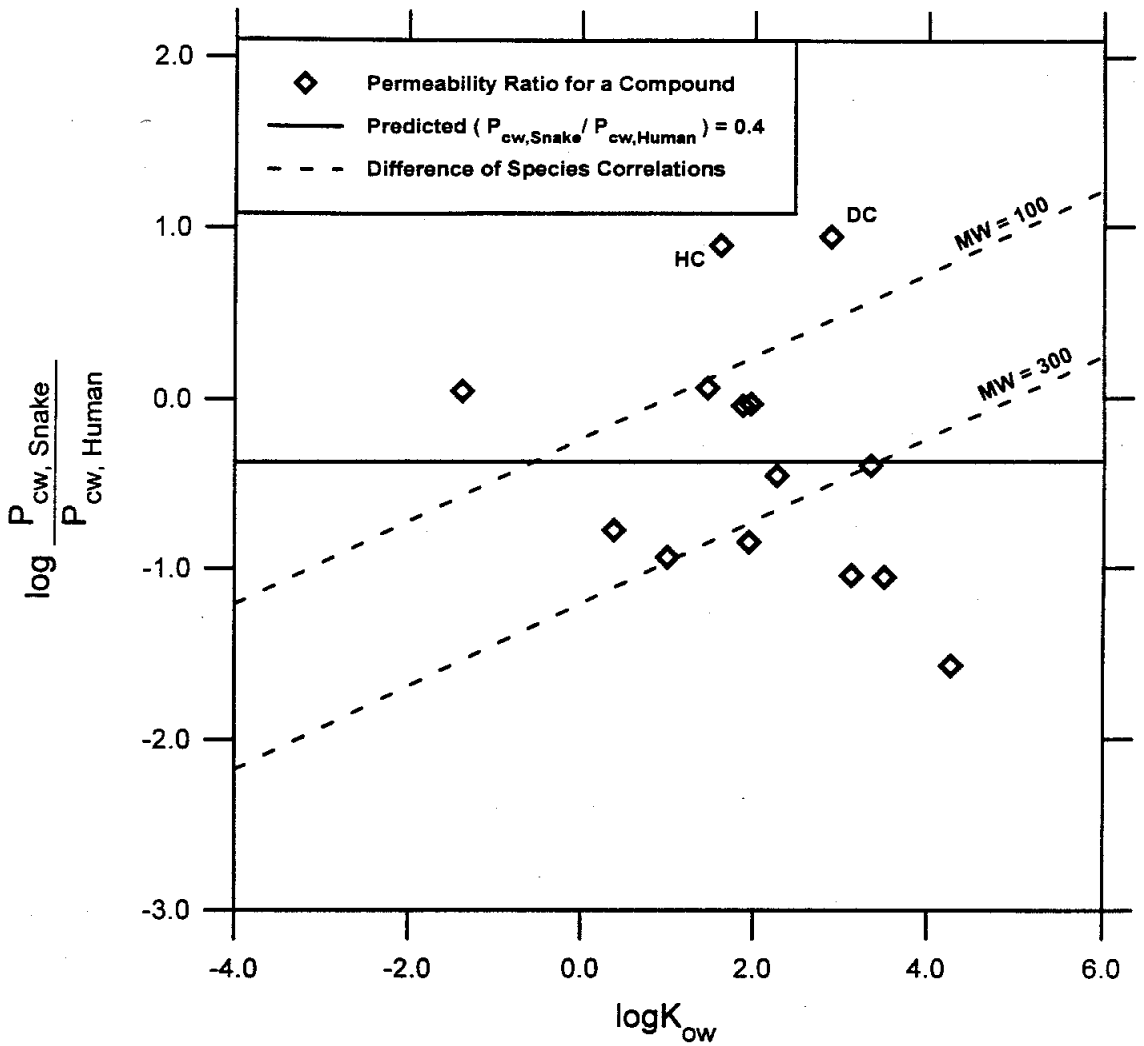

Figure 7.12 Ratios of average permeability coefficients for 14 compounds common to the validated snake and human databases plotted as a function of K_{ow} compared to the average ratio (solid horizontal line) and to the difference between the correlations developed from the snake database and the fully-validated human database (dashed lines) evaluated at MW = 100 and 300.

Figure 7.13 shows the data from Fig. 7.12 plotted as a function of MW. The solid line is the average value of the permeability coefficient ratios. Equation (7.18) is shown as the dashed lines for $\log K_{ow} = 2$ and $\log K_{ow} = 4$. Based on the entire valid snake database and the fully-validated human database, Eq. (7.18) predicts a significant decrease in the permeability coefficient ratio as MW increases. This general trend is supported by the data common to both databases except for the two labeled compounds deoxycorticosterone and hydrocortisone. The database of compounds common to both databases has a narrower range of MW which can conceal trends within the uncertainty of the data. The dashed predictions may provide the more credible prediction of the effect of MW on permeability coefficient ratios of shed snake skin to human skin.

The number of chemicals common to the rat (hairless and with hair) databases and the fully-validated human skin database are too small for meaningful conclusions. However, as a starting place we have linearly regressed the permeability ratios of those two databases using Eq. (7.7). Specifically, 12 chemicals are common to both the hairless rat and human skin validated databases (based on 15 hairless rat permeability coefficients and 28 human permeability coefficients). There was no significant effect of MW or $\log K_{ow}$ in the present database. The average ratio of hairless rat to human permeability coefficients was found to be 2.3 (mean value of $\log(P_{cw,HLRat}/P_{cw,Human}) = 0.365$), standard error of mean = 0.122, upper (75%) quartile = 0.68, lower (25%) quartile = 0.06).

Ten compounds are common to both the rat skin and human skin databases (based on 14 rat permeability coefficients and 32 human permeability coefficients) were analyzed with Eq. (7.7). For this small set of data, $\log K_{ow}$ was not significant but MW was significant with a 95% confidence. The apparent effect of MW probably occurs because most of the data are a series of homologous alkanols. The average ratio of rat to human permeability coefficients was found to be 1.9 (mean value of $\log(P_{cw,Rat}/P_{cw,Human})$

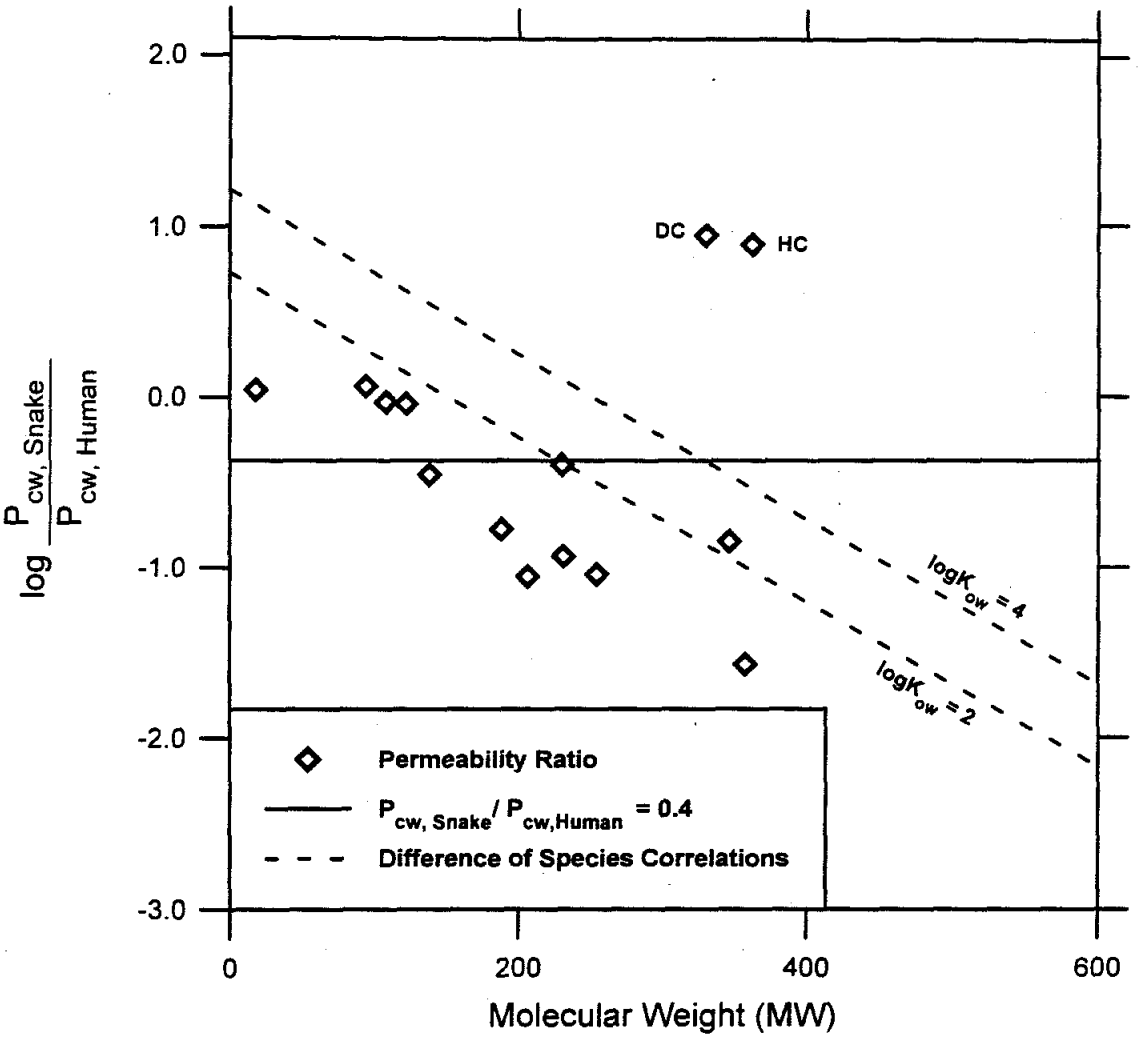

Figure 7.13 Ratios of average permeability coefficients for 14 compounds common to the validated snake and human databases plotted as a function of MW compared to the average ratio (solid horizontal line) and to the difference between the correlations developed from the snake database and the fully-validated human database (dashed lines) evaluated at $\log K_{ow} = 2$ and 4.

= 0.272), standard error of mean = 0.436, upper (75%) quartile = 0.51, lower (25%) quartile = -0.05).

7.5. Conclusions

Dermal absorption in different animal species has many qualitative similarities to dermal absorption in humans that can be observed through examination of permeability coefficients. However, for the purpose of estimating dermal absorption in humans, the many permeability coefficients determined in animal skins are of limited use until quantitative relationships to human skin are established. Based on the data collected so far, we have developed correlations of permeability coefficients as functions of $\log K_{ow}$ and MW for several animal species (hairless mouse, hairless rat, rat, snake). The correlation from hairless mouse skin is similar to a correlation of the same form for human skin. On average, hairless mouse skin is 3.1 times more permeable than human skin. This simple relationship is remarkably accurate, although the ratio may increase weakly for higher MW compounds. Our limited data sets indicate that a constant factor is not necessarily appropriate for other species of animals. In particular, there is evidence that the ratio of permeability coefficients may depend on MW or K_{ow} for some species. For example, shed snake skin depends more strongly on MW and $\log K_{ow}$ than human skin and thus the ratio of permeability coefficients for snake and human appears to vary with MW and $\log K_{ow}$. Based on our present data, the ratio of permeability coefficients for hairless rat and rat skins may depend on $\log K_{ow}$ and MW. However, the datasets for these animal species are so small that this conclusion can not be supported with confidence. Until more data are compiled to better define this relationship, we recommend using the average ratio of 2.3 for hairless rat skin and 1.9 for rat skin.

Historically, many researchers have ranked penetration rates measured in different animals implying that the same relative rankings would apply for other penetrants. This

assumption is clearly inappropriate when snake skin is considered and may also be inappropriate when hairless rat or rat skin is considered. Data like these demonstrated that the general application of relative penetration rates in various animal species would be careless. As the data in Chapters 5 and 7 indicate, the variability in measurements of permeability coefficients are sufficiently large that conclusions based on only a few measurements may be misleading. Hence, relationships of penetration rates in animal skins relative to human skins need to be determined from large numbers of measurements on many chemicals with varied properties.

7.6. Notation

D_c	=	Effective diffusivity of the absorbing chemical in the SC
D_o	=	Diffusion constant of hypothetical chemical having zero MV
f_{ui}	=	Fraction of the total chemical dose that is unionized in the vehicle
K_a	=	Acid dissociation constant for the absorbing chemical
K_{cw}	=	Equilibrium partition coefficient between the SC and water for the absorbing chemical
K_{ow}	=	Octanol-water partition coefficient of the penetrating chemical
L_c	=	Effective thickness of the SC
MV	=	Molecular volume of the absorbing chemical
MW	=	Molecular weight of the absorbing chemical
P_{cw}	=	Steady-state permeability of the SC from water
$P_{cw,\,i}$	=	Steady-state permeability of the SC from water for species i
pH	=	Negative logarithm of the hydrogen ion molarity $-\log_{10}[H^+]$
pK_a	=	Negative logarithm of the acidity equilibrium constant $-\log_{10}[K_a]$
SC	=	Stratum corneum

Superscripts

*	=	Regression constants not statistically different from zero at 95% confidence
#	=	Distinguish regression constants (different meaning when used with K_{ow})

7.7. References

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7.8. Appendix 7A: Tables of Permeability Coefficients

Table 7A.1 Hairless Mouse Skin Permeability Coefficients

Table 7A.2 Hairless Rat Skin Permeability Coefficients

Table 7A.3 Rat Skin Permeability Coefficients

Table 7A.4 Shed Snake Skin Permeability Coefficients

Table 7A.5 Permeability Coefficients for Various Animals

Table 7A.6 Stratum Corneum-Water Partition Coefficients Determined with Animal Skin

Table 7A.7 Temperature Effects on f_{ui} and Calculation of Unmeasured pH

Table 7A.1 Hairless Mouse Skin Permeability Coefficients

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ul}[d]	pH[e]	Skin[f]	Strain	Reference
E	Alanine (+ -)	-2.96	89.1	37	5.50E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Alanine (+ -)	-2.96	89.1	37	3.70E-05	ion	<0.1	6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Arginine (+ - +)	-4.20	174.2	37	1.00E-04	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Arginine (+ -)	-4.20	174.2	37	3.26E-04	ion	<0.1	10.8	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Asparagine (+ -)	-3.41	132.1	37	3.50E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Asparagine (+ -)	-3.41	132.1	37	4.10E-05	ion	<0.1	5.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Aspartic acid (- + -)	N/A	133.1	37	8.00E-06	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Aspartic acid (+ -)	N/A	133.1	37	8.60E-05	ion	<0.1	2.8	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Bevantolol (+)	[2.64]	345.4	37	1.32E-02	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
	Butanol	0.88	74.1	N/A	1.50E-02	1.50E-02	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Butanol	0.88	74.1	N/A	5.35E-03[l]	5.35E-03	1	ND	FULL	SKH-hr-1	Behl et al., 1980
	Butanol	0.88	74.1	20	2.90E-03	2.90E-03	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Butanol	0.88	74.1	25	4.40E-03	4.40E-03	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Butanol	0.88	74.1	37	1.54E-02	1.54E-02	1	ND	SC[h]	HRS/J	Flynn et al., 1981
	Chloramphenicol	1.14	323.1	31	1.12E-02	1.12E-02	1	ND	FULL	HR/HR	Aguiar & Weiner, 1969
	Chloramphenicol	1.14	323.1	37	1.81E-02	1.81E-02	1	ND	FULL	HR/HR	Aguiar & Weiner, 1969
	Chloramphenicol	1.14	323.1	45	3.72E-02	3.72E-02	1	ND	FULL	HR/HR	Aguiar & Weiner, 1969
	4-Chloro-3-cresol	3.10	142.6	37	2.35E-01	2.35E-01	1	ND	SC[h]	SKH-hr-1	Huq et al., 1986
	2-Chlorophenol	2.15	128.6	37	1.82E-01	1.82E-01	1	ND	SC[h]	SKH-hr-1	Huq et al., 1986
E	Cidofovir (- + -)	N/A[i]	279.0	35	2.47E-05	ion	<0.1	7	FULL	N/A	Aspe et al., 1995
	Cortisone	1.47	360.5	30	5.11E-04	5.11E-04	1	7.4	FULL	N/A	Katayama et al., 1992
E	Cyclopropanoyl propranolol (+)	[3.94]	327.0	37	1.29E-03	ion	<0.1	4	FULL	N/A	Ahmed et al., 1995
E	Cysteine (+ -)	-2.49	121.2	37	1.90E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Cysteine (+ -)	-2.49	121.2	37	3.40E-05	ion	<0.1	5.2	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
	Decanol	4.57	158.3	37	2.33E-01	2.33E-01	1	ND	EPID	HRS/J	Flynn et al., 1981
	2,4-Dichlorophenol	3.06	163.0	37	4.53E-01	4.53E-01	1	ND	SC[h]	SKH-hr-1	Huq et al., 1986
	2,4-Dimethylphenol	2.30	122.2	37	1.10E-01	1.10E-01	1	ND	FULL	SKH-hr-1	Huq et al., 1986
	2,4-Dinitrophenol	1.67	184.1	37	2.28E-01	2.28E-01	1	ND	SC[h]	SKH-hr-1	Huq et al., 1986
	2,4-Dinitrophenol	1.67	184.1	37	1.72E-01	1.72E-01	1	ND	FULL	SKH-hr-1	Jetzer et al., 1986
	β-Estradiol	4.01	272.4	37	[2.70E-02]	2.70E-02	1	ND	FULL	N/A	Kim et al., 1992

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ul}[d]	pH[e]	Skin[f]	Strain	Reference
	β-Estradiol	4.01	272.4	37	3.20E-02	3.20E-02	1	ND	SC	SKH-HR1	Liu et al., 1994
	Estrone	3.13	270.4	37	[3.96E-02]	3.96E-02	1	ND	FULL	N/A	Kim et al., 1992
	Estrone Ammonium Sulfate	3.13[j]	270.4[j]	37	[1.80E-04]	1.80E-04	1	ND	FULL	N/A	Kim et al., 1992
	Ethanol	-0.31	46.0	N/A	4.80E-03	4.80E-03	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Ethanol	-0.31	46.0	N/A	2.05E-03[l]	2.05E-03	1	ND	FULL	SKH-hr-1	Behl et al., 1980
	Ethanol	-0.31	46.0	20	7.20E-04	7.20E-04	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Ethanol	-0.31	46.0	25	1.23E-03	1.23E-03	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Ethanol	-0.31	46.0	37	4.88E-03	4.88E-03	1	ND	SC[h]	HRS/J	Flynn et al., 1981
E	Etorphine (+)	[1.41]	411.5	37	3.60E-03	ion	<0.1	7.3	FULL	SKH-hr-1	Jolicoeur et al., 1992
	Fentanyl	4.05	336.5	37	2.90E-02	3.97E-02	0.73	7.4	FULL	SKH-HR1	Roy et al., 1994
E	5-Fluorouracil (+ - + -)	-0.89	130.1	31	1.07E-04	ion	<0.1	4.75[k]	FULL	CBA/HL	Bond & Barry, 1988a
E	5-Fluorouracil (+ - + -)	-0.89	130.1	31	9.56E-05	ion	<0.1	4.75[k]	FULL	CBA/hl	Rigg & Barry, 1990
E	5-Fluorouracil (+ - + -)	-0.89	130.1	31	6.03E-05	ion	<0.1	4.75[k]	FULL	CBA/hl	Rigg & Barry, 1990
	Glucose	[-3.53]	180.2	N/A	9.50E-05	9.50E-05	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
E	Glutamic Acid (- + -)	-3.69	147.1	37	1.00E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Glutamic Acid (+ -)	-3.69	147.1	37	4.90E-05	ion	<0.1	2.8	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Glutamine (+ -)	-3.15	146.2	37	5.00E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Glutamine (+ -)	-3.15	146.2	37	3.20E-05	ion	<0.1	5.6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
	Glycerol	-1.76	92.1	N/A	1.40E-04	1.40E-04	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	1,2-Glyceryl dinitrate	[-0.22]	183.0	N/A	1.40E-03	1.40E-03	1	7.4	FULL	SKH:HR-1	Kikkoji et al., 1991
	1,3-Glyceryl dinitrate	[0.20]	183.0	N/A	1.20E-03	1.20E-03	1	7.4	FULL	SKH:HR-1	Kikkoji et al., 1991
E	Glycine (+ -)	-3.21	75.1	37	3.80E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Glycine (+ -)	-3.21	75.1	37	1.19E-04	ion	<0.1	6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
	Heptanol	2.72	116.0	N/A	9.30E-02	9.30E-02	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Heptanol	2.72	116.0	N/A	6.59E-02[l]	6.59E-02	1	ND	FULL	SKH-hr-1	Behl et al., 1980
	Heptanol	2.72	116.0	37	1.13E-01	1.13E-01	1	ND	EPID	HRS/J	Flynn et al., 1981
	Hexanol	2.03	102.2	N/A	4.80E-02	4.80E-02	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Hexanol	2.03	102.2	N/A	1.94E-02[l]	1.94E-02	1	ND	FULL	SKH-hr-1	Behl et al., 1980
	Hexanol	2.03	102.2	31	1.52E-02	1.52E-02	1	ND	FULL	CBA/HL	Bond & Barry, 1988b
	Hexanol	2.03	102.0	20	5.25E-03	5.25E-03	1	ND	FULL	HJS/J	Durrheim et al., 1980

	COMPOUND[a]	$logK_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ul}[d]	pH[e]	Skin[f]	Strain	Reference
	Hexanol	2.03	102.0	25	8.60E-03	8.60E-03	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Hexanol	2.03	102.2	37	5.88E-02	5.88E-02	1	ND	SC[h]	HRS/J	Flynn et al., 1981
E	Histidine (+ -)	-3.56	155.2	37	2.00E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Histidine (+ -)	-3.56	155.2	37	1.60E-05	ion	<0.1	7.6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
	Hydrocortisone	1.61	362.5	N/A	1.60E-04	1.60E-04	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Hydrocortisone	1.61	362.5	37	2.28E-04	2.28E-04	1	ND	FULL	SKH-hr-1	Behl et al., 1984
	Hydrocortisone	1.61	362.5	37	[3.60E-04]	3.60E-04	1	ND	FULL	N/A	Kim et al., 1992
	Hydrocortisone-21-acetate	1.11	404.0	N/A	1.70E-03	1.70E-03	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Hydrocortisone-21-butyrate	[2.22]	432.0	N/A	4.30E-02	4.30E-02	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Hydrocortisone-21-heptanoate	[3.81]	474.0	N/A	3.40E-01	3.40E-01	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Hydrocortisone-21-hexanoate	[3.28]	460.6	N/A	2.00E-01	2.00E-01	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Hydrocortisone-21-pentanoate	[2.75]	446.0	N/A	7.10E-02	7.10E-02	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Hydrocortisone-21-propionate	[1.69]	418.5	N/A	9.80E-03	9.80E-03	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Indomethacin	4.27	357.8	30	3.25E-04	3.35E-04	0.97	3	FULL	N/A	Katayama et al., 1992
E	Isoleucine (+ -)	-1.72	131.2	37	1.30E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Isoleucine (+ -)	-1.72	131.2	37	5.20E-05	ion	<0.1	6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Isovaleryl propranolol (+)	[5.04]	343.3	37	2.80E-03	ion	<0.1	4	FULL	N/A	Ahmed et al., 1995
E	Leucine (+ -)	-1.52	131.2	37	2.90E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Leucine (+ -)	-1.52	131.2	37	1.60E-05	ion	<0.1	6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Levobunolol (+)	2.40	291.0	37	1.55E-03	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
E	Lysine (+ - +)	-3.05	146.2	37	2.10E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Lysine (+ -)	-3.05	146.2	37	3.89E-04	ion	<0.1	9.8	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
	Mannitol	-3.10	182.2	30	7.80E-05	7.80E-05	1	7.4	FULL	N/A	Katayama et al., 1992
	Mannitol	-3.10	182.2	37	[1.08E-04]	1.08E-04	1	ND	FULL	N/A	Kim et al., 1992
	Methanol	-0.77	32.0	N/A	2.60E-03	2.60E-03	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Methanol	-0.77	32.0	N/A	2.81E-03[i]	2.81E-03	1	ND	FULL	SKH-hr-1	Behl et al., 1980
	Methanol	-0.77	32.0	37	3.20E-03[i]	3.20E-03	1	ND	SC[h]	SKH-hr-1	Behl et al., 1983b
	Methanol	-0.77	32.0	20	4.10E-04	4.10E-04	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Methanol	-0.77	32.0	25	9.30E-04	9.30E-04	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Methanol	-0.77	32.0	37	2.62E-03	2.62E-03	1	ND	SC[h]	HRS/J	Flynn et al., 1981

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ul}[d]	pH[e]	Skin[f]	Strain	Reference
E	Methionine (+ -)	-1.87	149.2	37	3.10E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Methionine (+ -)	-1.87	149.2	37	1.70E-05	ion	<0.1	5.6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Metoprolol (+)	1.88	267.4	37	2.75E-03	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
E	Morphine (+)	0.76	285.3	37	1.10E-04	ion	<0.1	7	FULL	SKH-HR1	Roy et al., 1994
E	Nadolol (+)	0.71	309.4	37	3.89E-03	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
	Nicorandil	[0.69]	211.2	37	2.66E-04	2.66E-04	1	[8.0]	FULL	WBN/kob	Sato et al., 1991
	Nitroglycerine	[0.98]	227.1	N/A	2.00E-02	2.00E-02	1	7.4	FULL	SKH:HR-1	Kikkoji et al., 1991
	2-Nitrophenol	1.79	139.1	37	1.01E-01	1.01E-01	1	ND	FULL	SKH-hr-1	Huq et al., 1986
	4-Nitrophenol	1.91	139.1	37	2.54E-02	2.54E-02	1	ND	SC[h]	SKH-hr-1	Huq et al., 1986
	4-Nitrophenol	1.91	139.1	37	1.22E-02	1.22E-02	1	ND	FULL	SKH-hr-1	Jetzer et al., 1986
	Nonanol	4.26	144.0	37	2.48E-01	2.48E-01	1	ND	EPID	HRS/J	Flynn et al., 1981
	Octanol	3.00	130.2	N/A	9.70E-02	9.70E-02	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Octanol	3.00	130.2	N/A	7.82E-02[i]	7.82E-02	1	ND	FULL	SKH-hr-1	Behl et al., 1980
	Octanol	3.00	130.0	20	1.89E-02	1.89E-02	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Octanol	3.00	130.0	25	3.15E-02	3.15E-02	1	ND	FULL	HJS/J	Durrheim et al., 1980
	Octanol	3.00	130.2	37	1.80E-01	1.80E-01	1	ND	EPID	HRS/J	Flynn et al., 1981
E	Oxprenolol (+)	2.10	265.3	37	5.25E-03	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	N/A	1.07E-02	ion	<0.1	N/A	FULL	N/A	Walker et al., 1983
E	Penbutolol (+)	4.15	291.0	37	2.57E-02	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
	Pentanol	1.56	88.0	N/A	2.20E-02	2.20E-02	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Pentanol	1.56	88.0	37	2.38E-02	2.38E-02	1	ND	SC[h]	HRS/J	Flynn et al., 1981
	Phenol	1.46	94.1	37	2.78E-02[i]	2.78E-02	1	ND	SC[h]	SKH-hr[-1]	Behl et al., 1983b
	Phenol	1.46	94.1	37	2.02E-02	2.02E-02	1	ND	SC[h]	SKH-hr-1	Huq et al., 1986
E	Phenylalanine (+ -)	-1.52	165.2	37	3.00E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Phenylalanine (+ -)	-1.52	165.2	37	2.44E-04	ion	<0.1	5.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
	Progesterone	3.87	314.5	37	[7.20E-02]	7.20E-02	1	ND	FULL	N/A	Kim et al., 1992
E	Proline (+ -)	-2.50	115.1	37	2.70E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Proline (+ -)	-2.50	115.1	37	3.30E-05	ion	<0.1	6.3	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
	Propanol	0.25	60.0	N/A	5.40E-03	5.40E-03	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
	Propanol	0.25	60.0	37	5.49E-03	5.49E-03	1	ND	SC[h]	HRS/J	Flynn et al., 1981
E	Propranolol (+)	2.98	259.3	37	9.25E-05	ion	<0.1	4	FULL	N/A	Ahmed et al., 1995

	COMPOUND[a]	$logK_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ui}[d]	pH[e]	Skin[f]	Strain	Reference
E	Propranolol (+)	2.98	259.3	37	7.41E-03	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
E	Serine (+ -)	-3.07	105.1	37	3.00E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Serine (+ -)	-3.07	105.1	37	3.60E-05	ion	<0.1	5.6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Sotalol (+)	[0.23]	272.4	37	3.72E-04	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
	Sufentanil	3.95	386.5	37	2.40E-02	2.53E-02	0.95	7.4	FULL	SKH-HR1	Roy et al., 1994
E	Tetraethylammonium Bromide (+)	-2.82	210.2	37	[1.08E-04]	ion	<0.1	ND	FULL	N/A	Kim et al., 1992
	Thiourea	-1.02	76.1	N/A	9.60E-05	9.60E-05	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
E	Threonine (+ -)	-2.94	119.1	37	1.30E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Threonine (+ -)	-2.94	119.1	37	1.18E-04	ion	<0.1	6.2	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Timolol (+)	1.83	316.4	37	1.55E-03	ion	<0.1	7.4	FULL	Skh:Sr-1	Ghosh et al., 1993
	2,4,6-Trichlorophenol	3.69	197.5	37	1.74E-01	1.74E-01	1	ND	FULL	SKH-hr-1	Huq et al., 1986
E	Tryptophan (+ -)	-1.06	204.2	37	1.50E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Tryptophan (+ -)	-1.06	204.2	37	1.90E-05	ion	<0.1	5.7	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Tyrosine (+ -)	-2.26	181.2	37	1.60E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Tyrosine (+ -)	-2.26	181.2	37	2.60E-05	ion	<0.1	5.6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
	Urea	-2.11	60.1	N/A	1.20E-04	1.20E-04	1	N/A	N/A	SKH-hr-1	Ackerman et al., 1987[g]
E	Valine (+ -)	-2.26	117.2	37	1.30E-05	ion	<0.1	7.4	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Valine (+ -)	-2.26	117.2	37	4.50E-05	ion	<0.1	6	FULL	hr/hr-c_3H/Tif Bom	Ruland & Kreuter, 1991
E	Vidarabine	-1.11	285.3	37	[7.20E-05]	7.66E-05	0.94	> [7]	FULL	N/A	Kim et al., 1992
	Water	-1.38	18.0	N/A	1.60E-03	1.60E-03	1	ND	FULL	SKH-hr-1	Behl et al., 1980
	Water	-1.38	18.0	31	3.36E-03	3.36E-03	1	ND	FULL	CBA/HL	Bond & Barry, 1988b
	Water	-1.38	18.0	31	2.19E-03	2.19E-03	1	ND	FULL	CBA/hl	Rigg & Barry, 1990
	Water	-1.38	18.0	N/A	3.51E-03	3.51E-03	1	ND	FULL	N/A	Walker et al., 1983

a. The compound investigated. Measurements indicated at left by an E are excluded measurements and those indicated by a P are provisional measurements. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, zwitterionic alanine with one positive and one negative charge is indicated as (+ -).

b. Reported $logK_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for bevantolol [2.64]), in which case they were calculated (Daylight, 1995).

c. Permeability coefficients contained within brackets were digitized from figures in the publication.

d. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 7A.7.

e. Reported solution pH unless contained within brackets (e.g. for nicorandil [8.0]) in which case the pH was calculated from the reported concentration and calculated pK_a values (see Table 7A.7). Compounds that are essentially undissociated are indicated by ND when

no pH was reported.

f. Type of skin used in the study: isolated stratum corneum (SC), epidermal membranes (EPID), or full-thickness skin (FULL).

g. Experimental procedure reported in the Ph.D. dissertations of Ackerman, Durrheim, and Smith.

h. Stratum corneum permeability coefficient was estimated from measurements made with other skin layers.

i. The calculated $\log K_{ow}$ of -1.29 (Daylight, 1995) is invalid (structural fragments of this molecule are not adequately represented by Daylight software).

j. The parameters $\log K_{ow}$ and MW are for estrone which was the penetrating species.

k. Information obtained through personal communication from Barry (1996).

l. The permeability coefficient for this chemical and a different copenetrating chemical were measured simultaneously (see also Appendix 7B).

Table 7A.2 Hairless Rat Skin Permeability Coefficients

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ui}[d]	pH[e]	Skin[f]	Strain	Reference
E	Alanine (+ -)	-2.96	89.1	37	1.68E-04	ion	<0.1	6	FULL	N/A	Wearley et al., 1990
	Aminopyrine	1.00	231.3	37[g]	[1.65E-03]	1.65E-03	1	7.94[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
	Antipyrene	0.38	188.2	37[g]	[4.00E-04]	4.00E-04	1	7.6[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
	Atenolol	0.16	266.3	37	1.04E-03	1.06E-03	0.98	[10.9]	FULL	WBN/ILA-Ht	Kobayashi et al., 1994
	Cyclobarbitone	1.77	236.3	37[g]	[4.26E-03]	4.26E-03	1	3.58[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	Diclofenac (-)	4.40	260.7	37[g]	[8.34E-04]	ion	<0.1	7.96[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	Diclofenac (-)	4.40	260.7	37	4.25E-04	ion	<0.1	7.7	FULL	WBN/kob	Okumura et al., 1989
E	Diltiazem (+)	[3.55]	414.5	37	[1.66E-05]	ion	<0.1	3.3	FULL	WBN/kob	Okumura et al., 1989
E	Disodium Cromoglycate (- -)	1.92	468.4	37	[1.05E-05]	ion	<0.1	5.7	FULL	WBN/kob	Okumura et al., 1989
E	Dopamine (+)	[-0.05]	153.2	37[g]	[9.40E-04]	ion	<0.1	3.26[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	Dopamine (+)	[-0.05]	153.2	37	9.04E-04	ion	<0.1	3.7	FULL	WBN/kob	Okumura et al., 1989
	Estradiol (Beta)	4.01	272.4	37[g]	[9.40E-02]	9.40E-02	1	ND	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	FD-4 (FITC Dextran)[j]	N/A	4400[k]	37	1.92E-05	1.92E-05	1	7.4	FULL	N/A	Ogiso et al., 1994
E	FD-10 (FITC Dextran)[j]	N/A	9600[k]	37	1.22E-05	1.22E-05	1	7.4	FULL	N/A	Ogiso et al., 1994
E	FD-70 (FITC Dextran)[j]	N/A	69000[k]	37	4.50E-06	4.50E-06	1	7.4	FULL	N/A	Ogiso et al., 1994
E	5-Fluorouracil (+ - + -)	-0.89	130.1	37[g]	[1.71E-04]	ion	<0.1	4.66[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
	Flurbiprofen	4.16	244.3	37[g]	[3.80E-01]	1.17E+00	0.32	4.7[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	Glycine (+ -)	-3.21	75.1	37	3.99E-05	ion	<0.1	6	FULL	N/A	Wearley et al., 1990
	Ibuprofen	3.50	206.3	37[g]	[3.60E-01]	6.21E-01	0.58	4.44[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
	Indomethacin	4.27	357.8	37[g]	[1.49E-01]	8.28E-01	0.18	5.15[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
	Indomethacin	4.27	357.8	37	1.00E-01	1.00E-01	1[l]	Range	FULL	WBN/ILA-Ht	Hayashi et al., 1992
	Indomethacin	4.27	357.8	37	1.54E-01	5.06E-01	0.30	4.9	FULL	WBN/kob	Okumura et al., 1989
E	Isoproterenol (+)	[0.08]	211.2	37[g]	[9.10E-04]	ion	<0.1	2.75[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	Isoproterenol (+)	[0.08]	211.2	37	4.46E-04	ion	<0.1	3.8	FULL	WBN/kob	Okumura et al., 1989
	Isosorbide dinitrate	1.31	236.1	37[g]	[1.77E-02]	1.77E-02	1	ND	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
	Ketoprofen	3.12	254.3	37[g]	[8.57E-02]	1.08E-01	0.79	3.72[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	Leucine (+ -)	-1.52	131.2	37	1.45E-04	ion	<0.1	6	FULL	N/A	Wearley et al., 1990

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ui}[d]	pH[e]	Skin[f]	Strain	Reference
E	Levodopa (+ -)	-2.74	197.0	37[g]	[3.10E-04]	ion	<0.1	5.42[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	Lignocaine (+)	2.26	234.3	37[g]	[2.72E-02]	ion	<0.1	6.82[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
E	Morphine (+)	0.76	285.3	37	1.50E-04	ion	<0.1	[4.9]	FULL	WBN/ILA-Ht	Kobayashi et al., 1994
E	Morphine (+)	0.76	285.3	37[g]	[4.30E-04]	ion	<0.1	4.22[h]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
	Nicorandil	[0.69]	211.2	37[g]	[3.64E-04]	3.64E-04	1	[8.0]	SC[i]	WBN/ILA-Ht	Hatanaka et al., 1990
	Nicorandil	[0.69]	211.2	37	7.27E-04	7.27E-04	1	[8.0]	FULL	WBN/kob	Sato et al., 1989
	Nifedipine	[2.20]	346.3	37	3.06E-02	3.06E-02	1	ND	FULL	WBN/ILA-Ht	Kobayashi et al., 1994
E	Papaverine (+)	[3.00]	339.4	37	[7.18E-05]	ion	<0.1	3	FULL	WBN/kob	Okumura et al., 1989
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	N/A	3.55E-04	ion	<0.1	ND	FULL	N/A	Walker et al., 1983
	Salicylic acid	2.26	138.1	25	1.50E-02	3.07E-02	0.49	3	FULL	N/A	Harada et al., 1993
E	Valine (+ -)	-2.26	117.2	37	7.68E-05	ion	<0.1	6	FULL	N/A	Wearley et al., 1990
E	Vinpocetine	N/A[m]	350.5	37	2.22E-02	N/A	N/A	> [7]	FULL	WBN/ILA-Ht	Kobayashi et al., 1994
	Water	-1.38	18.0	37	4.28E-03	4.28E-03	1	ND	FULL	WBN/kob	Okumura et al., 1989
	Water	-1.38	18.0	N/A	1.30E-03	1.30E-03	1	ND	FULL	N/A	Walker et al., 1983

a. The compound investigated. Measurements indicated at left by an E are excluded measurements and those indicated by a P are provisional measurements. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, zwitterionic alanine with one negative charge and one positive charge is indicated by (+ -).

b. Reported $\log K_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for dopamine [-0.05]), in which case they were calculated (Daylight, 1995).

c. Permeability coefficients contained within brackets were digitized from figures in the reference.

d. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 7A.7.

e. Reported solution pH unless contained within brackets (e.g., for atenolol [10.9]), in which case they were calculated from the concentration and calculated pK_a values (see Table 7A.7). Compounds that are essentially undissociated are identified with ND when no pH was reported.

f. Type of skin used in the study: isolated stratum corneum (SC), epidermis (EPID), split (SPLIT), or full-thickness skin (FULL).

g. The temperature was not provided. We assumed 37C, which is the temperature at which the K_{ow} is reported.

h. The pH of saturated solutions at this temperature were reported elsewhere (Morimoto et al., 1992).

i. Permeability coefficients of the stratum corneum membrane were calculated from measurements made on full-thickness skin and stripped full-thickness skin.

j. Fluorescein isothiocyanate-labeled dextrans
k. The average MW of a polydispersed mixture
l. The unionized permeability coefficient was determined from measurements covering a range of pH (i.e., fraction ionized).
m. The calculated $\log K_{ow}$ of 4.72 (Daylight, 1995) is invalid (structural fragments of this molecule are not adequately represented by Daylight software).

Table 7A.3 Rat Skin Permeability Coefficients

	COMPOUND[a]	$logK_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ui}[d]	pH[e]	Skin[f]	Strain	Reference
	Butanol	0.88	74.1	37	4.80E-03[i]	4.80E-03	1	ND	FULL	Sprague-Dawley	Behl et al., 1983a
	Diclofenac	4.40	260.7	37[g]	2.07E-01	2.07E-01[h]	0.92	3	N/A	N/A	Maitani et al., 1993
	Ethanol	-0.31	46.0	30	4.15E-04	4.15E-04	1	ND	FULL	Alpk/AP	Scott et al., 1991
	Hexanol	2.03	102.2	37	9.40E-03[i]	9.40E-03	1	ND	FULL	Sprague-Dawley	Behl et al., 1983a
	Mannitol	-3.10	182.2	30	[2.56E-04]	2.56E-04	1	ND	EPID	Alpk:APfSD	Dick & Scott 1992
	Mannitol	-3.10	182.2	30	1.34E-03	1.34E-03	1	ND	FULL	Alpk/AP	Scott et al., 1991
	Methanol	-0.77	32.0	37	2.35E-03[i]	2.35E-03	1	ND	FULL	Sprague-Dawley	Behl et al., 1983a
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	30	[4.81E-04]	ion	<0.1	ND	EPID	Alpk:APfSD	Dick & Scott 1992
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	30	3.46E-04	ion	<0.1	ND	FULL	Alpk/AP	Scott et al., 1991
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	N/A	2.67E-04	ion	<0.1	ND	FULL	N/A	Walker et al., 1983
	Phenol	1.46	94.1	37	1.14E-02	1.14E-02	1	ND	FULL	Wistar	Roberts & Anderson, 1975
	Salicylic acid	2.26	138.1	25	2.73E-02	2.73E-02	1[i]	Range	FULL	Wistar	Harada et al., 1992
	Urea	-2.11	60.1	37	3.41E-04	3.41E-04	1	7.1[j]	SC[j]	CD(SD)BR	Barber et al., 1992
	Water	-1.38	18.0	30[j]	2.97E-03	2.97E-03	1	ND	SC[j]	CD(SD)BR[k]	Barber et al., 1992
	Water	-1.38	18.0	30	[1.40E-03]	1.40E-03	1	ND	EPID	Alpk:APfSD	Dick & Scott 1992
	Water	-1.38	18.0	30	4.85E-04	4.85E-04	1	ND	FULL	Alpk/AP	Scott et al., 1991
	Water	-1.38	18.0	N/A	1.03E-03	1.03E-03	1	ND	FULL	N/A	Walker et al., 1983

a. The compound investigated. Measurements indicated at left by E are excluded measurements and those indicated by a P are provisional measurements. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, paraquat with two positive charges is indicated by (++).

b. Reported $logK_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for paraquat [-5.65]) in which case they were calculated (Daylight, 1995).

c. Permeability coefficients contained within brackets were digitized from figures in the publication.

d. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 7A.7.

e. Reported solution pH. Compounds that are essentially undissociated are indicated by ND when no pH was reported.

f. Type of skin used in the study: isolated stratum corneum (SC), epidermal membranes (EPID), or full-thickness skin (FULL).

g. The temperature was not reported. Solubilities of these compounds were determined at 37C.

h. The permeability coefficient of the unionized species was reported (Maitani et al., 1993).

i. Permeability coefficient has been corrected for the fraction unionized for measurements made over a range of pH.

j. Information obtained through personal communication from Barber (1996).
k. In addition to this strain (Sprague-Dawley (CD(SD)BR)), Fisher 344 CDF(F-344)/CrlBR rats were also used.
l. Permeability coefficients for this chemical and a different copenetrating chemical were measured simultaneously (see discussion in Appendix 7B).

Table7A.4 Shed Snake Skin Permeability Coefficients

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ul}[d]	pH[e]	Strain	Reference
	Acetanilide	0.45	135.2	32	9.77E-04	9.77E-04	1	7	Elaphe Obsoleta	Takahashi et al., 1993
	Aminopyrine	1.00	231.3	32	1.18E-04	1.18E-04	1	7	Elaphe Obsoleta	Takahashi et al., 1993
	Aniline	0.90	93.1	32	1.64E-02	1.64E-02	1	7	Elaphe Obsoleta	Takahashi et al., 1993
	Antipyrine	0.38	188.2	32	1.10E-05	1.10E-05	1	7	Elaphe Obsoleta	Takahashi et al., 1993
	Benzoic acid	1.87	122.1	37	2.43E-02	2.73E-02	0.89	3	Elaphe Obsoleta	Itoh et al., 1990b
	Butylparaben	3.57	194.2	37	2.26E-02	2.26E-02	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
	Clonidine	1.57	230.1	32	2.30E-04	2.30E-04	1[f]	4.6, 7	Elaphe Obsoleta	Fleeker et al., 1989
	Corticosterone	1.94	346.5	25	1.02E-04	1.02E-04	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
	Corticosterone	1.94	346.5	37	1.27E-04	1.27E-04	1	7.2	Elaphe Obsoleta	Itoh et al., 1990a
	m-Cresol	1.96	108.1	25	9.88E-03	9.88E-03	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
	m-Cresol	1.96	108.1	37	1.85E-02	1.85E-02	1	3	Elaphe Obsoleta	Itoh et al., 1990b
	Deoxycorticosterone	2.88	330.5	37	3.98E-03	3.98E-03	1	7.2	Elaphe Obsoleta	Itoh et al., 1990a
	Diazepam	2.99	284.8	32	3.07E-03	3.07E-03	1	7	Elaphe Obsoleta	Takahashi et al., 1993
	Ethylparaben	2.47	166.2	37	4.97E-03	4.97E-03	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
E	5-Fluorouracil (+ - + -)	-0.89	130.1	31	3.18E-04	ion	<0.1	4.75[g]	Elaphe Obsoleta	Rigg & Barry, 1990
E	5-Fluorouracil (+ - + -)	-0.89	130.1	31	4.59E-04	ion	<0.1	4.75[g]	Elaphe Obsoleta	Rigg & Barry, 1990
E	5-Fluorouracil (+ - + -)	-0.89	130.1	31	1.73E-04	ion	<0.1	4.75[g]	Python Molurus	Rigg & Barry, 1990
E	5-Fluorouracil (+ - + -)	-0.89	130.1	31	7.30E-05	ion	<0.1	4.75[g]	Python Molurus	Rigg & Barry, 1990
	Hydrocortisone	1.61	362.5	37	2.28E-05	2.28E-05	1	7.2	Elaphe Obsoleta	Itoh et al., 1990a
	p-Hydroxybenzoic acid	1.58	138.1	37	5.10E-04	5.54E-04	0.92	3	Elaphe Obsoleta	Itoh et al., 1990b
	m-Hydroxybenzyl alcohol	0.49	124.1	37	3.37E-04	3.37E-04	1	3	Elaphe Obsoleta	Itoh et al., 1990b
	m-Hydroxyphenylacetic acid	0.85	152.1	37	3.40E-04	3.54E-04	0.96	3	Elaphe Obsoleta	Itoh et al., 1990b
E	11α-Hydroxyprogesterone	N/A[h]	330.0	25	2.80E-04	2.80E-04	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
E	11α-Hydroxyprogesterone	N/A[h]	330.0	37	8.26E-04	8.26E-04	1	7.2	Elaphe Obsoleta	Itoh et al., 1990a
	Ibuprofen	3.50	206.3	37	8.81E-02	9.05E-02	0.97	3[i]	Elaphe Obsoleta	Itoh et al., 1990a
	Indomethacin	4.27	357.8	32	3.62E-03	3.62E-03	1[f]	2.9, 7	Elaphe Obsoleta	Fleeker et al., 1989

COMPOUND[a]	$logK_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ui}[d]	pH[e]	Strain	Reference
Ketoprofen	3.12	254.3	37	6.22E-03	6.46E-03	0.96	3	Elaphe Obsoleta	Itoh et al., 1990a
Methylparaben	1.96	152.1	25	4.69E-03	4.69E-03	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
Methylparaben	1.96	152.1	37	3.82E-03	3.82E-03	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
Naproxen	3.34	230.3	37	1.48E-02	1.53E-02	0.97	3	Elaphe Obsoleta	Itoh et al., 1990a
Phenol	1.46	94.1	25	5.23E-03	5.23E-03	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
Phenol	1.46	94.1	37	1.62E-02	1.62E-02	1	3	Elaphe Obsoleta	Itoh et al., 1990b
Propylparaben	3.04	180.2	37	8.75E-03	8.75E-03	1	7.2	Elaphe Obsoleta	Itoh et al., 1990b
Salicylic acid	2.26	138.1	25	8.00E-03	8.00E-03	1[f]	Range	Python Reticulatus	Harada et al., 1993
p-Toluidine	1.39	107.2	32	3.33E-02	3.33E-02	1	7	Elaphe Obsoleta	Takahashi et al., 1993
Water	-1.38	18.0	31	1.26E-03	1.26E-03	1	ND	Elaphe Obsoleta	Rigg & Barry, 1990
Water	-1.38	18.0	31	1.34E-03	1.34E-03	1	ND	Python Molurus	Rigg & Barry, 1990

a. The compound investigated. Measurements indicated at left by an E are excluded measurements and those indicated by a P are provisional measurements. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, 5-fluorouracil with two negative charges and two positive charges is indicated by (+ - + -).

b. Reported $logK_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets, in which case they were calculated (Daylight, 1995).

c. Permeability coefficients contained within brackets were digitized from figures in the reference.

d. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 7A.7.

e. Reported solution pH unless contained within brackets in which case the pH was calculated from the reported concentration and calculated pK_a values (see Table 7A.7). Compounds that are essentially undissociated are indicated by ND when no pH was reported.

f. Unionized species permeability coefficient was determined from measurements covering a range of pH (i.e., fraction unionized).

g. Information obtained through personal communication from Barry (1996).

h. The calculated $logK_{ow}$ of 6.86 (Daylight, 1995) is not valid.

i. The pH used in studying ibuprofen was not reported. We assume a pH of 3.0, which is the same pH at which penetration of the other carboxylic acids (indomethacin, ketoprofen, naproxen) and the distribution coefficients were measured.

Table 7A.5 Permeability Coefficients for Various Animals

	COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ui}[d]	pH[e]	Skin[f]	Strain	Reference
	Acyclovir	[-2.31]	225.0	37	1.50E-04	1.56E-04	0.96	[9.8]	FULL	Guinea Pig (Hartley)	Okamoto et al., 1991
P	Benzoic acid	1.87	122.1	N/A	4.66E-04	N/A	N/A	N/A	EPID	Pig (Ear)	Bhatti et al., 1988
	Butyl paraben	3.57	194.2	37	2.26E-03	2.26E-03	1	ND	FULL	Guinea Pig (Hartley)	Okamoto et al., 1991
E	DTMA[g] Chloride (+)	N/A	263.8	N/A	2.40E-05	ion	<0.1	7	FULL	Guinea Pig	Scala et al., 1968
	Ethanol	-0.31	46.0	30	5.12E-04	5.12E-04	1	ND	FULL	Marmoset	Scott et al., 1991
	Ethanol	-0.31	46.0	33	2.66E-03	2.66E-03	1	ND	FULL	Rabbit	Treherne, 1956
	Ethyl iodide	2.00	156.0	33	5.50E-03	5.50E-03	1	ND	FULL	Rabbit	Treherne, 1956
E	5-Fluorouracil (+ - + -)	-0.89	130.1	37	4.80E-04	ion	<0.1	[4.1]	FULL	Guinea Pig (Hartley)	Okamoto et al., 1991
	Glucose	[-3.53]	180.2	33	5.34E-05	5.34E-05	1	ND	FULL	Rabbit	Treherne, 1956
	Glycerol	-1.76	92.1	33	2.35E-04	2.35E-04	1	ND	FULL	Rabbit	Treherne, 1956
	Mannitol	-3.10	182.2	N/A	1.99E-03	1.99E-03	1	ND	EPID	Pig (Ear)	Bhatti et al., 1988
	Mannitol	-3.10	182.2	30	1.20E-04	1.20E-04	1	ND	FULL	Marmoset	Scott et al., 1991
E	6-Mercaptopurine (+ -)	0.01	152.2	37	2.32E-04	ion	<0.1	[4.6]	FULL	Guinea Pig (Hartley)	Okamoto et al., 1988
E	6-Mercaptopurine (+ -)	0.01	152.2	37	2.03E-04	ion	<0.1	[4.6]	SC[h,i]	Guinea Pig	Okamoto et al., 1989
E	6-Mercaptopurine (+ -)	0.01	152.2	37	2.50E-04	ion	<0.1	[4.6]	FULL	Guinea Pig (Hartley)	Okamoto et al., 1991
	Methanol	-0.77	32.0	33	2.50E-03	2.50E-03	1	ND	FULL	Rabbit	Treherne, 1956
	Nicorandil	[0.69]	211.2	37	5.08E-04	5.08E-04	1	[8.0]	FULL	Guinea Pig (Hartley)	Sato et al., 1989
	Nicorandil	[0.69]	211.2	37	2.85E-04	2.85E-04	1	[8.0]	FULL	Pig (Immature, LWD)	Sato et al., 1989
	Nicorandil	[0.69]	211.2	37	1.69E-04	1.69E-04	1	[8.0]	FULL	Dog	Sato et al., 1989
E	Nicotinic acid (-)	[0.77]	123.1	N/A	3.60E-04	ion	<0.1	7	FULL	Guinea Pig	Scala et al., 1968
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	30	1.01E-04	ion	<0.1	ND	FULL	Marmoset	Scott et al., 1991
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	N/A	7.99E-04	ion	<0.1	ND	FULL	Rabbit	Walker et al., 1983
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	N/A	1.96E-03	ion	<0.1	ND	FULL	Guinea Pig	Walker et al., 1983
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	N/A	3.53E-04	ion	<0.1	ND	FULL	Nude Rat	Walker et al., 1983
E	Paraquat Dichloride (+ +)	[-5.65]	257.3	N/A	3.72E-04	ion	<0.1	ND	FULL	Mouse	Walker et al., 1983
	Salicylic acid	2.26	138.1	25	1.16E-02	2.38E-02	0.49	3	FULL	Nude Mouse	Harada et al., 1993
E	Sulfanilic acid (+ -)	[-0.94]	173.8	37	8.20E-04	ion	<0.1	[3.4]	FULL	Guinea Pig (Hartley)	Okamoto et al., 1991
	Thiourea	-1.02	76.1	33	1.70E-04	1.70E-04	1	ND	FULL	Rabbit	Treherne, 1956

	COMPOUND[a]	$logK_{ow}$[b]	MW	T (C)	P_{cw} (rep)[c]	P_{cw} (adj)	f_{ui}[d]	pH[e]	Skin[f]	Strain	Reference
	Toluene	2.73	92.1	N/A	3.49E-03	3.49E-03	1	ND	EPID	Pig (Ear)	Bhatti et al., 1988
E	TPBS[j] Sodium salt) (-)	N/A	N/A	N/A	1.56E-04	ion	<0.1	7	FULL	Guinea Pig	Scala et al., 1968
	Urea	-2.11	60.1	33	1.40E-04	1.40E-04	1	ND	FULL	Rabbit	Treherne, 1956
	Water	-1.38	18.0	N/A	3.46E-03	3.46E-03	1	ND	EPID	Pig (Ear)	Bhatti et al., 1988
	Water	-1.38	18.0	30	1.80E-03	1.80E-03	1	ND	FULL	Pig	Galey et al., 1976
	Water	-1.38	18.0	30	8.12E-04	8.12E-04	1	ND	FULL	Marmoset	Scott et al., 1991
	Water	-1.38	18.0	N/A	2.53E-03	2.53E-03	1	ND	FULL	Rabbit	Walker et al., 1983
	Water	-1.38	18.0	N/A	4.42E-03	4.42E-03	1	ND	FULL	Guinea Pig	Walker et al., 1983
	Water	-1.38	18.0	N/A	1.52E-03	1.52E-03	1	ND	FULL	Nude Rat	Walker et al., 1983
	Water	-1.38	18.0	N/A	1.44E-03	1.44E-03	1	ND	FULL	Mouse	Walker et al., 1983

a. The compound investigated. Measurements indicated at left by E are excluded measurements and those indicated by a P are provisional measurements. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, 5-fluorouracil with two negative charges and two positive charges is indicated by (+-+-).

b. Reported $logK_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets (e.g., for nicorandil [0.69]), in which case they were calculated (Daylight, 1995).

c. Permeability coefficients contained within brackets were digitized from figures in the reference.

d. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 7A.7.

e. Reported solution pH unless contained within brackets in which case the pH was calculated from the reported concentration and calculated pK_a values (see Table 7A.7). Compounds that are essentially undissociated are indicated by ND when no pH was reported.

f. Type of skin used in the study: isolated stratum corneum (SC), epidermal membranes (EPID), or full-thickness skin (FULL).

g. Dodecyltrimethylammonium

h. Stratum corneum permeability coefficient is calculated from measurements with intact full-thickness and stripped full-thickness skin.

i. Permeability coefficient obtained by combining a SC-water partition coefficient, SC diffusion coefficient and SC thickness, which are consistent with measured penetration data.

j. Sodium Tetrapropylenebenzenesulfonate

Table 7A.6 Stratum Corneum-Water Partition Coefficients Determined with Animal Skin

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	K_{cw}[c]	Basis[d]	f_{ui}[e]	pH[f]	t_{equil} (hrs)[g]	Species	Reference
Acetamidophenol	0.51	151.2	25	5.0	Dry Mass	1	ND	24	Rhesus M.	Surber et al., 1990b
Acetamidophenol	0.51	151.2	25	4.5	Dry Mass	1	ND	24	HLGuinea P.	Surber et al., 1990b
Atenolol[h]	0.16	266.3	37	0.2	N/A	0.98	[10.9]	8	HLRat[i]	Kobayashi et al., 1994
Butanol	0.88	74.1	37	5.3	Excised Mass[j]	1	ND	Overnight	HLMouse	Flynn et al., 1981
Cyclopropanoyl Propranolol[k] (+)	[3.94]	327.0	37	0.6	Excised Mass	<0.1	4	6	HLMouse	Ahmed et al., 1995
Ethanol	-0.31	46.0	37	4.5	Excised Mass[j]	1	ND	Overnight	HLMouse	Flynn et al., 1981
Heptanol	2.72	116.0	37	24.1	Excised Mass[j]	1	ND	Overnight	HLMouse	Flynn et al., 1981
Hexanol	2.03	102.2	37	14.0	Excised Mass[j]	1	ND	Overnight	HLMouse	Flynn et al., 1981
Isovaleryl Propranolol[k] (+)	[5.04]	343.3	37	1.5	Excised Mass	<0.1	4	6	HLMouse	Ahmed et al., 1995
Lindane (C_w = 4ug/ml)	[3.72][l]	291.0	37	160.0	Excised Volume[m]	1	ND	18	Fisher Rat	Jepson et al., 1994
Lindane (C_w = 8ug/ml)	[3.72][l]	291.0	37	235.0	Excised Volume[m]	1	ND	18	Fisher Rat	Jepson et al., 1994
6-Mercaptopurine (+ -)	0.01	152.2	37	1.3	Wet Mass	<0.1	[4.6]	24	Guinea Pig	Okamoto et al., 1989
Morphine[h] (+)	0.76	285.3	37	0.0	N/A	<0.1	[4.9]	8	HLRat[i]	Kobayashi et al., 1994
Nicorandil	[0.65]	211.2	37	10.4	Dry Mass	1	[7-8]	24	HLRat	Sato et al., 1989
Nicorandil	[0.65]	211.2	37	8.6	Dry Mass	1	[7-8]	24	Guinea Pig	Sato et al., 1989
Nicorandil	[0.65]	211.2	37	4.5	Dry Mass	1	[7-8]	24	Dog	Sato et al., 1989
Nicorandil	[0.65]	211.2	37	7.9	Dry Mass	1	[7-8]	24	Pig	Sato et al., 1989
Nifedipine[h]	[2.20]	346.3	37	3.9	N/A	1	ND	8	HLRat[i]	Kobayashi et al., 1994
p-Nitrophenol	1.91	139.1	25	12.4	Dry Mass	1	4.2	> 24	Pig	Williams et al., 1994
Nonanol	4.26	144.0	37	118.0	Excised Mass[j]	1	ND	Overnight	HLMouse	Flynn et al., 1981
Octanol	3.00	130.2	37	68.5	Excised Mass[j]	1	ND	Overnight	HLMouse	Flynn et al., 1981
Parathion (C_w = 3.5ug/ml)	[3.83][l]	291.0	37	160.0	Excised Volume[m]	1	ND	18	Fisher Rat	Jepson et al., 1994
Parathion (C_w = 7ug/ml)	[3.83][l]	291.0	37	180.0	Excised Volume[m]	1	ND	18	Fisher Rat	Jepson et al., 1994
Pentanol	1.56	88.0	37	5.1	Excised Mass[j]	1	ND	Overnight	HLMouse	Flynn et al., 1981
Pentyloxyphenol	3.50	180.2	25	153.0	Dry Mass	1	ND	24	Rhesus M.	Surber et al., 1990b
Pentyloxyphenol	3.50	180.2	25	140.0	Dry Mass	1	ND	24	HLGuinea P.	Surber et al., 1990b
Phenol	1.46	94.1	25	11.7	Dry Mass	1	7.1	> 24	Pig	Williams et al., 1994

COMPOUND[a]	$\log K_{ow}$[b]	MW	T (C)	K_{cw}[c]	Basis[d]	f_{ui}[e]	pH[f]	t_{equil} (hrs)[g]	Species	Reference
Propanol	0.25	60.0	37	4.4	Excised Mass[j]	1	ND	Overnight	HLMouse	Flynn et al., 1981
Propranolol[k] (+)	2.98	259.3	37	0.1	Excised Mass	<0.1	4	6	HLMouse	Ahmed et al., 1995
Vinpocetine[h]	N/A[n]	350.5	37	9.5	N/A	N/A	> [7]	8	HLRat[i]	Kobayashi et al., 1994

a. The compound investigated. All positive (+) and negative (-) ionic charges (for the chemical at experimental conditions) are indicated. For example, 6-mercaptopurine with one negative and one positive charge is indicated by (+ -).
b. Reported $\log K_{ow}$ are taken from the Starlist (Hansch et al., 1995), unless contained within brackets, in which case they were calculated (Daylight, 1995).
c. Reported stratum corneum-water partition coefficients prior to any adjustment.
d. Basis on which the unadjusted SC-water partition coefficient was reported. SC concentration expressed relative to a gram of dry SC (Dry Mass), a gram of hydrated SC (Wet Mass), or a unit volume or mass of freshly excised SC (Excised Volume or Excised Mass). See Section 6.2.1.
e. Fraction unionized determined from pK_a values calculated in SPARC at 25C and adjusted to the experimental temperature as listed in Table 7A.7.
f. Reported solution pH unless contained within brackets in which case the pH was calculated from the reported concentration and calculated pK_a values (see Table 7A.7). Compounds that are essentially undissociated are indicated by ND when no pH was reported.
g. Time allowed for the absorbing chemical to equilibrate with the stratum corneum
h. Partition coefficients calculated as the ratio of concentrations in full-thickness skin and vehicle at the end of the penetration experiments
i. Full-thickness skin of WBN/ILA-HT hairless rats
j. Stratum corneum-water partition coefficients have units of cm^3/(g freshly excised epidermis).
k. The average partition coefficient of two stereoisomers
l. This $\log K_{ow}$ is a calculated value taken from the US EPA interim report on dermal exposure assessment (US EPA, 1992).
m. Partition coefficients are relative to the skin volume. Freshly excised full-thickness skin weighing 0.3 mg was used in the partitioning experiment.
n. The calculated $\log K_{ow}$ of 4.72 (Daylight, 1995) is invalid (structural fragments of this molecule are not adequately represented by Daylight software).

Table 7A.7 Temperature Effects on f_{ui} and Calculation of Unmeasured pH

COMPOUND[a]	$pK_a(25)$[b]	T (°C)	ΔH (kcal/mol)[c]	$pK_a(T)$[d]	C_w (mol/L)[e]	pH[f]	f_{ui}[g]	Reference
Acyclovir	8.69	37	10	8.40	0.002	[9.8]	0.96	Okamoto et al., 1991
Atenolol	9.52	37	10	9.23	0.038	[10.9]	0.98	Kobayashi et al., 1994
Benzoic Acid	3.91	37	0	3.91		3	0.89	Itoh et al., 1990b
Diclofenac	4.07	37	0	4.07		3	0.92	Maitani et al., 1993
Fentanyl	7.25	37	10	6.96		7.4	0.73	Roy at al., 1994
5-Fluorouracil (-+- → +-+-)	5.59	37	5	5.45	0.002	[4.1][h]	<0.1	Okamoto et al., 1991
5-Fluorouracil (- - → +- -)	11.95	37	5	11.81	0.002	[4.1][h]	<0.1	Okamoto et al., 1991
5-Fluorouracil (- - → –+-)	12.63	37	5	12.49	0.002	[4.1][h]	<0.1	Okamoto et al., 1991
Flurbiprofen	4.38	37	0	4.38		4.7	0.32	Hatanaka et al., 1990
p-Hydroxybenzoic Acid	4.05	37	0	4.05		3	0.92	Itoh et al., 1990b
m-Hydroxyphenylacetic Acid	4.41	37	0	4.41		3	0.96	Itoh et al., 1990b
Ibuprofen	4.55	37	0	4.55		4.44	0.58	Hatanaka et al., 1990
Ibuprofen	4.55	37	0	4.55		3	0.97	Itoh et al., 1990a
Indomethacin	4.54	37	0	4.54		5.15	0.18	Hatanaka et al., 1990
Indomethacin	4.54	37	0	4.54		4.9	0.30	Okumura et al., 1989
Ketoprofen	4.41	37	0	4.41		3.72	0.79	Hatanaka et al., 1990
Ketoprofen	4.41	37	0	4.41		3	0.96	Itoh et al., 1990a
6-Mercaptopurine (+- → -)	6.3	37	10	6.16	0.001	[4.6]	<0.1	Okamoto et al., 1988[i]
Morphine HCl (- → +-)	10.56	37	10	10.27	0.031	[4.9][j]	<0.1	Kobayashi et al., 1994
Morphine HCl (N → +)	9.40	37	10	9.11	0.031	[4.9][j]	<0.1	Kobayashi et al., 1994
Morphine HCl (N → -)	9.51	37	5	9.37	0.031	[4.9][j]	<0.1	Kobayashi et al., 1994
Naproxen	4.49	37	0	4.49		3	0.97	Itoh et al., 1990a
Nicorandil	2.87	37	5	2.72	0.188[k]	[8.0]	1	Hatanaka et al., 1990[l]
Sufentanil	6.44	37	10	6.15		7.4	0.95	Roy et al., 1994
Sulfanilic acid (- → +-)	3.44	37	10	3.15	0.002	[3.4]	<0.1	Okamoto et al., 1991
Sulfanilic acid (N → +)	3.98	37	10	3.69	0.002	[3.4]	<0.1	Okamoto et al., 1991
Sulfanilic acid (N → -)	2.39	37	0[m]	2.39	0.002	[3.4]	<0.1	Okamoto et al., 1991
p-Toluidine	5.20	32	7.5	5.07		7	1	Takahashi et al., 1993

COMPOUND[a]	$pK_a(25)$[b]	T (°C)	ΔH (kcal/mol)[c]	$pK_a(T)$[d]	C_w (mol/L)[e]	pH[f]	f_{ui}[g]	Reference
Vidarabine	5.91	37	5	5.77	N/A	> [7]	0.94	Kim et al., 1992
Vinpocetine	8.49	37	10	8.20	N/A	> [7]	N/A	Kobayashi et al., 1994

a. The compound investigated. Cations are indicated with (+), anions with (-), and zwitterions with (+-).
b. pK_a values calculated in SPARC at 25C using methods described in Section 5.2.1.
c. These heats of ionization are approximate values obtained from the literature (Sober, 1968). See also Sections 5.2.1 and 7.3.
d. Calculated using an integrated form of the van't Hoff equation, Eq. (5.2).
e. Solution concentration provided only when it was needed to calculate the pH.
f. The pH was reported in the original paper, unless contained within brackets (e.g. for acyclovir [9.8]) in which case it was calculated from $pK_a(T)$ and the solution concentration assuming that pH was 7.0 prior to chemical addition.
g. The fraction unionized was calculated using Eq. 7.8 when one pK_a is dominant. Otherwise it was determined from a more rigorous solution of simultaneous equilibrium (as described in Section 5.2.1).
h. The pH of 5-fluorouracil was calculated assuming that the drug was added to solution in its net-neutral form.
i. This result also applies to the Okamoto et al., 1989 and Okamoto et al., 1991 investigations.
j. Morphine hydrochloride (protonated morphine) was initially added to solution.
k. The concentration of a saturated solution of nicorandil was reported by Morimoto et al., 1992.
l. The calculated natural pH and fraction unionized were the same for Sato et al., 1989 and Sato et al., 1991.
m. This dissociation for sulfanilic acid was not adjusted for effects of temperature.

7.9. Appendix 7B: Documentation on Permeability Coefficients

This appendix contains specific information about the permeabilities included in the database. Details are arranged alphabetically by the last name of the leading author of the investigation. Conclusions made about the ionization of chemicals are based on calculations made in SPARC (SPARC, 1995), unless otherwise stated.

Ackermann et al., 1987

Permeability coefficients were taken without alteration from Table 1.

Aguiar and Weiner, 1967

The permeability coefficient of chloramphenicol at 3 temperatures were taken from Table II.

Ahmed et al., 1995

Permeability coefficients were taken directly from Table 4. The R and S-stereoisomer permeability coefficients were averaged. These compounds were essentially all ionized.

Aspe et al., 1995

The permeability of cidofovir was taken as the average of three permeability coefficients, from a solution at pH = 7, reported in Table 1.

Behl et al., 1980

Behl *et al.* studied the effect of prolonged contact of hairless mouse skin with water on permeability coefficients. The authors showed that permeability coefficients increase after extended periods of hydration. Since other permeability coefficients in the database we have assembled were measured on unhydrated skins or skins that were hydrated for short periods, the permeability coefficients with the shortest hydration time (0.3-0.8 hours) from Table I were selected for the valid database. Permeability coefficients were determined with either water or ethanol as a copenetrant. The concentrations were dilute (alcohol concentrations $< 10^{-4}$M) and were probably below damaging concentrations. Six reported measurements were averaged for methanol, two

for ethanol, two for butanol, and permeability coefficients were reported singly for hexanol, heptanol and octanol.

Behl et al., 1983a

The permeability coefficient for methanol was taken from Table 1. The average of permeability coefficients measured in skin without prior hydration from sets #1 and #2 were used (see discussion of hydration time in documentation of Behl *et al.* (Behl *et al.*, 1980)). The average permeability coefficient, for zero hydration time, was taken from Table II for butanol and from Table III for hexanol. Permeability coefficients of butanol and hexanol were determined with methanol as a copenetrant and the methanol permeability coefficient is the average of one permeability coefficient where butanol was the copenetrant (set #1) and one where hexanol was the copenetrant (set #2). The concentrations were dilute (alcohol concentrations $< 10^{-4}$M) and were probably below damaging concentrations.

Behl et al., 1983b

The stratum corneum permeability coefficient of methanol and phenol were taken from Table IX. The abdominal skin and dorsal skin measurements were not statistically different and were averaged. Phenol and methanol were copenetrants. The concentrations were dilute (alcohol concentrations $< 10^{-4}$M) and were probably below damaging concentrations.

Behl et al., 1984

The permeability of hydrocortisone was taken from Table I. The phase I, combined site (C) permeability coefficients for mice of age 35, 516, and 657 days were averaged. (Permeability coefficient data for mice of 5, 15, and 20 days old were larger and less certain and not added to the database.) The phase I permeability is the permeability coefficient observed for periods less than that required for hydration damage of skin (see discussion of hydration time in documentation of Behl *et al.* (Behl *et al.*, 1980)).

Bhatti et al., 1988

The permeability coefficients through epidermal membranes were taken from Table I. A permeability coefficient for paraquat was not included in these databases because the membrane was reported to be damaged.

Bond and Barry, 1988a

The permeability coefficient of 5-fluorouracil was taken unaltered from Table I.

Bond and Barry, 1988b

Hexanol and water permeabilities were taken from text on page 488.

Durrheim et al., 1980

Permeabilities of methanol, ethanol, butanol, hexanol, and octanol were taken from Table III at the temperatures 20°C and 25°C. All measurements are for permeation through full-thickness skin.

Fleeker et al., 1989

The permeability coefficients of the unionized form of clonidine was taken from Table I, and the permeability coefficient of the unionized form of indomethacin was taken from Table IV.

Flynn et al., 1981

Permeability coefficients for normal alcohols (C_1 through C_{10}) were reported for full-thickness skin, heat separated epidermis, heat separated dermis, and tape-stripped full thickness skin. Data for methanol through hexanol were reported in Table IV as resistances through the SC calculated from the difference of resistances in the full-thickness skin and heat separated dermis. Data for heptanol through decanol were taken from the values for heat separated epidermis reported in Table 3.

Galey et al., 1976

The permeability of full-thickness pig skin to water was taken from Table II.

Ghosh et al., 1993

Permeability coefficients were taken without alteration from Table 1.

Harada et al., 1993

Salicylic acid permeability coefficients for all species were obtained from measurements reported in Table 1. Unionized permeability coefficients were calculated from reported flux values reported by dividing by a concentration of 500 μg/mL and then adjusting by the fraction unionized. We judged the pH = 2.0 medium to be too severe for stratum corneum, and excluded this measurement. All other pH levels, for which the compound was < 90% ionized as estimated from pK_a values calculated by SPARC (i.e., 3.0, 3.5), were equally weighted in this average. For the hairless rat and the nude mouse the pH = 3.5 measurement was not reported so the value is based only on measurements made at pH = 3.0.

Hatanaka et al., 1990

All permeability coefficients were digitized from Figure 13. Measurements were made on intact full-thickness and stripped full-thickness skin. According to the authors, the effect of permeation resistance in the dermis was corrected by calculating the permeability coefficient of the stratum corneum from the permeation data of stripped skin. These same data also appear in a paper comparing these data with permeability coefficients measured in human skin (Morimoto *et al.*, 1992).

Hayashi et al., 1992

The permeability coefficient for the unionized from of indomethacin was converted from the value given (in cm/s) in Table I.

Huq et al., 1986

These authors calculated the SC permeability coefficient from the differences in resistances presented by whole skin and tape-stripped (no SC) skin. The permeability coefficients in the database were taken from the SC permeability values reported in Table 4. SC-permeability values were not calculated for three chemicals (2-nitrophenol, 2,4-dimethylphenol and 2,4,6-trichlorophenol) because the permeability coefficient from tape-stripped skin was not available. The permeability coefficient values for these three chemicals were taken from the whole skin values in Table 1.

Itoh et al., 1990a

Permeability coefficients for black rat snake were taken directly from Table II for ibuprofen, naproxen, ketoprofen, deoxycorticosterone, 11α-Hydroxyprogesterone, corticosterone, and hydrocortisone. Permeability coefficients for indomethacin and progesterone are not included because they were not measured from aqueous solution. Permeability coefficients for the parabens are not included because they are identical to those reported by Itoh *et al.* (Itoh *et al.*, 1990b).

Itoh et al., 1990b

The permeability coefficients at 25°C of phenol, m-cresol, methylparaben, 11α-hydroxyprogesterone, and corticosterone were taken from Table II. The 37°C permeability coefficients of ethylparaben, propylparaben, butylparaben, phenol, benzoic acid, p-hydroxybenzoic acid, m-cresol, m-HBAl (m-hydroxybenzyl alcohol), and m-hydroxyphenyl acetic acid were taken from Table III. The 37°C permeability coefficient of methylparaben was not taken from Table III, but from Table VI (where the values reported in Table III are averaged with several additional values).

Jetzer et al., 1986

Permeation of 2,4-dinitrophenol and 4-nitrophenol, which are different from those reported by Huq *et al.* (Huq *et al.*, 1986), are taken from Table I. The value for 4-nitrophenol is the average value of permeability coefficients determined at two concentrations.

Jolicoeur et al., 1992

The permeability coefficient of etorphine was taken from Table I. Three measurements on hairless mouse skin were averaged.

Katayama et al., 1994

Permeability of mannitol, cortisone, and indomethacin (data at pH = 3 were used) were taken directly from Table 1.

Kikkoji et al., 1991

Permeability coefficients for full-thickness skin were reported in Table II.

Kim et al., 1992

Permeability coefficients through whole skin are digitized from Figure 3. These data were originally reported in three other papers (which are reference numbers 3, 4 and 5).

Kobayashi et al., 1994

Permeability coefficients presented in Table II (in cm/s) from an aqueous vehicle were converted from cm/s to cm/hr.

Liu et al., 1994

A stratum corneum permeability coefficient for β-estradiol was reported in the text on page 1781. The permeability coefficient was originally reported in another paper (reference number 1 in this paper).

Maitani et al., 1993

The permeability coefficient of diclofenac was taken from Table II, with unit conversion from cm/s to cm/hr. The 0% v/v ethanol value was selected.

Ogiso et al., 1994

The permeability coefficients for three high MW compounds (FITC dextrans FD-4 (MW = 4400), FD-10 (MW = 9600), and FD-70 (MW = 69000)) were reported in Table 1.

Okamoto et al., 1988

The permeability coefficient of 6-mercaptopurine was taken directly from Table II. The control value (guinea pig skin without pretreatment) was selected.

Okamoto et al., 1989

In this paper, partition coefficients between stratum corneum and vehicle and stripped skin and vehicle were directly determined. Then the penetration profile of 6-mercaptopurine through the stripped skin was fitted to the solution of the one-membrane

differential mass balance to determine the diffusion coefficient and thickness of the stripped skin. The diffusion coefficient and thickness of the SC were then determined by fitting the penetration profile of 6-mercaptopurine through intact (unstripped) skin to the solution of the two-membrane differential mass balance to which the previously determined parameters (partition coefficients, diffusion and thickness for the stripped skin) were applied. They report their results as the SC-water partition coefficient, a SC diffusion coefficient, and SC thickness. The permeability coefficient is then calculated from D_cK_{cw}/L_c.

Okamoto et al., 1991

The authors determined (D_c/L_c^2) and ($K_{cw}L_c$) by analyzing the cumulative appearance of penetrating chemical in the receptor fluid using the complete solution of a one-membrane differential material balance. Permeability coefficients included in the database were calculated by multiplying these reported parameters (i.e., (D_c/L_c^2) times ($K_{cw}L_c$)) which are reported in Table III. The measurements in this paper are different than those reported in their other two papers (Okamoto *et al.*, 1988; Okamoto *et al.*, 1989).

Okumura et al., 1989

Permeability coefficients in this paper are different from permeability coefficients in another related paper (Hatanaka *et al.*, 1990) because different hairless rat strains were used. The permeability coefficients for diclofenac sodium, dopamine hydrochloride, isoproterenol hydrochloride, indomethacin, and water were reported in the text on page 1406 in units of cm/s. Permeability coefficients for disodium cromoglycate, diltiazem hydrochloride, and papaverine hydrochloride were digitized from Figure 3a and converted into units of cm/hr.

Rigg and Barry, 1990

Water and 5-fluorouracil permeability coefficients are reported for hairless mouse skin and shed skin of two different snakes (P. molurus, and E. obsoleta). Water permeabilities were determined by diffusion cell experiments lasting about 6 hours. These investigators studied the effect of pretreatment of skin with water for 1 to 8 days, which did affect permeability coefficients in hairless mouse (although apparently not for snake skin). The water permeability coefficient in hairless mouse was taken from Table 1 for the 1 day pretreatment with water (the shortest pretreatment time studied). The water permeability coefficients for snake skin were calculated as the average of the

permeability coefficients reported in Table 1 for water pretreatment times of 1 to 8 days. For P. molurus permeability coefficients were reported for both dorsal and ventral skin and were different. The values for dorsal skin were arbitrarily chosen for the database. The normal saline and control permeability coefficients of 5-fluorouracil were taken respectively from Table 2 and Table 3 for all species. Again, the dorsal skin of P. molurus was used. These two 5-fluorouracil permeability coefficients were not averaged since the Table 2 (normal saline control) permeability coefficient was pretreated with saline for 12 hours and the Table 3 permeability coefficient (control) were not pretreated with water.

Roberts and Anderson, 1975

The permeability coefficient of phenol from a water vehicle was taken from Table 2 and converted to appropriate units.

Roy et al., 1994

The permeability of fentanyl, sufentanil, and morphine were taken directly from Table 1.

Ruland and Kreuter, 1991

The permeability coefficients of 20 amino acids at physiological pH (7.4) and at the pH of the isoelectric point were taken without alteration from Table 2. Based on pK_a values calculated by SPARC and the pH reported in this paper, we calculate that the ionic condition of the amino acids was zwitterionic for all but four measurements. The exceptions were aspartic acid at pH = 7.4 and glutamic acid at pH = 7.4 (which were both net anionic), and lysine at pH = 7.4 and arginine at pH = 7.4 (which were both net positive).

Sato et al., 1989

Permeability coefficients for nicorandil in hairless rat, guinea pig, dog, and pig skin were taken directly from Table 1.

Sato et al., 1991

The permeability coefficient of nicorandil in hairless mouse given in Table 1 was not reported previously (Sato *et al.*, 1989).

Scala et al., 1968

Permeability coefficients measured using whole skin were presented in Table II and converted to consistent units. The permeability coefficients are based on penetration over 10-15 hours.

Scott et al., 1991

Permeability coefficients for rat skin were taken directly from Table I. Permeability coefficients for marmoset skin were calculated as the average for measurements made with abdomen, back, thigh, and thorax skin of the marmoset.

Takahashi et al., 1993

Permeability coefficients were taken directly from Table 1.

Tojo et al., 1987

The authors calculated stratum corneum permeability coefficients from the measurements on full-thickness skin and from stripped full-thickness skin (no SC) reported in Table II by assuming a bilaminate model.

Treherne, 1956

Permeability coefficients through whole skin were taken from Table II with conversion from cm/min to cm/hr.

Walker et al., 1983

Permeability coefficients were taken without alteration from Table 1.

Wearley et al., 1990

Permeability coefficients for the amino acids alanine, glycine, leucine, and valine were calculated from the flux measurements reported in Table III divided by the concentration (10 mM).

7.10. Appendix 7C: Review of Prior Investigations

1. Marzulli *et al.*, 1969

Marzulli and colleagues (unpublished results of Marzulli, reviewed in Marzulli *et al.* (Marzulli *et al.*, 1969)) reported that the back skin of weanling pigs most closely approximates human forearm skin with regard to its resistance to penetration of several classified chemical warfare agents and agent stimulants. The forearm of the chimpanzee was less permeable than that of man, whereas the back skins of monkey, dog, cat, horse, rabbit, goat, guinea pig, and mouse were, in that order, increasingly more permeable than the forearm skin of man. The more permeable skins also showed a greater enhancement effect of vehicles and agent stimulants. The type of vehicle and other details of these, probably *in vitro* experiments, were not provided.

2. Bartek *et al.*, 1972

Bartek and colleagues determined *in vivo* dermal absorption by analyzing chemical in urine in rats, rabbits, miniature swine, and man. Five chemicals were studied (haloprogin, N-acetylcysteine, cortisone, testosterone, caffeine, and butter yellow) by depositing chemical on skin using acetone which evaporated (Bartek *et al.*, 1972). The absorption data demonstrate that skin permeability for different animal species increases in the following order: man, pig, rat, and rabbit. The authors also reference Tregear (Tregear, 1966) as determining that the skin of pig, guinea pig, rat, and rabbit were increasingly more permeable than human skin *in vitro* to organic solutes and ions. The Tregear results were not available for our analysis.

3. Chowhan and Pritchard, 1978

Chowhan and Pritchard studied the penetration of naproxen from oil-in-water cream vehicles through human, rat, and rabbit skins (Chowhan and Pritchard, 1978). If naproxen in the oil-in-water cream, which was adjusted to a pH of 7.5, ionizes the same as it would in aqueous solution, then essentially all of the naproxen would be ionized. The *in vitro* mean flux measured in the control experiments indicates that excised human skin was the least permeable (mean flux = 1.66 $\mu g/cm^2/hr$), rat skin was more permeable (mean flux = 3.75 $\mu g/cm^2/hr$), and excised rabbit skin was the most permeable (mean flux = 5.86 $\mu g/cm^2/hr$) to naproxen. The lag time for naproxen penetrating through human skin was approximately 100 hours, while the lag time for naproxen penetrating through rat and rabbit skin was approximately 14 hours. These lag times suggest, at least in the conventional interpretation, that species differences arise from differences in the skin thickness or the diffusion coefficient. Other data in this study suggested that the effect of surfactants on the penetration of compounds similar to naproxen can be quite different in human and animal skin.

4. Wester and Noonan, 1980

Wester and Noonan reviewed several *in vivo* and *in vitro* experimental investigations of the relationship of animal and human skin permeability coefficients (Wester and Noonan, 1980). An analysis by Wester and Maibach is so similar to this one that it will not be discussed separately (Wester and Maibach, 1986).

The authors reviewed several *in vivo* investigations of dermal absorption of acetone deposited chemicals: (1) the Bartek and colleagues investigation already discussed (Bartek *et al.*, 1972), (2) an experimentally similar investigation by Bartek and La Budde which found that DDT, lindane, parathion, and malathion penetrated much more rapidly in rabbit than in pig, squirrel monkey, or man which are similar, (3) an

investigation of the dermal absorption of hydrocortisone, testosterone, and benzoic acid which showed the rhesus monkey and man to have similar barriers to penetration for these compounds (Wester and Maibach, 1975a; Wester and Maibach, 1975b; Wester and Maibach, 1976; Wester and Maibach, 1977), and (4) an investigation of the dermal absorption of neat hydrocortisone and benzoic acid which showed that guinea pig and human skin were similar, and analogous measurements for testosterone which showed that guinea pig skin was more permeable than human skin (Andersen *et al.*, 1980). The authors summarize these results with a recommendation that percutaneous absorption in the pig and monkey (rhesus or squirrel) is in most cases similar to man, whereas in the rat and especially in the rabbit, skin penetration is greater than that observed in man. The amount of absorption in the guinea pig was similar to man for hydrocortisone and benzoic acid, but higher for testosterone.

The authors also reviewed three *in vitro* rankings of skin permeability in different species, two of which we have already discussed: (1) from experiments which he performed, Tregear provided the following ranking of permeabilities: rabbit > rat > guinea pig > man (Tregear, 1966), (2) Marzulli and colleagues observed the following ranking of permeabilities: mouse > guinea pig > goat > rabbit > horse > cat > dog > monkey > weanling pig > man > chimpanzee (Marzulli *et al.*, 1969), and (3) McGreesh observed the following ranking of permeabilities: rabbit > rat > guinea pig > cat > goat > monkey > dog > pig (McGreesh, 1965). Also reviewed was the study of Campbell and others who found that human skin was less permeable than rat or rabbit to scopolamine while the relative order of rat and rabbit skin permeabilities depends both on skin location (back and side) and the method used to remove the hair (Campbell *et al.*, 1976). They concluded that, considering the different compounds used in each study to rank the species and the differences in origin of the skin sample (back, forearm), the studies generally showed the skin of common laboratory animals (rat, rabbit, and guinea pig) is

more permeable than the skin of man. They also cited notable discrepancies to this trend. For example, the *in vitro* findings of Stoughton who observed that human skin and hairless mouse skin have similar absorption for several compounds disagrees with the large differences observed by other researchers ((Marzulli *et al.*, 1969); among others).

5. Bronaugh *et al.*, 1982

Bronaugh, and colleagues compared the percutaneous absorption of three chemicals (benzoic acid, acetylsalicylic acid, and urea) through the skin of four animals (Hormel miniature pigs, Osborne-Mendel rats, NIH hairless mice, and Swiss mice), and human skin using an *in vitro* diffusion cell technique (Bronaugh *et al.*, 1982). The compounds were delivered in a petrolatum vehicle. They concluded that the animal model of choice depends upon the compound. For benzoic acid and acetylsalicylic acid, permeability coefficients measured in pig and rat are similar to those in human skin, while hairless mouse and mouse skins are much more permeable than human skin. These investigators recommended that slowly absorbed (hydrophilic) compounds such as urea should be investigated with animals that are not densely haired, as the haired species (mouse and rat) absorbed urea more readily than either of the lesser haired species (pig, human, hairless mouse). They also compare several different studies which we discussed previously (i.e., (Andersen *et al.*, 1980; Bartek *et al.*, 1972; Chowhan and Pritchard, 1978; Stoughton, 1975; Tregear, 1966)). Based on their comparison, which was also published in the US EPA's interim report on dermal absorption (US EPA, 1992), they observed that values for even the most permeable animal skins are often well within an order of magnitude of values for human skin. However, this data comparison combines observations with very different experimental techniques which might not be appropriate. The authors also present results of measured skin layer thickness and hair follicle density in these different species.

6. Hawkin and Reifenrath, 1986

Hawkin and Reifenrath made *in vitro* percutaneous absorption measurements using neat compounds applied to skin and allowed to evaporate to air circulated over the applied dose (Hawkins and Reifenrath, 1986). For studies on benzo(a)pyrene absorption without sodium azide (NaN_3) they found that mouse skin (6% of dose absorbed) was more permeable than human skin (1% of dose absorbed) which was more permeable than pig skin (0.6% of dose absorbed). In a similar study using eleven compounds (DDT, benzo(a)pyrene, fluocinonide acetonide, progesterone, lindane, testosterone, parathion, diisopropyl fluorophosphonate, malathion, benzoic acid, and caffeine) they found that there was no statistically significant difference between human and pig skin.

7. Sato *et al.*, 1989

Sato and others investigated the *in vitro* permeability of nicorandil from saturated aqueous solution into several species and found that penetration rates had the following order: hairless rat > guinea pig > pig > dog (Sato *et al.*, 1989). They further suggest that the main factor for the species differences in the skin permeability of nicorandil would be the difference in partitioning of the drug from vehicle to the SC. Sato and colleagues recommended one of the few quantitative methods for inferring human permeability coefficients from animal data. Diffusion coefficients through the skin barrier and partition coefficients from the drug donor compartment to skin of the drug, in each species, were calculated by curve fitting the *in vitro* permeation data to a diffusion equation describing the drug permeation through a homogeneous membrane. They propose that differences in penetration rates arise from differences in the partition coefficient rather than from differences in the diffusion coefficient. They suggest that calculated diffusion coefficients determined with skin from different species should be

averaged to determine an overall average diffusion coefficient that is representative also of human SC. Three steps are required to obtain the SC-water partition coefficient: (1) partition coefficients are measured for human SC (they used callus) and SC for various animals, and (2) then measured partition coefficients for the animals are regressed to the calculated partition coefficients for the same species, and (3) the measured human SC-water partition coefficient is used to predict, via the regression equation, a calculated SC-water partition coefficient. Thickness of human SC is measured microscopically. Permeability is calculated using the mean of calculated diffusion coefficients, the calculated SC-water partition coefficient, and a microscopically measured human SC thickness.

8. Dick and Scott, 1992

Dick and Scott measured skin permeability of several lipophilic (aldrin, carbaryl, and fluaziflop-butyl) and hydrophilic (water, mannitol, and paraquat) compounds in pig ear skin, rat dorsal skin, and human abdominal skin (Dick and Scott, 1992). Although different concentrations and vehicles make a comparison of the compounds difficult, all the data can be used for interspecies comparison, as the vehicle and penetrant concentration were the same for all species studied. There is a discrepancy between values shown in Figure 4 and Table 1 which appear to be an error in the typesetting of Table 1 (see discussion in Appendix 5B and Appendix 7B). The qualitative conclusion that pig ear skin and rat dorsal skin are both more permeable than human abdominal skin is not affected by this error.

9. US EPA, 1992

The panel of authors who wrote the interim report on dermal absorption (US EPA, 1992) reviewed several earlier experimental studies comparing permeability coefficients

of human and animal skin, especially the review by Bronaugh *et al.* (Bronaugh *et al.*, 1982) (which was already discussed). No new data were presented in this report. The opinion of this panel was that the numerical differences between human skin and animal skin permeability coefficients vary with the test compound. Thus, they concluded that it was not possible to find a constant factor for adjusting the permeability coefficient from a specified animal to reliably represent the permeability coefficient for human skin. A major conclusion of this report was that animal skins are generally more permeable than human skin and that dermal absorption data from animals could be used as a conservative estimate of absorption in humans.

8. A COMPARISON OF THE US EPA MODEL FOR ESTIMATING DERMAL ABSORPTION OF AQUEOUS CONTAMINANTS WITH A LIMITED DATASET

8.1. Abstract

Bogen and colleagues conducted a limited experimental investigation of the uptake of chloroform (CF) and trichloroethylene (TCE) into full-thickness human cadaver skin (Bogen *et al.*, 1996). They compared these data to a membrane model for predicting percutaneous absorption of aqueous organic chemicals (US EPA, 1992), and a pharmacokinetic model. Based on this comparison, they claim that the membrane model is not consistent with their data. Specifically, they argue that: (1) the membrane model under predicts short-term dermal absorption of CF and TCE, and (2) cumulative unsteady-state uptake is a linear function of time, as predicted by the pharmacokinetic model, instead of the $\sqrt{t}$ dependency predicted by the membrane model.

We investigate the Bogen *et al.* (1996) data to find that the conditions under which Bogen *et al.* claims are valid have little relevance to data analysis or estimating the dermally absorbed dose. In fact, both models are consistent with the data. At this time, the physical relevance of the model to the physiological characteristics of skin is the most discriminating criteria that can be applied to determine the appropriateness of the two models.

Totally predictive estimates are generated with the membrane model and predictive correlations for the skin permeability coefficient and skin-water partition

coefficient. Estimates generally lie within two standard deviations of dermal uptake measured at all times, indicating that steady state parameters may have relevance to the unsteady-state period.

8.2. Introduction

Concern has been expressed about the dermal absorption of pervasive aqueous pollutants from frequent, although brief exposures to contaminated water, as might occur through daily bathing or swimming (Flynn, 1990; US EPA, 1992). Reasonably estimating the amount of chemical absorbed by the skin is important for determining safe levels of contamination in terrestrial waters or chemically disinfected tap water.

Mathematical representations of the skin have been developed to model the dermal absorption process. Two common approaches for mathematically modeling dermal absorption involve representing the skin as a membrane or as a compartment (i.e., a well-stirred tank). Most models predict the same behavior at long times when the rate of dermal absorption is at steady-state. However, during the short-time period of unsteady-state dermal absorption there are discrepancies between models. Membrane models predict unsteady-state absorption varies with the square-root of time ((Cleek and Bunge, 1993; US EPA, 1992), among many others). Compartment models can be designed to have different time dependencies for unsteady-state absorption (McCarley and Bunge, 1997). One compartment model, which assumes that the rate of dermal absorption is constant, has appeared in numerous publications, including a recent paper by Bogen *et al.* (1996).

Bogen *et al.* (1996) conducted an *in vitro* experimental investigation of the short-term uptake of chloroform (CF) and trichloroethylene (TCE) into full-thickness human cadaver skin. Based on their data, Bogen *et al.* (1996) claim that a "[membrane]-type model" (i.e., Bogen *et al.*'s regression fit of the unsteady-state membrane model) ". . . is not consistent with [the] data" and "may tend to underestimate human dermal exposures

to organic water contaminants such as CF and TCE". They also claim that the "pattern of AMS (accelerator mass spectrometry)-measured uptakes differed significantly from the pattern of values predicted by the EPA-type model" (i.e., Bogen *et al.*'s fit of the short-time membrane model) "as proportional to the square root of exposure time" (Bogen *et al.*, 1996). Bogen *et al.* (1996) further claim that the first-order, one-compartment model they present is consistent with their data and better describes the pattern of measured uptakes of CF and TCE. In the next section we will use their data to examine these claims further.

Predictive estimates can be made when mathematical models of dermal absorption are combined with transport parameters determined experimentally or estimated using predictive correlations developed using experimentally determined transport parameters for other chemicals. The transport parameters required for most models are the stratum corneum (SC) permeability coefficient from aqueous solution, P_{cw} (which measures how easily a chemical penetrates skin), the SC-water partition coefficient, K_{cw}, (which measures the affinity of skin for a chemical relative to waters affinity for that chemical), and the SC thickness which is generally 10-20 μm (Kalia *et al.*, 1996). Fortunately, P_{cw} and K_{cw} have been measured for many chemicals and predictive methods are available for estimating these parameters for other chemicals.

Almost all *in vitro* dermal absorption data (e.g., P_{cw}) have been measured at steady-state by conducting the experiment for a long enough time for the rate of dermal absorption to become constant. However, for shorter exposures, including those of interest in risk assessment, the rate of absorption may not be constant. This is called the unsteady-state period. Two important questions are (1) how long until steady state is established, and (2) whether unsteady-state dermal uptake (i.e., the cumulative amount entering the skin during short exposures) can be accurately predicted by correlations derived from steady-state penetration (i.e., a constant rate of chemical penetration through

the skin) experiments. Data measuring unsteady-state dermal uptake into skin are limited. The TCE and CF data reported by Bogen *et al.* (1996) are among the few *in vitro* unsteady-state uptake studies available for comparison to predictive models.

We use the data of Bogen *et al.* (1996) to address three questions which are important for the modeling of dermal absorption: (1) how did Bogen *et al.* (1996) come to the conclusion that the membrane model was inconsistent with the data, (2) is the conclusion justified, and (3) can transport parameters determined at steady state be used to estimate unsteady-state absorption.

8.3. Background

8.3.1. Experimental Procedure

Bogen *et al.* (1996) measured short term (exposure periods less than one hour) percutaneous absorption of CF and TCE into human cadaver skin. Since several of the experimental details are important to the discussion here, we will reiterate their procedures. Radiolabeled CF ([^{14}C]-CF with activity of 5.0 mCi/mmol) and TCE ([1,2-^{14}C]-TCE with activity 13.1 mCi/mmol) were diluted in methanol to form stock solutions with concentrations of 30 μCi/mL for CF and 55μCi/mL for TCE. Exposure solutions were prepared from the stock solutions without the addition of untagged CF and TCE. Full-thickness skin of a single adult Caucasian cadaver was divided into 12 pieces. Six pieces of full-thickness skin were placed on top of bottles containing diluted aqueous stock solution of ^{14}C-labeled trichloroethylene ($5.0 \pm \leq 0.20$ μg/L) and the other six pieces of skin were placed on top of bottles containing dilute aqueous stock solution of ^{14}C-labeled chloroform ($5.0 \pm \leq 0.20$ μg/L). The stratum corneum was mounted with the SC facing the solution which entirely filled the bottles (i.e., there was no head space). The bottles were capped, turned upside down, and placed onto magnetic stirrers at a rotation velocity of 430 rpm (a magnetic stir bar was placed inside the bottle before the

skin was mounted). After the prescribed exposure time (1, 5, 15, 30, or 60 minutes), one bottle each of TCE and CF was removed from the magnetic stirrer and turned right side up, and the skin was removed and blotted dry. Three to five samples were then taken from this skin using a small hole punch (0.054 cm^2). Each of these punch samples was separately analyzed for ^{14}C by accelerator mass spectrometry (AMS). Data for a few time points are based on only one or two analysis; the authors do not indicate what happened to the unreported samples. Consequently, the standard deviations reported by Bogen *et al.* (1996) represent the variability in replicate sampling and analysis from a single skin sample. Dermal absorption was calculated from the amount, in fmol, of ^{14}C appearing in the skin after a known exposure time adjusted by the activity. Table 8.1 contains the CF and TCE dermal absorption data and a discussion of calculations.

8.3.2. The Models

There have been two common approaches for mathematically representing the skin: (1) as a passive membrane in which the concentration variations across the skin are accounted for, and (2) as a well-stirred compartment in which only an average value for the concentration is considered. Models for both of these mathematical representations of the skin can be developed for two conditions occurring at the interface between the skin and either the cutaneous blood (for *in vivo* experiments) or the receptor chamber (for *in vitro* experiments). The infinite sink condition is when there is no resistance to transfer of the chemical from the interior surface of the skin into either the cutaneous blood or the receptor chamber. The no-flux condition is when chemical accumulates within the skin, because it can not pass out of the skin (i.e., there is not flux out of the skin), until the entire membrane becomes saturated.

Models representing both the compartment and membrane representations of skin have been compared (but incompletely) with experimentally measured uptake of CF and TCE (Bogen *et al.*, 1996): (1) Bogen *et al.* (1996) discussed a model (hereafter referred to

Table 8.1 Percutaneous Absorption of Chloroform and Trichloroethylene

Exposure Time (min)	Chloroform[a] Absorbed Dose (fmol ^{14}C)	$M_{in}/(A\ C_w^o)$[b] [=] cm Mean	S.D.	Regression Relative Weight
0	0.16, 0.12, 0.16, 0.12, 0.18, 0.12	0.0008	0.00015	1664
1	0.467, 0.394, 0.515, 0.204, 0.306	0.0021	0.00069	75.4
5	0.708, 1.13, 1.12, 1.09	0.0056	0.00112	28.4
15	3.16, 2.94, 2.49	0.0158	0.00188	10.1
30	3.88	0.0214	N/A	N/A[c]
60	6.39, 6.26, 8.20	0.0382	0.00597	1

Exposure Time (min)	Trichloroethylene[a] Absorbed Dose (fmol ^{14}C)	$M_{in}/(A\ C_w^o)$[b] [=] cm Mean	S.D.	Regression Relative Weight
0	0.16, 0.12, 0.16, 0.12, 0.18, 0.12	0.0003	6.2E-5	35630
1	0.844, 0.560, 0.362, 0.999	0.0016	0.00066	310
5	6.90, 4.73, 6.76, 4.54	0.0133	0.00295	15.6
15	9.56, 2.35, 12.0	0.0185	0.01164	1
30	18.3, 19.7, 14.6	0.0407	0.00611	3.6
60	24.6, 20.1	0.0518	0.00738	2.5

a. Absorption data provided by Bogen (personal communication, Aug. 1996)

b. Calculated assuming an activity of 5.0 mCi/mmol for CF and an activity of 13.1 mCi/mmol for TCE. From the isotope specific decay constant for ^{14}C (0.000121 yr^{-1} calculated from the half life of ^{14}C reported in the CRC Handbook of Chemistry and Physics as 5730 years (Weast, R.C., 1968)) we calculate that there are 1.3856×10^{14} dpm for each mole of ^{14}C. The required conversion between activities expressed in mCi and disintegrations expressed in dpm is 1 mCi = 2.22×10^9 dpm. With these we are able to relate measured disintegrations to moles of ^{14}C in the skin plugs (A = $0.054 cm^2$). The initial aqueous concentrations which we calculated are 3365 fmol ^{14}C/mL for CF and 7986 fmol ^{14}C/mL for TCE.

c. The 30 min CF measurement was excluded from the weighted regressions because there was no sample variance.

as compartment model) that treated the skin as a single well-stirred compartment with flux in from the vehicle and out into the cutaneous blood; (2) the membrane model of skin (which Bogen *et al.* referred to as the US EPA model) treats dermal absorption of moderately lipophilic compounds as permeation through a single pseudo-homogeneous membrane separating an aqueous solution of constant concentration from an infinite sink (US EPA, 1992). In this section, we present equations for the average SC concentration and mass absorbed under infinite-sink and no-flux conditions, for the compartment and membrane models of the skin.

Bogen *et al.* (1996) only presented the compartment model equations for average SC concentration. We have derived from their compartment model equations describing the mass absorbed into the SC. The assumptions of their compartment model are: (1) the arterial blood concentration is zero, (2) the SC-water partition coefficient is consistent with the rate into the skin from the vehicle (k_1) and the rate out of the skin into the vehicle (k_{-1}) (i.e., $K_{cw} = k_1/k_{-1}$), and (3) the venous blood concentration leaving the SC (C_b) is assumed to be in equilibrium with the average concentration in the SC ($\langle C_c \rangle$) as represented by the SC-venous blood partition coefficient (K_{cb}) (i.e., $\langle C_c \rangle = (K_{cb}\, C_b)$).

In the US EPA document, only equations for the dermally absorbed mass are listed for the membrane model (US EPA, 1992). These equations and equations for the concentration within a membrane are listed by Crank (Crank, 1975).

Table 8.2 lists equations for the normalized average SC concentration, $\langle C_{sc} \rangle/(K_{cw}\, C_w^{o})$, and normalized cumulative mass absorbed into the SC, $M_{in}/(A\, L_c\, K_{cw}\, C_w^{o})$, for the compartment model when there is finite, non-zero cutaneous blood flow. The normalized average SC concentration and normalized mass absorbed in the skin are expressed in terms of the rate in from the vehicle (k_1) and the rate out by transfer to the blood (k_2). Specifically, they define these transfer coefficients as $k_1 = P_{cw}/(L_c\, K_{cw})$ and $k_2 = Q/(A\, L_c\, K_{cw})$ were Q is the rate of blood perfusion through skin (mL/s) and A is the exposed area.

Table 8.2 Compartment Model with Finite Cutaneous Blood Flow where $\left[\tilde{C}_c = \langle C_c \rangle / (K_{cw} C_w^o)\right]$ and $\left[\tilde{M}_{in} = M_{in} / (A\, L_c\, K_{cw}\, C_w^o)\right]^a$

Case	$\tilde{C}_c$	$\tilde{M}_{in}$
General	$\tilde{C}_c = \frac{k_1}{k_1 + k_2}\left[1 - \exp(-(k_1 + k_2)\, t)\right]$ (8.1)	$\tilde{M}_{in} = \frac{k_1 k_2}{k_1 + k_2} t + \frac{k_1^2\left[1 - e^{-(k_1 + k_2)t}\right]}{(k_1 + k_2)^2}$ (8.2)
Short Time	$\tilde{C}_c = k_1 t$ (8.3)	$\tilde{M}_{in} = k_1 t$ (8.4)
Long Time	$\tilde{C}_c = \frac{k_1}{k_2 + k_2}$ (8.5)	$\tilde{M}_{in} = \frac{k_1 k_2}{k_1 + k_2} t + \frac{k_1^2}{(k_1 + k_2)^2}$ (8.6)

a. For the compartment model $k_1 = P_{cw}/(L_c K_{cw}) = D_c/(L_c)^2$ and $k_2 = Q/(A\, L_c\, K_{cw})$

The general equations for normalized average SC concentration (i.e., Eq. (8.1)) and normalized cumulative mass absorbed (i.e., Eq. (8.2)) are applicable at all times. In the short-time limit, the normalized average SC concentration and normalized cumulative mass absorbed are equal and vary linearly in time as given by Eqs. (8.3) and (8.4). In the long-time limit, the normalized average concentration becomes constant as indicated by Eq. (8.5) and the normalized mass absorbed varies linearly in time as given by Eq. (8.6).

Next, we consider the membrane and compartment models under infinite sink conditions. Table 8.3 lists membrane model and compartment model equations for the normalized average SC concentration and normalized cumulative mass absorbed under the conditions of infinite sink. Equations (8.7) and (8.13), from Crank (Crank, 1975), can be used to estimate the normalized average concentration in, and normalized mass absorbed into the SC for all times. In the short-time limit, equations for the normalized average SC concentration (i.e., Eq. (8.9)) and normalized cumulative mass absorbed (i.e., Eq. (8.15)) are equivalent, and both the concentration and mass absorbed are proportional to $\sqrt{t}$. In the long-time limit, the membrane model predicts that the normalized average concentration becomes 1/2, as indicated in Eq. (8.11), and that the normalized cumulative mass absorbed varies linearly in time, as indicated by Eq. (8.17). Compartment model results for the normalized average SC concentration and normalized cumulative mass absorbed were derived from Eqs. (8.1) or (8.2), respectively, by allowing the cutaneous blood flow to be much larger than permeability across the skin (i.e., $k_2 >> k_1$). Equations (8.8), (8.10) and (8.12) show that the normalized average concentration predicted by the compartment model is always zero under the infinite-sink condition. Equations (8.14), (8.16) and (8.18) show that the normalized cumulative mass absorbed varies linearly in time, and that a single slope is able to represent absorption into the SC under infinite-sink conditions.

Table 8.3 Infinite-Sink Condition, where $\left[\tilde{M}_{in} = M_{in} / (A\, L_c\, K_{cw}\, C_w^o)\right]$ and $\left[\tilde{C}_c = \langle C_c \rangle / (K_{cw}\, C_w^o)\right]$

	Case	Membrane Model	Compartment Model[a]
$\tilde{C}_c$	General	$\tilde{C}_c = \frac{1}{2} - \frac{4}{\pi^2}\sum_{n=0}^{\infty}\frac{\exp\left(-(2n+1)^2\pi^2 D_c t / L_c^2\right)}{(2n+1)^2}$ (8.7)	$\tilde{C}_c = \frac{k_1}{k_2} = \frac{P_{cw} A}{Q} = 0$ (8.8)
	Short Time[a]	$\tilde{C}_c = 2\sqrt{D_c t / (L_c^2 \pi)}$ (8.9)	$\tilde{C}_c = \frac{k_1}{k_2} = \frac{P_{cw} A}{Q} = 0$ (8.10)
	Long Time	$\tilde{C}_c = \frac{1}{2}$ (8.11)	$\tilde{C}_c = \frac{k_1}{k_2} = \frac{P_{cw} A}{Q} = 0$ (8.12)
$\tilde{M}_{in}$	General	$\tilde{M}_{in} = \frac{D_c t}{L_c^2} + \frac{1}{3} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{\exp\left(-\frac{n^2\pi^2 D_c t}{L_c^2}\right)}{n^2}$ (8.13)	$\tilde{M}_{in} = \frac{D_c t}{L_c^2}$ (8.14)
	Short Time[a]	$\tilde{M}_{in} = 2\sqrt{D_c t / (L_c^2 \pi)}$ (8.15)	$\tilde{M}_{in} = \frac{D_c t}{L_c^2}$ (8.16)
	Long Time	$\tilde{M}_{in} = \frac{D_c t}{L_c^2} + \frac{1}{3}$ (8.17)	$\tilde{M}_{in} = \frac{D_c t}{L_c^2}$ (8.18)

a. For the Membrane Model short time is defined as $t \leq 0.4\, L_c^2/D_c$.

Finally, we consider the membrane and compartment models under the no-flux condition. Table 8.4 lists the membrane model and compartment model results for the normalized average SC concentration and normalized cumulative mass absorbed under the conditions of no-flux. Under the no-flux condition, normalized cumulative mass absorbed exactly equals normalized average SC concentration (i.e., $\widetilde{C}_c = \widetilde{M}_{in}$) for both the membrane and compartment models. The general membrane model result for the no-flux condition (i.e., Eq. (8.20)) was obtained from Crank (Crank, 1975). The short-time limit is given by Eq. (8.22). The general result for the compartment model under the no-flux condition was derived from Eq. (8.1) (and can also be derived from Eq. (8.2)) by letting the rate out of the skin by transfer to the blood (k_2) become zero (i.e., $Q = 0$). The short time limit of the no-flux condition is expressed by Eq. (8.23) for the compartment model. At the long-
time limit, the membrane and compartment models both predict that the SC becomes saturated and cumulative dermal absorption stops (i.e., the normalized cumulative mass absorbed becomes constant at one).

To estimate M_{in} based on either Eqs. (8.13) or (8.14), or Eqs. (8.20) or (8.21) the SC thickness (L_c) must be known along with the diffusion coefficient through the SC (D_c) and the SC-water partition coefficient (K_{cw}) of the absorbing chemical. The thickness of the SC varies, but, is commonly reported to be 10-20 μm (Kalia *et al.*, 1996). When D_c and K_{cw} are not available, Cleek and Bunge proposed a method (Cleek and Bunge, 1993) for estimating D_c / L_c and K_{cw} based on the correlation for permeability coefficients (i.e., $K_{cw} D_c / L_c$) as given by Potts and Guy (Potts and Guy, 1992). Potts and Guy showed that the permeability of the SC can be reasonably represented as a function of molecular weight (MW) and lipophilic character (i.e., the logarithmically-transformed octanol-water partition coefficient, $\log K_{ow}$) according to Eq. (8.19) (US EPA, 1992):

$$\log P_{cw}\,(\mathrm{cm/hr}) = -2.72 - 0.0061\,\mathrm{MW} + 0.71 \log K_{ow} \tag{8.19}$$

Table 8.4 No-Flux Condition, where $\left[\tilde{M}_{in} = M_{in} / (A\, L_c\, K_{cw}\, C_w^o)\right]$ and $\left[\tilde{C}_c = \langle C_c \rangle / (K_{cw}\, C_w^o)\right]$

Case	Membrane Model	Compartment Model
General	$\tilde{M}_{in} = \tilde{C}_c = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{\exp\left(\frac{-(2n+1)^2 \pi^2 D_c t}{4 L_c^2}\right)}{(2n+1)^2}$ (8.20)	$\tilde{M}_{in} = \tilde{C}_c = 1 - \exp\left(-\frac{D_c t}{L_c^2}\right)$ (8.21)
Short Time[a]	$\tilde{M}_{in} = \tilde{C}_c = 2\sqrt{D_c t / (L_c^2 \pi)}$ (8.22)	$\tilde{M}_{in} = \tilde{C}_c = \frac{D_c t}{L_c^2}$ (8.23)
Long Time	$\tilde{M}_{in} = \tilde{C}_c = 1$ (8.24)	$\tilde{M}_{in} = \tilde{C}_c = 1$ (8.25)

a. For the Membrane Model short time is defined as $t \leq 0.4\, L_c^2/D_c$.

Cleek and Bunge proposed separating Eq. (8.19) into two equations, which were given in the US EPA report on dermal absorption (US EPA, 1992) as:

$$\log(D_c / L_c, cm / hr) = -2.72 - 0.0061\,MW \tag{8.26}$$

$$\log K_{cw} = 0.71 \log K_{ow} \tag{8.27}$$

In other papers, a slightly different equation was generated from a modified version of the Flynn database (Cleek and Bunge, 1993; Bunge *et al.*, 1994):

$$\log P_{cw}\,(cm / hr) = -2.80 - 0.0060\,MW + 0.74 \log K_{ow} \tag{8.28}$$

The analogous separation of this equation into two equations was (Cleek and Bunge, 1993; Bunge *et al.*, 1994):

$$\log(D_c / L_c, cm / hr) = -2.80 - 0.0060\,MW \tag{8.29}$$

$$\log K_{cw} = 0.74 \log K_{ow} \tag{8.30}$$

8.4.Data Analysis

8.4.1. Previous Analysis

In the experimental system used by Bogen *et al.* (1996), the absorbing chemical has no way to leave the skin (i.e., the measurements are for absorption under the no-flux condition). Consequently, it is appropriate to use the no-flux form of the two models as listed in Table 8.4. For the short exposure times of one hour used in these experiments the no-flux form (Eq. (8.20)) or the infinite sink form (Eq. (8.13)) of the membrane model give nearly identical results (i.e., the time period of absorption is too short for the finite limits of the skin to be noticed). As a result, either the complete membrane model Eq. (8.13) or the short-time limit of the membrane model (i.e., Eqs. (8.15) or (8.22)) are appropriate for these chemicals, and the analysis will be identical to that using the no-flux equation (Eq. (8.20)).

Bogen *et al.* (1996) chose the short-time limit of the membrane no-flux result (i.e., Eq. (8.22)) and the general-time compartment no-flux result for analysis. An additional regression constant, k_o, was added to both models to account for non-zero levels of background ^{14}C radiation. The final functional form of the compartment model used in the analysis by Bogen *et al.* (1996) was,

$$\frac{M_{in}}{A\,C_w^o} = L_c\,K_{cw}\,\tilde{C}_c + k_o = \frac{P_{cw}}{k_1}\left[1-\exp(-k_1\,t)\right] + k_o \tag{8.31}$$

and the final functional form of the membrane model used in the Bogen *et al.* (1996) analysis was:

$$\frac{M_{in}}{A\,C_w^o} = a\sqrt{t} + k_o \tag{8.32}$$

It follows from Eq. (8.22) that the parameter *a* [in cm/hr$^{1/2}$] is equivalent to:

$$a = 2K_{cw}\sqrt{\frac{D_c}{\pi}} = \frac{2\,P_{cw}\,L_c}{\sqrt{\pi\,D_c}} \tag{8.33}$$

In their analysis, Bogen *et al.* (1996) used Eq. (8.26) to estimate (D_c/L_c), and assumed a skin thickness of 10 μm to obtain the following expression for the parameter *a*:

$$a = P_{cw}\,10^{0.00305\,MW}\sqrt{2/3\ \mathrm{hr}^{-1}} \tag{8.34}$$

Permeability coefficients calculated from this correlation are consistent with the Eq. (8.19) and a skin thickness of 10 μm. An alternate equation relating P_{cw} and *a* is,

$$a = 2\sqrt{\frac{K_{cw}\,L_c\,P_{cw}}{\pi}} \tag{8.35}$$

where P_{cw} and K_{cw} are measured directly or can be obtained from correlations like (8.19) and (8.27), respectively.

As described earlier, Bogen *et al.* (1996) fit $M_{in}/(A\,C_w^o)$ ($= L_cK_{cw}\tilde{M}_{in}$) for CF and TCE to Eq. (8.31) for the compartment model and Eq. (8.32) and (8.34) for the membrane model in reciprocal-variance weighted regressions. Since the membrane

model was linear in $\sqrt{t}$, least squares general-linear-model regression was used for the membrane model to determine k_o and P_{cw}. Since the compartment model was nonlinear, the compartment model parameters k_o, P_{cw} and k_1 were obtained by Levenberg-Marquardt X^2-minimization. Parameter estimates and goodness-of-fit statistics are summarized in Table 8.5 for both models. Bogen *et al.* (1996) assessed goodness-of-fit as the probability that X^2 is greater than a χ^2 distribution evaluated with df = (# data points (= 22 for CF or 23 for TCE)) - n_p where $n_p = 3$ for the compartment model and $n_p = 2$ for the membrane model, respectively. Comparison of means was done by a 2-tail T-test. Bogen *et al.* (1996) justified the reciprocal-variance weighted regressions on the basis of variance non-homogeneity (heteroscedasticity) which was demonstrated by performing a Bartlett's test of the CF data ($\chi^2 = 33.6$, df = 4, $p < 10^{-6}$) and the TCE data ($\chi^2 = 46.6$, df = 5, $p < 10^{-8}$).

Based on the statistics in Table 8.5 (i.e., $p < 0.05$), Bogen *et al.* claimed that the membrane model was not consistent with the CF and TCE data. In the next section we examine this claim in more detail.

8.4.2. Current Analysis

8.4.2.1. Reanalysis of the Models with the Data

Our goal in this section is to investigate the claims made by Bogen *et al.* (1996) concerning the appropriateness of the membrane model as a representation for skin. We will examine both the membrane and compartment models by three standards: (1) by their ability to describe the extent and pattern (or general shape) of the data, (2) by their ability to calculate permeability coefficients that agree with other experimentally determined permeability coefficients for these chemicals, and (3) by their physical relevance to the actual characteristics of skin.

Table 8.5 Fitted Parameter Values Reported by Bogen *et al.* (1996)

Chemical	Model Form	P_{cw} (cm/hr)	k_1 (hr^{-1})	K_{cw} (unitless)	χ^2	df	p-value
CF	compartment	0.067	1.3	26	17.5	19	0.55
CF	membrane	0.022[a]	N/A	N/A	81.7	20	$<10^{-8}$
TCE	compartment	0.12	2.0	30	27.9	19	0.086
TCE	membrane	0.029[a]	N/A	N/A	132.0	20	~0

a. The permeability coefficient is based on Eq. (8.34) and assumes that the US EPA version of the Potts and Guy correlation (Eq. (8.19)) is correct and has built into it a skin thickness of $L_c = 10$ μm.

One standard used to compare the membrane and compartment models is their ability to fit the extent and pattern of the measured CF and TCE uptake data. First, we repeat the weighted regression analysis of all data that Bogen *et al.* (1996) performed. Next, we consider the analysis of the CF and TCE uptake measurements more thoroughly (1) by performing unweighted regression of the mean values, and (2) by performing weighted regressions of the CF and TCE uptake data when certain highly weighted data points are excluded.

Weighted and unweighted regressions for both the compartment and membrane models were performed using JMP (JMP, 1995). The CF and TCE uptake data were analyzed with Eq. (8.32) (linear in $\sqrt{t}$) for the membrane model and Eq. (8.31) (nonlinear equation) for the compartment model. Linear regression (in $\sqrt{t}$), was used for the membrane model, and nonlinear regression (using the Gauss-Newton method with stephalving to search for the least-squares estimate (JMP User's Guide, (SAS Institute, 1995)) was used for the compartment model.

The CF and TCE data were analyzed with weighted regression analysis using the membrane and compartment models. Weighting, based on the inverse of the sample standard deviation squared (generally for 3-6 samples), favored the early data points excessively. The zero time measurement, as shown in Table 8.1 was weighted particularly heavy (1,660 times more significant than the one-hour measurement for CF and 35,600 times more significant than the 15-minute measurement for TCE). This is not totally unexpected since the level of background radiation should be nearly constant. Further, the one minute measurement was also weighted very heavily (75 times more significant than the one-hour measurement for CF and 310 times more significant than the 15-minute measurement for TCE). The 30-minute data point for CF was excluded from the weighted regressions since there were no replicates. Coefficients determined by the weighted regression analysis for both models are summarized along with the root

mean square error (RMSE), which quantifies goodness-of-fit, in Table 8.6 for CF and Table 8.7 for TCE. RMSE is zero when the model perfectly correlates the data and low RMSE values mean that a model has good predictive power. Permeability coefficients were calculated for the membrane model using Eq. (8.35) and Eqs. (8.19) and (8.36), and assuming a skin thickness of 20 μm.

Equations (8.31) and (8.32) were fit to the CF and TCE data when the mean values at each time point were unweighted. Coefficients and RMSE for the unweighted regressions are summarized in Table 8.6 for CF and Table 8.7 for TCE. The membrane and compartment models were also used to analyze the CF and TCE data when the data were weighted and either the 0-minute, 1-minute, or 5-minute data point was excluded. Coefficients and RMSE for each of these weighted regressions are also summarized in Table 8.6 for CF and Table 8.7 for TCE.

Figures 8.1 and 8.2 show the TCE and CF uptake data along with weighted-regression fits to all data for both models (curves a and b), new estimates based on unweighted regressions for both models (curves c and d), and the predictive estimate from the membrane model (i.e., curve e). The weighted fit of the compartment model (i.e., curve a) represents the long-time data better than the weighted fit of the membrane model (i.e., curve b). This fact led Bogen *et al.* (1996) to conclude that the membrane model was inadequate for describing this data. However, the unweighted fits of the membrane model (i.e., curve d), for both CF and TCE, are comparable to the fits of the compartment model and precisely describes the shape (i.e., general pattern) and magnitude of the data. Absorption predicted by the membrane model (i.e., curve e), based on permeability coefficients calculated using Eq. (8.19), is consistent with the shape of the data and estimates generally lie within two standard deviations of measured absorption for both CF and TCE. The permeability coefficients calculated using Eq. (8.19) and $\log K_{ow} = 1.97$ for CF (Hansch *et al.*, 1995) and $\log K_{ow} = 2.61$ for TCE

Table 8.6 Model Fits to *In Vitro* Measured Chloroform Uptake Data.

	Membrane				Compartment			
Regression	a (cm/hr$^{1/2}$)	k_o (cm)	P_{cw} (cm/hr)	RMSE	k_1 (hr^{-1})	P_{cw} (cm/hr)	k_o (cm)	RMSE
Unweighted	0.0376	-0.0025	0.0141[c]	0.00298	0.842	0.0539	0.0013	0.00179
Weighted[a,b]	0.0234	0.0007	0.0088[c]	0.01445	1.296	0.0676	0.0008	0.00218
Weighted[a,b] (0 min excluded)	0.0358	-0.0030	0.0134[c]	0.00837	1.268	0.0667	0.0009	0.00375
Weighted[a,b] (1 min excluded)	0.0259	0.0008	0.0097[c]	0.01373	1.269	0.0670	0.0008	0.00340
Weighted[a,b] (5 min excluded)	0.0266	0.0007	0.0100[c]	0.01547	1.518	0.0725	0.0008	0.00096
Predictive	0.0239[c]	N/A	0.00892[d]	0.0144	0.171[e]	0.00892[d]	N/A	0.1043

a. Weight is based on reciprocal variance according to Table 8.1
b. 30 min measurement excluded because there was no replication (no variance)
c. Calculated from Eqs. (8.35), (8.19) and (8.27) with a skin thickness of 20 μm.
d. Calculated from Eq. (8.19). The value calculated from Eq. (8.29) is 0.00874 (cm/hr).
e. Calculated as $k_1 = P_{cw}/(L_c K_{cw})$ with $L_c = 20$ μm and K_{cw} from Table 8.5.

Table 8.7 Model Fits to *In Vitro* Measured Trichloroethylene Uptake Data.

	Membrane				Compartment			
Regression	a (cm/hr$^{1/2}$)	k_o (cm)	P_{cw} (cm/hr)	RMSE	k_1 (hr^{-1})	P_{cw} (cm/hr)	k_o (cm)	RMSE
Unweighted	0.0551	-0.0031	0.0186[b]	0.00437	1.821	0.1124	0.0007	0.00405
Weighted[a]	0.0320	0.0003	0.0108[b]	0.03459	2.058	0.1241	0.0003	0.01163
Weighted[a] (0 min excluded)	0.0621	-0.0064	0.0209[b]	0.00732	3.304	0.1762	-0.0012	0.00899
Weighted[a] (1 min excluded)	0.0512	0.0003	0.0172[b]	0.00749	3.001	0.1589	0.0003	0.00794
Weighted[a] (5 min excluded)	0.0302	0.0003	0.0102[b]	0.0388	1.453	0.1018	0.0003	0.00805
Predictive	0.0638[b]	N/A	0.0215[c]	.0245	0.358[d]	0.0215[c]	N/A	0.1781

a. Weight is based on reciprocal variance according to Table 8.1
b. Calculated from Eqs. (8.35), (8.19) and (8.27) with a skin thickness of 20 μm.
c. Calculated from Eq. (8.19). The value calculated using Eq. (8.29) is 0.02203 (cm/hr).
d. Calculated as $k_1 = P_{cw}/(L_c K_{cw})$ with $L_c = 20$ μm and K_{cw} from Table 8.5.

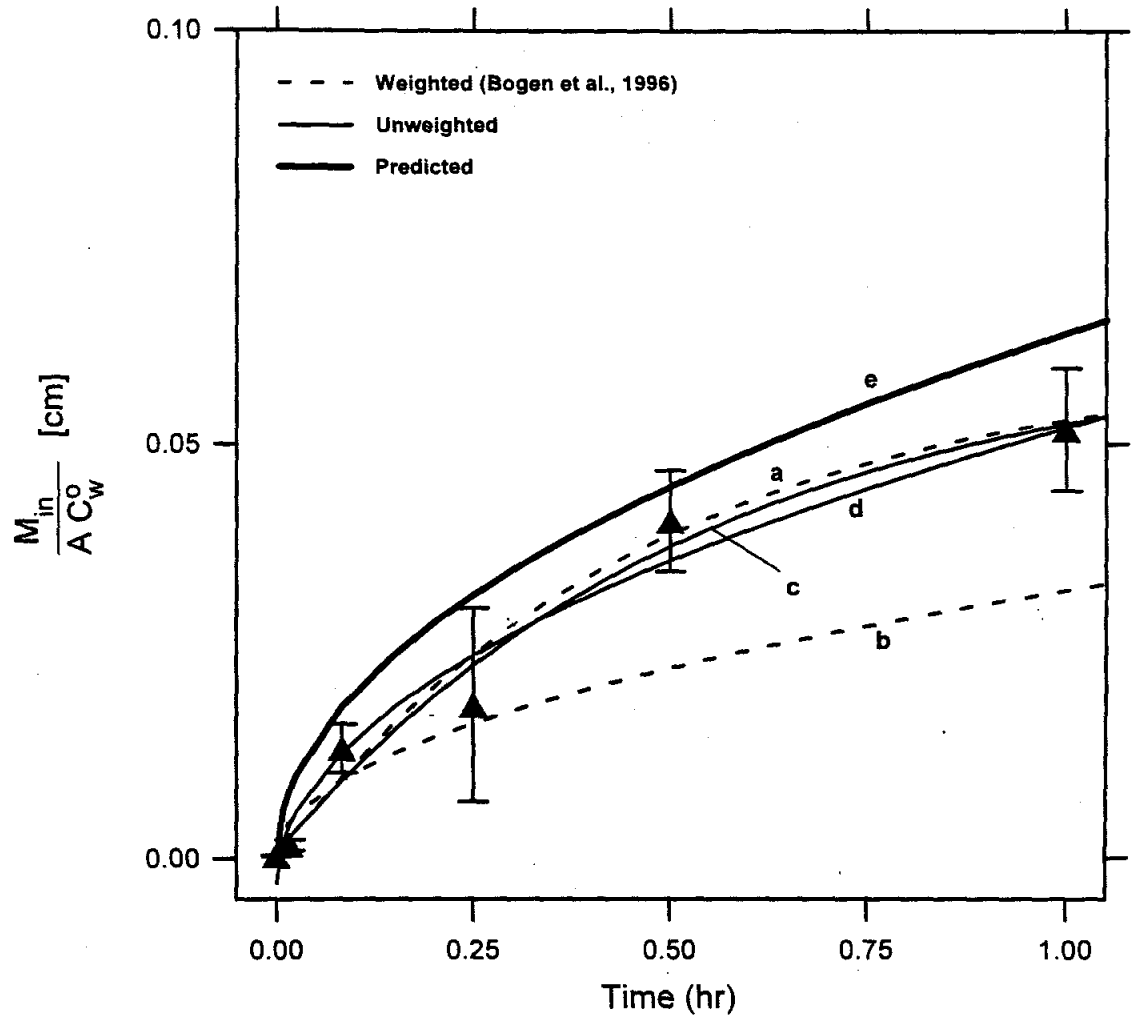

Figure 8.1 Comparison of the cumulative uptake of dilute aqueous TCE into full thickness human cadaver skin (Bogen *et al.*, 1996) with uptakes predicted by compartment (Bogen *et al.*, 1996) and membrane (US EPA, 1992) models of dermal absorption. Error bars indicate plus or minus one sample standard deviation from the mean. Model estimates are as follows:

(a) Compartment model weighted fit (P_{cw} = 0.124 cm/hr, k_1 = 2.06 hr^{-1}, k_o = 0.0003)
(b) Membrane model weighted fit (P_{cw} = 0.011 cm/hr and k_o = 0.0003)
(c) Compartment model unweighted fit (P_{cw} = 0.11 cm/hr, k_1 = 1.82 hr^{-1}, k_o = 0.0007)
(d) Membrane model unweighted fit (P_{cw} = 0.019 cm/hr and k_o = -0.0031)
(e) Membrane model with predictive Eqs. (8.19) and (8.27) (P_{cw} = 0.0215 cm/hr)

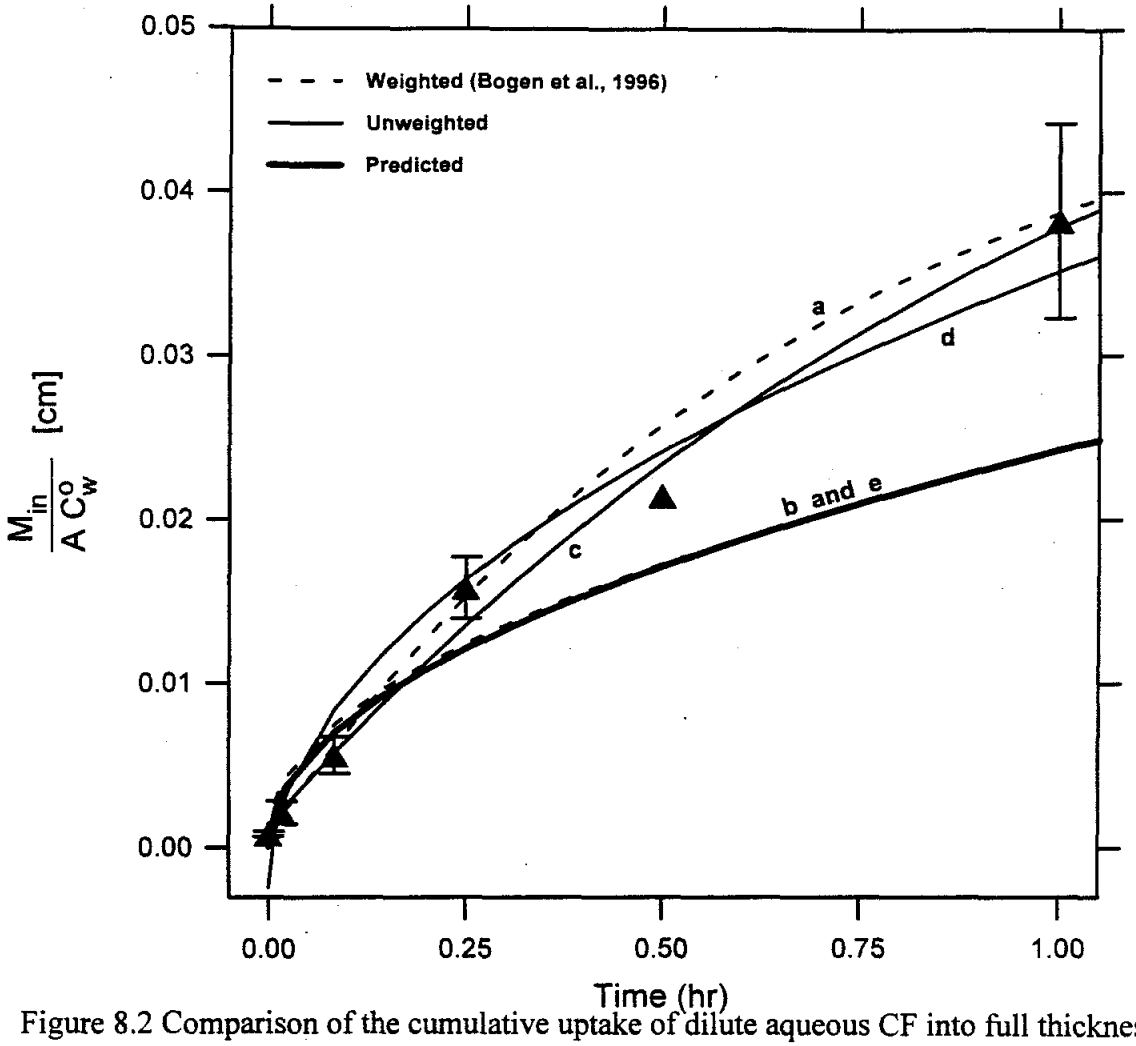

Figure 8.2 Comparison of the cumulative uptake of dilute aqueous CF into full thickness human cadaver skin (Bogen *et al.*, 1996) with uptakes predicted by compartment (Bogen *et al.*, 1996) and membrane (US EPA, 1992) models of dermal absorption. Error bars indicate plus or minus one sample standard deviation from the mean. Model estimates are as follows:

(a) Compartment model weighted fit (P_{cw} = 0.068 cm/hr, k_1 = 1.30 hr^{-1}, k_o = 0.0008)
(b) Membrane model weighted fit (P_{cw} = 0.009 cm/hr and k_o = 0.0007)
(c) Compartment model unweighted fit (P_{cw} = 0.05 cm/hr, k_1 = 0.84 hr^{-1}, k_o = 0.0014)
(d) Membrane model unweighted fit (P_{cw} = 0.014 cm/hr and k_o = -0.0025)
(e) Membrane model with predictive Eqs. (8.19) and (8.27) (P_{cw} = 0.0089 cm/hr)

(Hansch *et al.*, 1995) are 0.00892 cm/hr for CF and 0.02146 cm/hr for TCE. These predicted permeability coefficients are similar to the permeability coefficients calculated from unweighted fits of the CF and TCE data to the membrane model: 0.0141 cm/hr for CF and 0.0186 cm/hr for TCE (from Tables 8.6 and 8.7, respectively). The predicted absorption of TCE is higher and the predicted absorption of CF is lower, but not significantly different, than measured uptake for these chemicals. As shown in Chapters 4 and 5, measured and predicted permeability coefficients frequently deviate by an order of magnitude, in part because of poor reproducibility.

The compartment model is able to represent both the early-time and late-time data satisfactorily even when one of these periods is heavily weighted, because, it has two adjustable parameters, k_1 which is specified primarily by the early-time data points, and P_{cw} which is specified primarily by the later data points. Because the two parameters allow regression of the later time points to be essentially unaffected by the regression of the early time points the compartment model can fit all data even when one of these periods is highly weighted. The membrane model, with only one adjustable parameter that must be determined using all time points, is more sensitive to heavy weighting in one of these periods. Importantly, neither model gives a significantly preferable fit to the data when the regressions are performed on the unweighted mean data.

How relevant is the weighted regression analysis? Bogen *et al.* (1996) provided statistical justification for weighting (on the basis of variance non-homogeneity revealed through Bartlett's test), but, these rather significant (i.e., greater than linear) increases in variance are unexpected and difficult to explain experimentally. Variance in the data at each time point will arise from three sources: (1) variability in sample size (i.e., variability in the punch sampling), (2) random error in the AMS measurement, and (3) nonuniformity in chemical absorption. There is every reason to expect that variability in punch sampling and error in the AMS analysis should be time independent. However, if

the skin was spatially compromised, due to abrasion with the stirbar or stretching or fatiguing the skin, we might expect nonuniformity in chemical absorption which would increase with exposure time. Damage would probably not be uniform, hence, punch samples from different regions of the skin sample might reflect different amounts of damage and increase the standard deviations. If damage is the cause of the increased variability in the later time points, the mean absorption values of the later time points might be elevated relative to undamaged skin. This is consistent with weighted and unweighted fits of the membrane model based on early time data, which slightly under predict the amount of absorption at the longest time points.

Membrane model fits for five different regression strategies, are compared to measured uptake of TCE in Figure 8.3. This figure compares the unweighted analysis of the TCE uptake data to weighted analysis when all data are analyzed and when (sequentially) the highly weighted 0-minute, 1-minute, or 5-minute data points are excluded from the weighted regression. When all data were unweighted (i.e., curve a), when the data were weighted and the 0-minute data point was excluded (i.e., curve c), or when the data were weighted and the 1-minute data point was excluded (i.e., curve d) the membrane model was able to represent the shape of the data and estimates were consistently within one standard deviation of measured uptake. Only when all data were weighted (i.e., curve b) or when all data except the 5-minute data point were weighted (i.e., curve e) are the estimates lower and significantly different from measured uptake. Clearly, the unweighted regressions of the membrane model and several weighed regressions that are shown provide reasonable estimates for the measured uptake of TCE.

Membrane model fits for five different regression strategies, are compared to measured uptake of CF in Figure 8.4. These plots compare the unweighted analysis of the CF uptake data to weighted analysis when all data are analyzed and when (sequentially) the highly weighted 0-minute, 1-minute, or 5-minute data points are

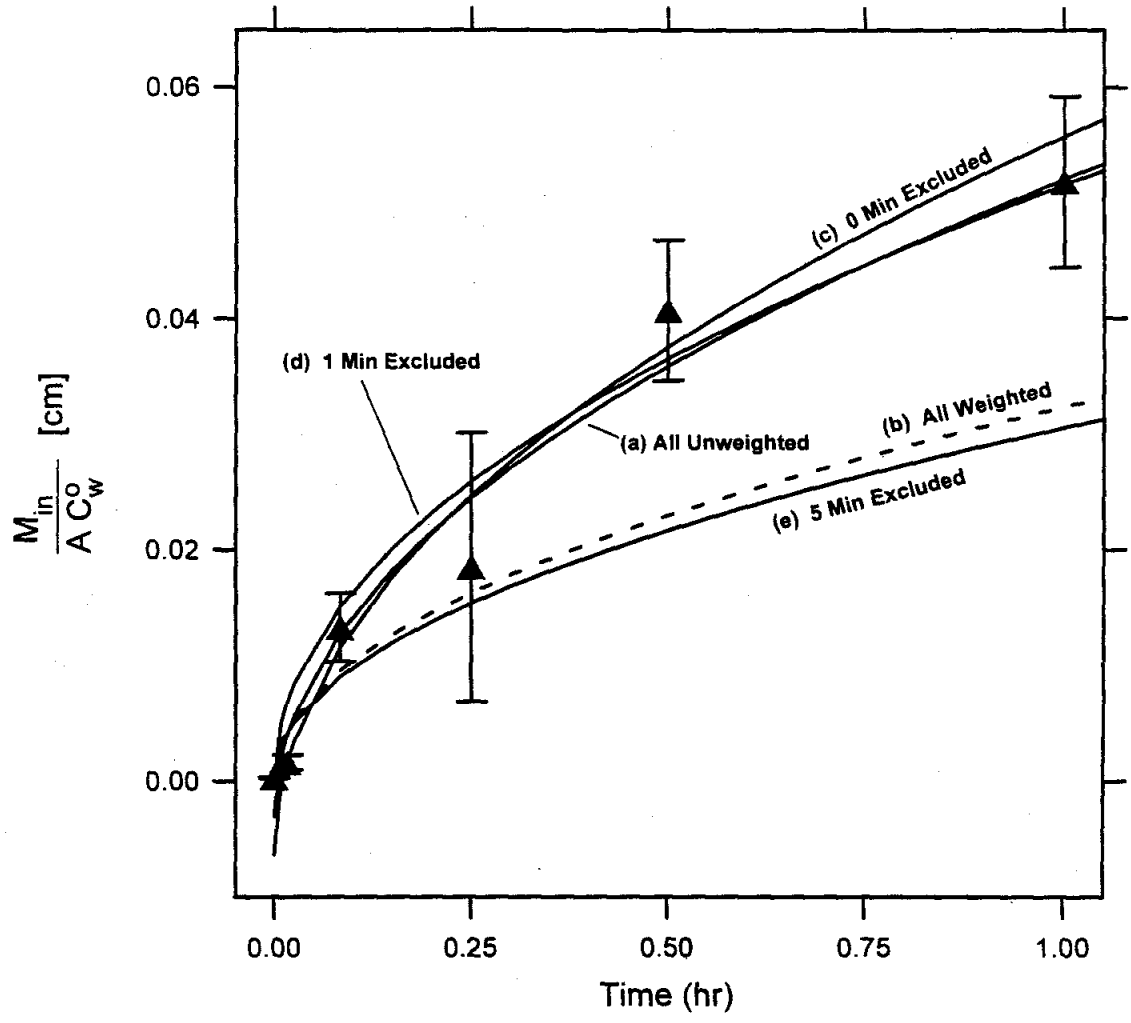

Figure 8.3 Analysis of the cumulative uptake of TCE using a membrane model of dermal absorption (US EPA, 1992) with different regression strategies. Error bars indicate plus or minus one sample standard deviation from the mean. Regression estimates are as follows:

(a) Unweighted fit to all data (P_{cw} = 0.0186 cm/hr and k_o = -0.0031)
(b) Weighted fit to all data (P_{cw} = 0.0108 cm/hr and k_o = 0.0003)
(c) Weighted fit to all but zero minute data point (P_{cw} = 0.0209 cm/hr and k_o = -0.0064)
(d) Weighted fit to all but one minute data point (P_{cw} = 0.0172 cm/hr and k_o = 0.0003)
(e) Weighted fit to all but five minute data point (P_{cw} = 0.0102 cm/hr and k_o = 0.0003)

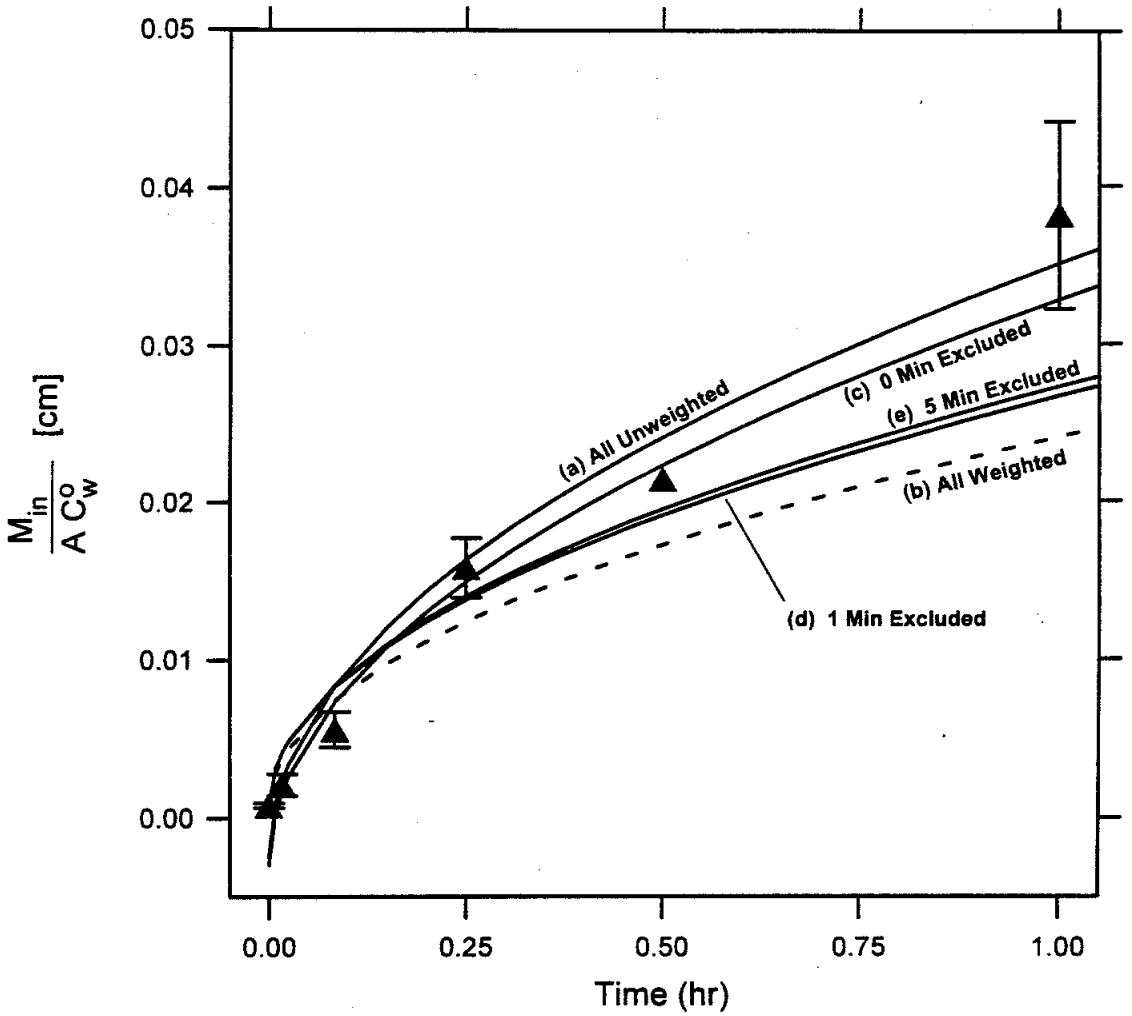

Figure 8.4 Analysis of the cumulative uptake of CF using a membrane model of dermal absorption (US EPA, 1992) with different regression strategies. Error bars indicate plus or minus one sample standard deviation from the mean. Regression estimates are as follows:

(a) Unweighted fit to all data (P_{cw} = 0.0141 cm/hr and k_o = -0.0025)
(b) Weighted fit to all data (P_{cw} = 0.0088 cm/hr and k_o = 0.0007)
(c) Weighted fit to all but zero minute data point (P_{cw} = 0.0134 cm/hr and k_o = -0.0030)
(d) Weighted fit to all but one minute data point (P_{cw} = 0.0097 cm/hr and k_o = 0.0008)
(e) Weighted fit to all but five minute data point (P_{cw} = 0.0100 cm/hr and k_o = 0.0007)

excluded from weighted regression. When all data were unweighted (i.e., curve a) and when the data were weighted with the 0-minute data point excluded (i.e., curve c) the membrane model was able to represent the shape of the data and estimates were consistently within one standard deviation of measured uptake (curve c lies just outside one standard deviation of the 5-minute measurement). Only when all data were weighted (i.e., curve b), when the data were weighted and the 1-minute data point was excluded (i.e., curve d), or when all data except the 5-minute data point were weighted (i.e., curve e) are the estimates lower and different from measured uptake. Even these regressions (i.e., curves b, d, and e) predict the general shape of the data. Clearly, the membrane model is adequate for representing the measured uptake of CF when the data are unweighted or when the 0-minute measurement is excluded from weighted regressions.

Bogen *et al.* (1996) used the weighted fit to all data as evidence that the membrane model did not follow the general pattern of absorption that was measured and said that this fit was *the best possible fit* of a membrane-type model (i.e., one that predicts that absorption varies with $\sqrt{t}$) to the data. Clearly, several alternative fits of the membrane model provide fits that are superior to the highly restrictive fit used by Bogen and colleagues. Furthermore, the conclusion that the membrane model does not have the general shape of the measured uptake (i.e., a square-root-of-time dependency is inappropriate) hinges on the confidence placed in the difference of sample variances for different times. Based on the close fit of the unweighted regressions and several weighted regressions with excluded data points to the TCE and CF uptake data, the membrane model appears to have a functional dependency (i.e., $\sqrt{t}$ dependency) which is able to adequately represent the data.

Figures 8.5 and 8.6 compare the TCE and CF uptake data to membrane model (i.e., Eq. (8.35)) and compartment model (i.e., Eq. (8.21)) estimates, when P_{cw} is calculated with Eq. (8.19) and K_{cw} is calculated with Eq. (8.27), for skin of thickness 10

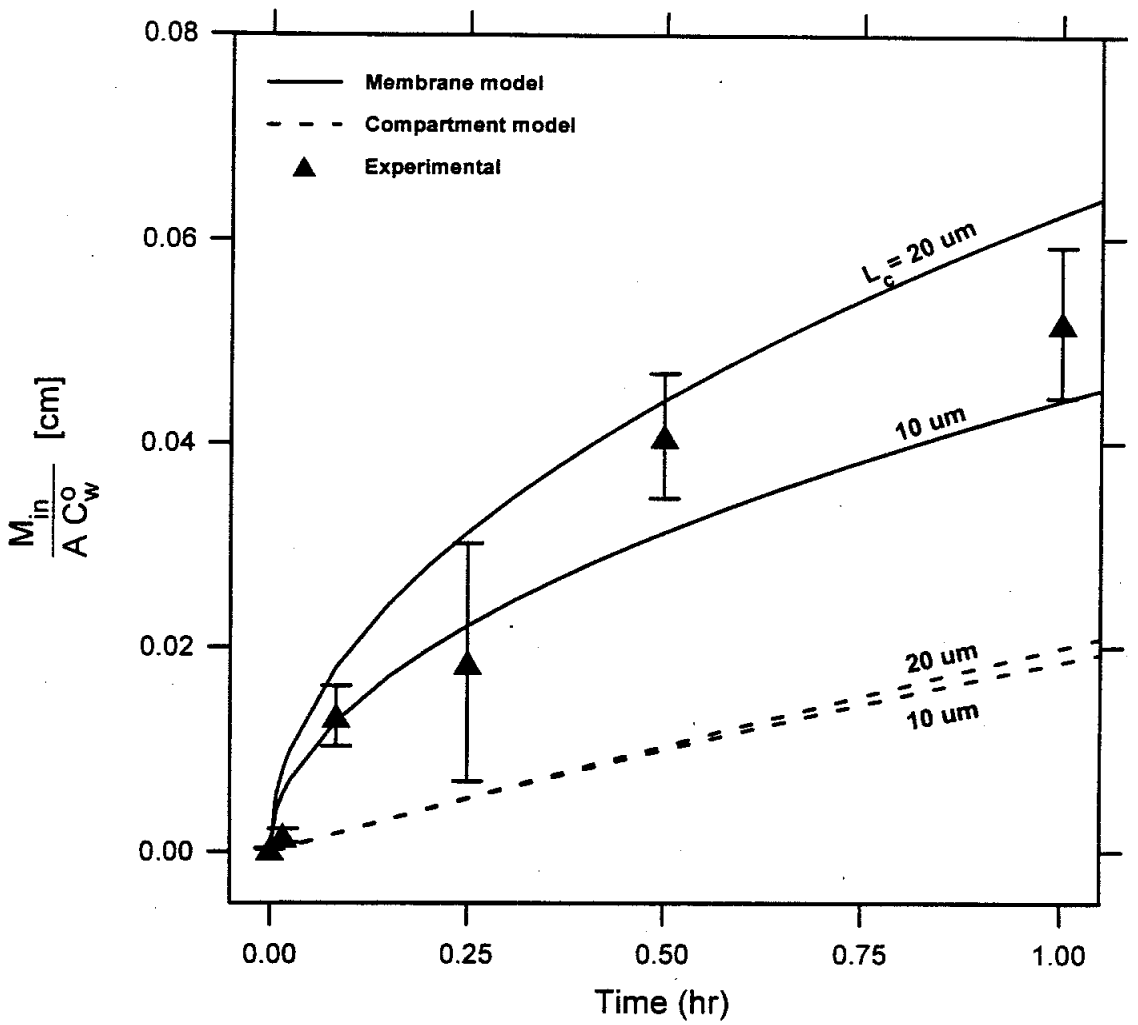

Figure 8.5 Predictions of TCE absorption (Bogen *et al.*, 1996) with the no-flux short-time membrane model (Eq. (8.22)) and the no-flux compartment model (Eq. (8.21)) using Eqs. (8.19) and (8.27). Error bars indicate plus or minus one sample standard deviation from the mean.

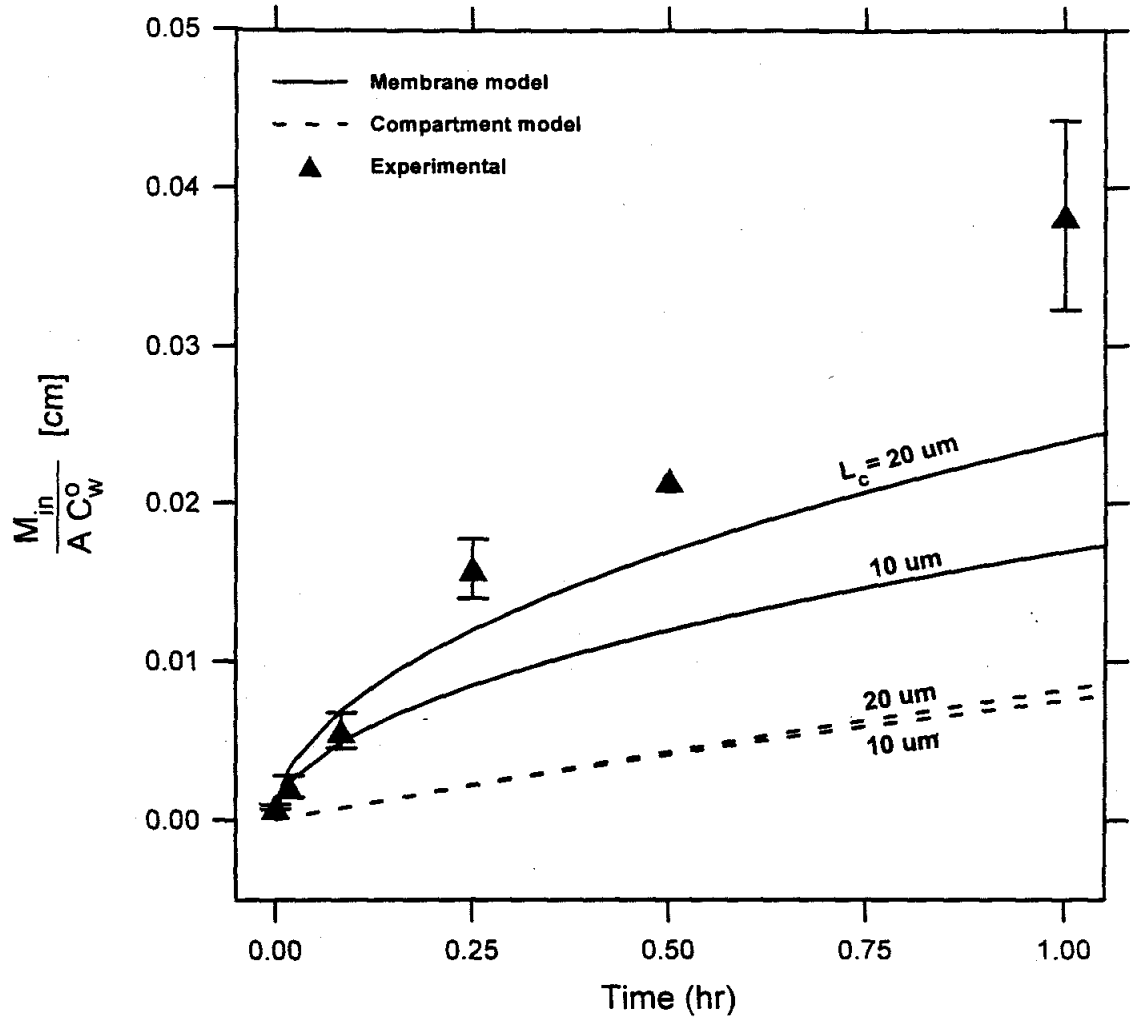

Figure 8.6 Prediction of CF absorption (Bogen *et al.*, 1996) with the no-flux short-time membrane model (Eq. (8.22)) and the no-flux compartment model (Eq. (8.21)) using Eqs. (8.19) and (8.27). Error bars indicate plus or minus one sample standard deviation from the mean.

and 20 μm. Membrane model estimates for TCE are within one standard deviation of measured uptake at most time points. For CF, membrane model estimates under predict measured uptake at several times by more than two standard deviations, although estimates for skin with thickness 20 μm lie within two standard deviations of nearly all measurements.

Compartment model estimates based on the same P_{cw} and K_{cw} under predict measured uptake of both TCE and CF and are not as close to measurements as membrane model estimates. For both TCE and CF, the general curvilinear shape of the measured uptake data is better predicted by the membrane model, where uptake varies as $\sqrt{t}$, than by the compartment model where the prediction is nearly linear for these values of P_{cw} and K_{cw}. The range of skin thickness (i.e., 10-20 μm) shown are very relevant. Scheuplein suggests that 20 μm is a reasonable upper bound on SC thickness (Scheuplein and Blank, 1971) and trans-epidermal water loss experiments suggest that L_c lies between 10-20 μm (Kalia *et al.*, 1996). Based solely on the fact that the correlations are totally predictive and were not trained to the data in any way, Figures 8.5 and 8.6 indicate that the membrane model may provide reasonable estimates of dermal absorption for these and similar chemicals.

Is there a difference between the *in vitro* unsteady-state uptake experiments performed by Bogen *et al.* and the *in vitro* steady-state penetration experiments upon which all predictive correlations are based? There is not enough data available to say definitively, although the CF and TCE datasets do not suggest that there is. Predictions using the membrane model, a predictive permeability coefficient correlation developed from steady-state permeability coefficients, and Eq. (8.27) are very close to measured uptake data and are comparable to or better than actual regression fits to the uptake data. *In vitro* measurement of the permeability coefficients in a standard diffusion cell experiment would have provided a more direct test of the approach.

Although the measured uptake of CF and TCE is not significantly different from predictions using the membrane model and P_{cw} from Eq. (8.19) and K_{cw} from Eq. (8.30) for SC of thickness 10-20 μm, there are at least three possible explanations why measured uptake may be higher than predicted: (1) methanol in the stock solution may be enhancing skin penetration, (2) the skin may be progressively damaged (e.g., perhaps by the stirbar) throughout the experiment, and (3) the skin from this donor was more permeable to CF and TCE than average human skin used in experiments upon which Eq. (8.19) was developed.

Experimentally determined steady-state permeability coefficients for CF and TCE can be compared with those calculated for CF and TCE when either the membrane or compartment model are applied to the unsteady-state uptake measurements of Bogen *et al.* (1996). Nakai *et al.* (1996) measured a steady-state, human-skin permeability coefficient of 0.160 cm/hr for CF and 0.120 cm/hr for TCE. These permeability coefficients have been determined by the usual diffusion cell procedure for determining steady-state permeability coefficients and satisfy a set of validation criteria (see also Chapter 5). Permeability coefficients calculated by the compartment model compared to Nakai *et al.* (1996) experimental values (i.e., experimental permeability/permeability calculated by the compartment model) are 2.36 (= 0.16/0.0676) for CF and 1.03 for TCE when the compartment model is weighted to all data, and 2.97 for CF and 1.07 for TCE when the compartment model is unweighted to all data. Permeability coefficients calculated by the membrane model compared to Nakai *et al.* (1996) experimental values (i.e., experimental permeability/permeability calculated by the membrane model) are 18.2 for CF and 11.1 for TCE when the membrane model is weighted to all data and 11.4 for CF and 6.45 for TCE when the membrane model is unweighted to all data.

The experimental permeability coefficients measured by Nakai *et al.* (1996) are higher than those calculated using either the membrane or compartment models, although

the compartment model estimates are closer. It should be noted that the Nakai *et al.* (1996) permeability coefficient for CF (i.e., 0.160 cm/hr) is high (by more than one order of magnitude) relative to permeability coefficients estimated using Eq. (5.27) (i.e., 0.0095 cm/hr) for other chemicals with the same size (i.e., molecular weight) and lipophilicity (i.e., K_{ow}) as indicated in Table 5A.1. The Nakai *et al.* (1996) permeability coefficient for TCE (i.e., 0.120 cm/hr) is also higher than an estimated permeability coefficient using Eq. (5.27) (i.e., 0.0176 cm/hr), but by slightly less than an order of magnitude. This trend might be expected, since Eq. (5.27), which was developed from a dataset of primarily hydrocarbons, may systematically under-estimate permeability coefficients for halogenated organic chemicals which are more dense than simple hydrocarbons.

Undeniably, permeability coefficients calculated using the compartment model are in better agreement than the membrane model with one set of steady state permeability measurements. Is this adequate evidence for concluding that (1) this particular compartment model better represents skin than does a membrane model, and (2) that permeability coefficients calculated by the compartment model are correct? It is important to consider the uncertainties in dermal uptake measurements as observed in replicated measurements for the same chemical. Figure 5.6, in Chapter 5, designated replicate permeability coefficients measured for the same chemical by different investigations, and showed that replicated measurements frequently differ by an order of magnitude or more. As a consequence, permeability coefficients estimated using Eqs. (8.19) and (8.28), which are based on a large number of measurements, averages most of the experimental variability, and become more relevant than individual measurements. This fact, and since the measurements of Nakai *et al.* are suspected to be higher than those determined in an 'average laboratory' by more than an order of magnitude for CF and slightly less for TCE, one should conclude that permeability coefficients calculated using the compartment model are better able to represent the Nakai *et al.* (1996)

permeability coefficients, but that this may not be true of the compartment model in general.

8.4.2.2. Physical Relevance of the Models

Physiologically, skin is more closely represented as a membrane than a well-stirred compartment. In skin, penetrant concentration depends upon position in addition to time. Concentration profiles in the skin, which are experimentally confirmed in numerous tape-stripping experiments (Pirot *et al.*, 1997) can be described by membrane models but never by pharmacokinetic models which can only describe the average concentration. Unless rate constants are carefully chosen, pharmacokinetic models will make physically irrational predictions. For example, in the limit of infinite blood flow (i.e., infinite Q), the compartment model (i.e., Eq. (8.8)) predicts that the SC concentration is zero.

The compartment model has computational advantages over the membrane model for conditions of partial blood flow. Blood flow limitations have been incorporated into membrane models (McCarley and Bunge, 1997), but, these equations are cumbersome and seldom used for routine analysis.

8.5. Conclusions

The Bogen *et al.* (Bogen *et al.*, 1996) data was inadequate for discriminating between the membrane and compartment model representations of skin, because both models can be made to closely describe the shape and magnitude of that data. Using both models, we found that permeability coefficients calculated from the unsteady-state CF and TCE uptake data by the compartment model were closer, but not significantly closer, to steady-state, *in vitro* measurements for CF and TCE. The physical relevance of a model is an important consideration for model choice. Although both the membrane model and the compartment model are sufficient for the routine analysis and fitting of

experimental data, only models with real physical relevancy should be used in the predictive capacity required for risk assessment. Physiologically, skin is a membrane and not a well-stirred compartment. Although both the $\sqrt{t}$ and Bogen *et al.*'s form of the compartment model can fit Bogen *et al.*'s TCE and CF uptake data, only the $\sqrt{t}$ -model is able to reasonably predict these same data from a correlation of steady-state permeability measurements for other chemicals. Indeed, incorporating the same estimates for permeability coefficients, partition coefficients and skin thickness into Bogen's compartment model poorly represents their uptake results. A similar but larger set of data, for more chemicals, is needed to provide a more decisive test of unsteady-state dermal absorption models.

8.6. Notation

A	=	Surface area of chemical exposure
C_w^0	=	Aqueous concentration of the absorbing chemical; assumed to remain constant during the exposure period, t_{exp}
C_b	=	Venous blood concentration leaving the SC
$\langle C_c \rangle$	=	Average concentration in the SC
D_c	=	Effective diffusivity of the absorbing chemical in the SC
k_o	=	Model regression constant
k_1	=	Rate into the skin from the vehicle
k_{-1}	=	Rate out of the skin into the vehicle
k_2	=	Rate out of skin by transfer to the blood
K_{cb}	=	SC-venous blood partition coefficient
K_{cw}	=	Equilibrium partition coefficient between the SC and water for the absorbing chemical
K_{ow}	=	Octanol-water partition coefficient of the penetrating chemical
L_c	=	Effective thickness of the SC
M_{in}	=	Cumulative mass absorbed into the SC during an exposure period, t_{exp}
MW	=	Molecular weight of the absorbing chemical
P_{cw}	=	Steady-state permeability of the SC from water
Q	=	Rate of blood perfusion through skin (mL/s)
SC	=	Stratum corneum
t	=	Time of exposure to absorbing compound

8.7. References

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9. ESTIMATION OF THE STRATUM CORNEUM - VIABLE EPIDERMIS PERMEABILITY COEFFICIENT RATIO

9.1. Abstract

The ratio of permeability coefficients in the stratum corneum and viable epidermis of skin, B, is an important parameter for modeling the dermal absorption of lipophilic compounds, that appears in membrane-based mathematical models. An intuitive method has been proposed for estimating this parameter, but, this method has never been tested against experimental B-values. An ideal database of measured B-values for lipophilic and diverse compounds does not exist, but, we have assembled and critically evaluated 70 B-values measured for organic compounds with various lipophilicities ($-3.53 < \log K_{ow} < 4.57$) in animal and human skin that has some utility in characterizing the B-parameter. These measurements indicate that the intuitive equation has too strong a dependency upon lipophilicity and on average underestimates the B-parameter by approximately one order of magnitude. An empirical correlation, developed using this database, should be useful for estimating the B-parameter, in terms of the lipophilicity and molecular weight (MW) of the penetrant, in the interim while more data is collected:

$$\log B = -1.31(0.12) + 0.48(0.04) \log K_{ow} - 0.004(0.001)\ MW$$

As well as being better able to represent the transport resistance, this investigation also gives us a more fundamental mechanistic understanding of penetration through the viable epidermis.

9.2. Introduction

Dermally absorbed chemical must traverse two skin layers to reach the capillary network which provides access to the systemic circulation. The outermost layer, the

stratum corneum (SC) and the inner layer, the viable epidermis (VE) are physicochemically and structurally different. The SC is thinner than the VE (10 - 20 μm compared to approximately 100 μm), more resistant to diffusion than the VE (typically the diffusion coefficient in the VE (D_e) is about 1000 times that in the SC (D_c)) and preferential towards lipophilic chemicals compared to the VE (i.e., the SC-VE partition coefficient, K_{ce}, is greater than 1). As a result, the relative magnitude of the mass transfer resistance for crossing the SC compared to the VE depends on the lipophilic nature of the penetrating chemical.

Mathematical models representing both the SC and VE contributions to dermal absorption require information about the relative penetrability of the SC and VE layers. In the mathematical development by Cleek and Bunge (Cleek and Bunge, 1993), two parameters, B and G, arise which describe respectively the relative permeability of the SC and VE (P_{cv} and P_{ev}), and the relative lag time for traversing the SC and VE ($t_{lag,\,sc}$ and $t_{lag,\,ve}$). That is,

$$B = \frac{P_{cv}}{P_{ev}} = \frac{D_c L_e K_{cv}}{D_e L_c K_{ev}} \tag{9.1}$$

$$G = \frac{t_{lag,sc}}{t_{lag,ve}} \tag{9.2}$$

where D_i is the diffusion coefficient in layer i, L_i is the apparent thickness of layer i, and $K_{i/v}$ is the partition coefficient between layer i and the vehicle. Provided the SC is intact and undamaged, Cleek and Bunge (Cleek and Bunge, 1993) showed that G is large enough that its independent effect is lost. However, B can vary from small for hydrophilic chemicals to large for lipophilic chemicals. Cleek and Bunge (Cleek and Bunge, 1993) demonstrated that dermal absorption (i.e., uptake) is effected by B when B ≥ about 0.1 and only for exposure times longer than the time required to reach steady state (i.e., approximately > $0.4t_{lag,sc}$).

Lacking experimental data, Bunge and Cleek (Bunge and Cleek, 1995) recommended estimating B as:

$$B = \frac{P_{cw}\sqrt{MW}}{2.6\ (cm/hr)} \tag{9.3}$$

where MW is the molecular weight of the penetrating chemical, and the SC permeability coefficient, P_{cw}, is calculated according to a version of the Potts and Guy correlation that was reported by Cleek and Bunge (Cleek and Bunge, 1993) and Bunge *et al.* (Bunge *et al.*, 1994):

$$\log P_{cw}(cm/hr) = -2.8(0.08) - 6.0(0.6)\ (10^{-3})\ MW + 0.74(0.07)\log K_{ow} \quad (n > 90,\quad r^2 = 0.67) \tag{9.4}$$

The key assumptions of Eq. (9.3) are (1) that the vehicle has not significantly altered the permeabilities of the SC and VE, (2) that the phase properties of the VE are similar to water (i.e., $K_{e/w} \approx 1$), (3) that the diffusion coefficient of a chemical in the VE varies with MW as theoretically predicted for large molecules diffusing in a liquid continuum (i.e., P_{ew} decreases as MW increases according to $1/(MW)^{0.5}$), and (4) that $D_e/L_e \approx 10^{-4}$ cm/sec (0.36 cm/hr) for a molecule of MW = 50.

Experimental values for B are not generally available. However, several studies of dermal absorption have collected enough information to calculate B. In this chapter, experimental B values have been collected for testing Eq. (9.3) with P_{cw} specified by Eq. (9.4).

9.3. *Results and Discussion*

9.3.1. Validation of the Data

Optimally, P_{cv} should be determined with isolated SC (e.g., VE removed by trypsin digestion), and P_{ev} should be determined with only VE (e.g., the dermis is removed by trypsin digestion and then the SC is removed completely by tape-stripping).

This is almost never done. Frequently, *in vitro* permeability measurements are made on split or full thickness skin which includes the dermis in addition to the SC and VE.

The presence of part or all of the dermis can add an additional resistance to the penetration of lipophilic penetrants. It is not known whether the thicker dermis layer yields a stronger or weaker resistance to penetration than the thinner, denser VE. The dermis resistance to penetration *in vivo* is small because this layer is well perfused with blood capillaries, but, this is not true *in vitro* where this layer is primarily penetrated by Fickian diffusion. This means that for estimating *in vivo* dermal absorption the resistance of the dermis should preferentially not be incorporated into the B-parameter.

Since B is a ratio of permeability coefficients, it would not be surprising for some of the factors which influence SC permeability (e.g., vehicle, temperature, exposure time before steady state, ionized status of the penetrant, and species skin type) to also influence B. Chapter 5 reviews in more detail various factors that influence the SC permeability coefficient. Factors influencing the VE permeability coefficient are not well documented. Unless the vehicle has altered physicochemically the SC, the B-parameter should be independent of the vehicle. It seems plausible that factors influencing SC permeability coefficients influence B less because these factors influence the VE permeability coefficient in the same way. As an example, SC and VE permeability coefficients probably both increase with increasing temperature producing a B-value with a weaker temperature dependency.

Meaningful B-parameters should be calculated from steady-state SC and VE permeability coefficients. Ionized status of the penetrant probably effects B because ionized species have lower rates of penetration through the SC, while ionized and unionized species probably penetrate the hydrophilic VE with more comparable rates. Thus, B-values for ionogenic compounds in this study were calculated by adjusting the SC permeability coefficient for ionization as long as 10% or more of the compound is

unionized. B-values should be different when measured in the skins of different animal species and this will be examined empirically using the data we compile.

Few satisfactory measurements of the relative permeability of the SC and VE are available for comparison, particularly for lipophilic penetrants, but an initial collection of 63 values is provided in Table 9.1. The mean MW was 148 (ranging from a maximum of 585 to a minimum of 18 with an upper quartile of 184 and a lower quartile of 60). The mean $\log K_{ow}$ was 1.40 (ranging from a maximum of 4.57 to a minimum of -3.53 with an upper quartile of 3.00 and a lower quartile of -0.31). Experimental B-values are available for many of the normal alcohols and various steroids but for few other compounds. B-parameters measured with human skin are distinguished from those measured with animal skin, and the B-values are classified according to the number and type of skin layers used in determining them. We do not withhold B-values from analysis because additional layers of skin are present in the measurement of P_{cw} or P_{ew} but we identify the layers present in our database. Footnotes document relevant information including the designation of B-values calculated from permeability coefficients from non-aqueous vehicles (i.e., neat alcohol, 40% polyethylene glycol, and 25% ethanol). The B-parameters for amphetamine (Galey *et al.*, 1976), morphine (Roy *et al.*, 1994), and tetraethylammonium bromide (Kim *et al.*, 1992) were excluded because more than 90% of the molecules were ionized for each of these species. The B-parameters for deoxycorticosterone, 17α-hydroxydesoxycorticosterone, and 11α-hydroxyprogesterone (Tojo *et al.*, 1987) and nandrolone (Foreman and Kelly, 1976) were reserved for future analysis when acceptable $\log K_{ow}$ can be determined or calculated for these compounds.

B-values were calculated as P_1/P_2, where P_1 is the permeability coefficient chosen to approximate P_{cv} and P_2 is the permeability coefficient chosen to approximate P_{ev}. Table 9.1 lists the skin layers present during the measurement of P_1 (labeled as Skin P_1) and P_2 (labeled as Skin P_2). To illustrate this idea, consider the B-value calculated for n-

butanol (Flynn *et al.*, 1981). As shown in Table 9.1, the permeability coefficient P_1 reported by Flynn *et al.* (Flynn *et al.*, 1981) was measured across isolated SC and P_2 was measured across VE alone. That is, the layers of skin were consistent with the definition of B as P_{cv}/P_{ev}, and $P_{cv}/P_{ev} = P_1/P_2$. Now consider another B-value calculated for n-butanol (Scheuplein and Blank, 1973). From Table 9.1, P_1 reported by Scheuplein and Blank (Scheuplein and Blank, 1973) was measured across epidermis (SC and VE) and P_2 was measured across dermis alone. Since P_{ev} was not measured, it was not possible to calculate $B = P_{cv}/P_{ev}$, so $B = P_1/P_2$ was used as an approximation. In all calculated B-values, P_1 was measured across skin which included SC and P_2 was measured across skin which did not include SC. For partially ionized compounds, P_1 was divided by the fraction of compound unionized (f_{ui}). This adjustment for ionization is discussed more completely in Chapter 5.

Table 9.2 contains additional relevant information about the B-database including f_{ui}, and the permeability coefficients P_1 and P_2. The data in Tables 9.1 and 9.2 are documented and described more fully in Appendix 9A where investigations are arranged alphabetically by the last name of the lead author.

9.3.2. Estimation of the B-parameter

Using a standard multiple linear regression program, JMP (SAS Institute, 1995), all valid (not provisional and not excluded) B-parameters listed in Table 9.1, including values from both human and animal skins, were analyzed for effects of MW and $\log K_{ow}$:

$$\begin{aligned} &\log B = -1.258(0.11) + 0.461(0.041)\log K_{ow} - 0.0035(0.0007)\,MW \\ &(n = 63,\ r^2 = 0.682,\ r^2(\text{adj.}) = 0.671,\ \text{RMSE} = 0.55,\ \text{F-Ratio} = 64.3) \end{aligned} \tag{9.5}$$

Standard errors are reported alongside the regression coefficients in parenthesis. Both of the predictor variables, $\log K_{ow}$ and MW, were relevant at the 95% level of significance. Equation (9.5) shows that 68.2% of the variability in logB can be explained by variation in $\log K_{ow}$ and MW alone. The r^2(adj.) statistic is analogous to r^2 but allows for more

Table 9.1 The B-Parameter Database

	Compound	MW	$\log K_{ow}$[a]	T (C)	B[b]	Species[c]	Skin P_1[d]	Skin P_2[e]	Reference
E	Amphetamine (pH = 7.0, f_{ui} < 0.1)	135.2	1.76	30	N/A[f]	Human	FULL	D	Galey et al., 1976
	n-Butanol	74.1	0.88	37	0.041	HL Mouse	SC	VE	Flynn et al., 1981
	n-Butanol	74.1	0.88	25	0.060[g]	Human	EPID	D	Scheuplein & Blank, 1973
	n-Butanol	74.1	0.88	25	0.083	Human	EPID	D	Scheuplein & Blank, 1973
	4-Chloro-3-cresol	142.6	3.10	37	0.778	HL Mouse	SC	VE+D	Huq et al., 1986
	2-Chlorophenol	128.6	2.15	37	0.569	HL Mouse	SC	VE+D	Huq et al., 1986
	Corticosterone	346.5	1.94	37	0.133[i]	HL Mouse	SC[h]	VE+D	Tojo et al., 1987
	n-Decanol	158.3	4.57	37	1.899	HL Mouse	SC[h]	VE+D	Flynn et al., 1981
P	Deoxycorticosterone	330.4	N/A	37	0.640[i]	HL Mouse	SC[h]	VE+D	Tojo et al., 1987
	2,4-Dichlorophenol	163.0	3.06	37	2.559	HL Mouse	SC	VE+D	Huq et al., 1986
	2,4-Dinitrophenol	184.1	1.67	37	0.323	HL Mouse	SC	VE+D	Huq et al., 1986
	β-Estradiol	272.4	4.01	30	0.071	Human	FULL	D	Galey et al., 1976
	β-Estradiol	272.4	4.01	37	[0.333]	HL Mouse	SC[h]	VE+D	Kim et al., 1992
	β-Estradiol	272.4	4.01	37	0.017[j]	Human	SC	VE	Liu et al., 1994
	Estrone	270.4	3.13	37	[0.458]	HL Mouse	SC[h]	VE+D	Kim et al., 1992
	Ethanol	46.0	-0.31	37	0.012	HL Mouse	SC	VE	Flynn et al., 1981
	Ethanol	46.0	-0.31	25	0.103[g]	Human	EPID	D	Scheuplein & Blank, 1973
	Ethanol	46.0	-0.31	25	0.023	Human	EPID	D	Scheuplein & Blank, 1973
	Ethanol	46.0	-0.31	33	0.032	Rabbit	FULL	D	Treherne, 1956
	Fentanyl (pH = 7.4, f_{ui} =0.73)	336.5	4.05	37	0.248	HL Mouse	SC[h]	VE	Roy et al., 1994
	Fentanyl (pH = 8.0, f_{ui} = 0.92)	336.5	4.05	37	0.016	Human	SC[k]	D	Roy & Flynn, 1990
	Glucose	180.2	[-3.53]	33	0.001	Rabbit	FULL	D	Treherne, 1956
	Glycerol	92.1	-1.76	33	0.004	Rabbit	FULL	D	Treherne, 1956
	n-Heptanol	116.0	2.72	37	0.714	HL Mouse	SC[h]	D	Flynn et al., 1981
	n-Heptanol	116.0	2.72	25	0.357[g]	Human	EPID	D	Scheuplein & Blank, 1973
	n-Heptanol	116.0	2.72	25	1.280	Human	EPID	D	Scheuplein & Blank, 1973
	n-Heptanol	116.0	2.72	30	0.183[k,l]	Human	SC	VE+D	Blank et al., 1967
	n-Hexanol	102.2	2.03	31	[0.518]	HL Mouse	SC[h]	VE+D	Bond & Barry, 1988

	Compound	MW	$\log K_{ow}$[a]	T (C)	B[b]	Species[c]	Skin P_1[d]	Skin P_2[e]	Reference
	n-Hexanol	102.2	2.03	31	[1.048]	Human	SC[h]	VE+D	Bond & Barry, 1988
	n-Hexanol	102.2	2.03	37	0.172	HL Mouse	SC	VE	Flynn et al., 1981
	n-Hexanol	102.2	2.03	25	0.149[g]	Human	EPID	D	Scheuplein & Blank, 1973
	n-Hexanol	102.2	2.03	25	0.650	Human	EPID	D	Scheuplein & Blank, 1973
	Hydrocortisone	362.5	1.61	37	0.021[i]	HL Mouse	SC[h]	VE+D	Tojo et al., 1987
	Hydrocortisone	362.5	1.61	37	[0.004]	HL Mouse	SC[h]	VE+D	Kim et al., 1992
P	17α-Hydroxydesoxycorticosterone	346.5	N/A	37	0.146[i]	HL Mouse	SC[h]	VE+D	Tojo et al., 1987
P	11α-Hydroxyprogesterone	330.5	N/A	37	0.125[i]	HL Mouse	SC[h]	VE+D	Tojo et al., 1987
	17α-Hydroxyprogesterone	330.5	3.17	37	0.176[i]	HL Mouse	SC[h]	VE+D	Tojo et al., 1987
	Methanol	32.0	-0.77	37	0.006	HL Mouse	SC	VE	Flynn et al., 1981
	Methanol	32.0	-0.77	37	0.003	HL Mouse	SC	VE	Behl et al., 1983
	Methanol	32.0	-0.77	25	0.452[g]	Human	EPID	D	Scheuplein & Blank, 1973
	Methanol	32.0	-0.77	25	0.009	Human	EPID	D	Scheuplein & Blank, 1973
	Methanol	32.0	-0.77	33	0.022	Rabbit	FULL	D	Treherne, 1956
E	Morphine (pH = 5.0, f_{ui} <0.1)	285.3	0.76	37	N/A[f]	HL Mouse	SC[h]	VE+D	Roy et al., 1994
P	Nandrolone	274.4	N/A	22	0.126[m]	Human	SC[h]	VE+D	Foreman & Kelly, 1976
	4-Nitrophenol	139.1	1.91	37	0.078	HL Mouse	SC	VE+D	Huq et al., 1986
	n-Nonanol	144.0	4.26	37	2.009	HL Mouse	SC[h]	VE+D	Flynn et al., 1981
	n-Octanol	130.2	3.00	37	0.761	HL Mouse	SC[h]	VE+D	Flynn et al., 1981
	n-Octanol	130.2	3.00	25	0.500[g]	Human	EPID	D	Scheuplein & Blank, 1973
	n-Octanol	130.2	3.00	25	2.000	Human	EPID	D	Scheuplein & Blank, 1973
	Ouabain	584.6	-1.70	30	7.4E-5	Human	FULL	D	Galey et al., 1976
	n-Pentanol	88.0	1.56	37	0.064	HL Mouse	SC	VE	Flynn et al., 1981
	n-Pentanol	88.0	1.56	25	0.064[g]	Human	EPID	D	Scheuplein & Blank, 1973
	n-Pentanol	88.0	1.56	25	0.250	Human	EPID	D	Scheuplein & Blank, 1973
	Phenol	94.1	1.46	37	0.060	HL Mouse	SC	VE+D	Huq et al., 1986
	Phenol	94.1	1.46	37	0.006	HL Mouse	SC	VE	Behl et al., 1983
	Progesterone	314.5	3.87	37	1.900[i]	HL Mouse	SC[h]	VE+D	Tojo et al., 1987
	Progesterone	314.5	3.87	37	[0.837]	HL Mouse	SC[h]	VE+D	Kim et al., 1992

Compound	MW	$\log K_{ow}$[a]	T (C)	B[b]	Species[c]	Skin P_1[d]	Skin P_2[e]	Reference
n-Propanol	60.0	0.25	37	0.014	HL Mouse	SC	VE	Flynn et al., 1981
n-Propanol	60.0	0.25	25	0.089[g]	Human	EPID	D	Scheuplein & Blank, 1973
n-Propanol	60.0	0.25	25	0.039	Human	EPID	D	Scheuplein & Blank, 1973
n-Propanol	60.0	0.25	30	0.007[k,l]	Human	SC	VE	Blank et al., 1967
Sufentanil (pH = 7.4, f_{ui} = 0.95)	386.5	3.95	37	0.722	HL Mouse	SC[h]	VE+D	Roy et al., 1994
Sufentanil (pH = 8.0, f_{ui} = 0.98)	386.5	3.95	37	0.028	Human	SC[k]	VE	Roy & Flynn, 1990
Tetraethylammonium bromide	210.2	-2.82	37	N/A[f]	HL Mouse	SC[h]	VE+D	Kim et al., 1992
Thiourea	76.1	-1.02	33	0.003	Rabbit	FULL	D	Treherne, 1956
Urea	60.1	-2.11	33	0.002	Rabbit	FULL	D	Treherne, 1956
Water	18.0	-1.38	31	[0.017]	HL Mouse	SC[h]	VE+D	Bond & Barry, 1988
Water	18.0	-1.38	31	[0.035]	Human	SC[h]	VE+D	Bond & Barry, 1988
Water	18.0	-1.38	25	0.008	Human	EPID	D	Scheuplein & Blank, 1973
Water	18.0	-1.38	30	0.007	Human	FULL	D	Galey et al., 1976

E

a. Reported $\log K_{ow}$ are taken from Hansch (Hansch et al., 1995), unless contained within brackets, in which case they were calculated (Daylight, 1995).
b. Calculated as P_1/P_2, where P_1 was divided by f_{ui} as an adjustment for ionization. Values contained within brackets were digitized.
c. Species that the skin was excised from to measure the permeability coefficients.
d. Layer (or layers) of skin used to approximate SC; SC for stratum corneum, EPID for stratum corneum and viable epidermis, FULL for full-thickness skin.
e. Layer (or layers) of skin used to approximate VE; VE for viable epidermis, D for dermis, VE+D for stripped full-thickness skin.
f. Penetrant was more than 90% ionized.
g. Vehicle was neat (i.e., pure) alcohol.
h. P_1 inferred from measurements made with full-thickness skin and stripped full-thickness skin.
i. Vehicle was a 40% (v/v) polyethylene glycol aqueous solution.
j. Skin-chemical contact times were brief, perhaps unsteady state.
k. P_1 inferred from measurements made with epidermis (SC and VE) and VE alone.
l. Stratum corneum was delipidized with solvents.
m. Vehicle was a 25% ethanol solution.

Table 9.2 Intermediate Permeability Coefficients Used to Calculate the B-Parameters

	Compound	f_{ui}[a]	P_1[b] (cm/hr)	P_2[c] (cm/hr)	B[d]	Species	Reference
E	Amphetamine (pH = 7.0, f_{ui} < 0.1)	<0.1	1.40E-5	9.54E-2	N/A[e]	Human	Galey et al., 1976
	n-Butanol	1	1.54E-2	3.77E-1	0.041	HL Mouse	Flynn et al., 1981
	n-Butanol	1	6.00E-5	1.00E-3	0.060[f]	Human	Scheuplein & Blank, 1973
	n-Butanol	1	2.50E-3	3.00E-2	0.083	Human	Scheuplein & Blank, 1973
	4-Chloro-3-cresol	~1[g]	2.35E-1	3.02E-1	0.778	HL Mouse	Huq et al., 1986
	2-Chlorophenol	~1[g]	1.82E-1	3.20E-1	0.569	HL Mouse	Huq et al., 1986
	Corticosterone	1	6.00E-4[h]	4.50E-3	0.133[i]	HL Mouse	Tojo et al., 1987
	n-Decanol	1	2.45E-1[h]	1.29E-1	1.899	HL Mouse	Flynn et al., 1981
P	Deoxycorticosterone	1	5.60E-3[h]	8.70E-3	0.640[i]	HL Mouse	Tojo et al., 1987
	2,4-Dichlorophenol	~1[g]	4.53E-1	1.77E-1	2.559	HL Mouse	Huq et al., 1986
	2,4-Dinitrophenol	~1[g]	2.28E-1	7.05E-1	0.323	HL Mouse	Huq et al., 1986
	β-Estradiol	1	3.90E-3	5.51E-2	0.071	Human	Galey et al., 1976
	β-Estradiol	1	3.60E-2[h]	1.08E-1	[0.333]	HL Mouse	Kim et al., 1992
	β-Estradiol	1	9.80E-3	5.67E-1	0.016[j]	Human	Liu et al., 1994
	Estrone	1	5.77E-2[h]	1.26E-1	[0.458]	HL Mouse	Kim et al., 1992
	Ethanol	1	4.88E-3	4.05E-1	0.012	HL Mouse	Flynn et al., 1981
	Ethanol	1	7.20E-4	7.00E-3	0.103[f]	Human	Scheuplein & Blank, 1973
	Ethanol	1	8.00E-4	3.50E-2	0.023	Human	Scheuplein & Blank, 1973
	Ethanol	1	2.66E-3	8.46E-2	0.032	Rabbit	Treherne, 1956
	Fentanyl (pH = 7.4, f_{ui} =0.73)	0.73	2.90E-2[h]	1.60E-1	0.248	HL Mouse	Roy et al., 1994
	Fentanyl (pH = 8.0, f_{ui} = 0.92)	0.92	3.00E-3	2.02E-1[k]	0.016	Human	Roy & Flynn, 1990
	Glucose	1	5.34E-5	4.20E-2	0.001	Rabbit	Treherne, 1956
	Glycerol	1	2.35E-4	5.46E-2	0.004	Rabbit	Treherne, 1956
	n-Heptanol	1	1.59E-1[h]	2.23E-1	0.714	HL Mouse	Flynn et al., 1981
	n-Heptanol	1	2.50E-5	7.00E-5	0.357[f]	Human	Scheuplein & Blank, 1973
	n-Heptanol	1	3.20E-2	2.50E-2	1.280	Human	Scheuplein & Blank, 1973
	n-Heptanol	1	4.44E-2	2.43E-1	0.183[l]	Human	Blank et al., 1967
	n-Hexanol	1	1.78E-2[h]	3.44E-2	[-0.518]	HL Mouse	Bond & Barry, 1988

	Compound	f_{ui}[a]	P_1[b] (cm/hr)	P_2[c] (cm/hr)	B[d]	Species	Reference
	n-Hexanol	1	5.58E-2[h]	5.32E-2	[1.048]	Human	Bond & Barry, 1988
	n-Hexanol	1	5.88E-2	3.41E-1	0.172	HL Mouse	Flynn et al., 1981
	n-Hexanol	1	5.20E-5	3.50E-4	0.149[f]	Human	Scheuplein & Blank, 1973
	n-Hexanol	1	1.30E-2	2.00E-2	0.650	Human	Scheuplein & Blank, 1973
	Hydrocortisone	1	5.91E-5[h]	2.80E-3	0.021[i]	HL Mouse	Tojo et al., 1987
	Hydrocortisone	1	3.60E-4[h]	9.00E-2	[0.004]	HL Mouse	Kim et al., 1992
P	17α-Hydroxydesoxycorticosterone	1	7.80E-4[h]	5.30E-3	0.146[i]	HL Mouse	Tojo et al., 1987
P	11α-Hydroxyprogesterone	1	9.80E-4[h]	7.80E-3	0.125[i]	HL Mouse	Tojo et al., 1987
	17α-Hydroxyprogesterone	1	2.80E-3[h]	1.59E-2	0.176[i]	HL Mouse	Tojo et al., 1987
	Methanol	1	2.62E-3	4.33E-1	0.006	HL Mouse	Flynn et al., 1981
	Methanol	1	3.20E-3	1.25E+0	0.003	HL Mouse	Behl et al., 1983
	Methanol	1	1.04E-2	2.30E-2	0.452[f]	Human	Scheuplein & Blank, 1973
	Methanol	1	5.00E-4	5.30E-2	0.009	Human	Scheuplein & Blank, 1973
	Methanol	1	2.54E-3	1.18E-1	0.022	Rabbit	Treherne, 1956
E	Morphine (pH = 5.0, f_{ui} < 0.1)	<0.1	7.10E-5[h]	3.80E-2	N/A[e]	HL Mouse	Roy et al., 1994
P	Nandrolone	1	8.74E-4[h]	6.93E-3	0.154[m]	Human	Foreman & Kelly, 1976
	4-Nitrophenol	~1[g]	2.54E-2	3.27E-1	0.078	HL Mouse	Huq et al., 1986
	n-Nonanol	1	2.67E-1[h]	1.33E-1	2.009	HL Mouse	Flynn et al., 1981
	n-Octanol	1	1.71E-1[h]	2.25E-1	0.761	HL Mouse	Flynn et al., 1981
	n-Octanol	1	1.00E-5	2.00E-5	0.500[f]	Human	Scheuplein & Blank, 1973
	n-Octanol	1	5.20E-2	2.60E-2	2.000	Human	Scheuplein & Blank, 1973
	Ouabain	1	3.96E-6	5.33E-2	0.000	Human	Galey et al., 1976
	n-Pentanol	1	2.38E-2	3.73E-1	0.064	HL Mouse	Flynn et al., 1981
	n-Pentanol	1	5.10E-5	8.00E-4	0.064[f]	Human	Scheuplein & Blank, 1973
	n-Pentanol	1	6.00E-3	2.40E-2	0.250	Human	Scheuplein & Blank, 1973
	Phenol	1	2.02E-2	3.38E-1	0.060	HL Mouse	Huq et al., 1986
	Phenol	1	2.78E-2	4.50E+0	0.006	HL Mouse	Behl et al., 1983
	Progesterone	1	3.30E-2[h]	1.74E-2	1.900[i]	HL Mouse	Tojo et al., 1987
	Progesterone	1	1.32E-1[h]	1.58E-1	[0.837]	HL Mouse	Kim et al., 1992

Compound	f_{ui}[a]	P_1[b] (cm/hr)	P_2[c] (cm/hr)	B[d]	Species	Reference
n-Propanol	1	5.50E-3	3.80E-1	0.014	HL Mouse	Flynn et al., 1981
n-Propanol	1	1.60E-4	1.80E-3	0.089[f]	Human	Scheuplein & Blank, 1973
n-Propanol	1	1.20E-3	3.10E-2	0.039	Human	Scheuplein & Blank, 1973
n-Propanol	1	1.71E-3	2.30E-1	0.007[l]	Human	Blank et al., 1967
Sufentanil (pH = 7.4, f_{ui} = 0.95)	0.95	2.40E-2[h]	3.50E-2	0.722	HL Mouse	Roy et al., 1994
Sufentanil (pH = 8.0, f_{ui} = 0.98)	0.98	5.50E-3	2.01E-1[k]	0.028	Human	Roy & Flynn, 1990
E Tetraethylammonium bromide	<0.1	1.10E-4[h]	1.08E-1	N/A[e]	HL Mouse	Kim et al., 1992
Thiourea	1	1.73E-4	6.66E-2	0.003	Rabbit	Treherne, 1956
Urea	1	1.42E-4	7.20E-2	0.002	Rabbit	Treherne, 1956
Water	1	1.83E-3[h]	1.10E-1	[0.017]	HL Mouse	Bond & Barry, 1988
Water	1	3.04E-3[h]	8.69E-2	[0.035]	Human	Bond & Barry, 1988
Water	1	5.00E-4	6.00E-2	0.008	Human	Scheuplein & Blank, 1973
Water	1	1.80E-3	2.20E-1	0.007	Human	Galey et al., 1976

a. Reported f_{ui} determined from pK_a values calculated in SPARC at 25 °C and adjusted for temperature using general methods.
b. Permeability coefficient used to approximate P_{cv}.
c. Permeability coefficient used to approximate P_{ev}.
d. Calculated as P_1/P_2, where P_1 was divided by f_{ui} as an adjustment for ionization. Values contained within brackets were digitized.
e. Penetrant was more than 90% ionized, so P_1 was not adjusted for ionization.
f. Vehicle was neat (i.e., pure) alcohol.
g. The pH was provided but it was mentioned that pH < pK_a so that the phenols studied are mostly unionized.
h. P_1 inferred from measurements made with full-thickness skin and stripped full-thickness skin.
i. Vehicle was a 40% (v/v) polyethylene glycol aqueous solution.
j. Skin-chemical contact times were brief, perhaps unsteady state.
k. P_1 inferred from measurements made with epidermis (SC and VE) and VE alone.
l. Stratum corneum was delipidized with solvents.
m. Vehicle was a 25% ethanol solution.

relevant comparisons between models with different numbers of fitted parameters (JMP User's Guide, (SAS Institute, 1995)). Specifically, $(1 - r^2)$ = error sum of squares/total sum of squares and $(1 - r^2(\text{adj.}) = (1 - r^2)(n - 1)/(n - p)$ where n = # of data points and p = # of parameters. RMSE is the root mean square error of the model, which is zero when the model perfectly correlates the data. When presented in an equation, F-Ratio is the model F-Ratio (sum of squares for the model divided by the degrees of freedom for the model) / (sum of squares for the error divided by the degrees of freedom for the error), and when presented for a term such as the MW or $\log K_{ow}$, the F-Ratio is the effect F-Ratio (sum of squares for the effect divided by the degrees of freedom for the effect) / (sum of squares for the error divided by the degrees of freedom for the error). The model F-ratio = 1 when there is zero correlation with the parameters and is large for correlations with good predictive power. Because the number of fitted parameters is in the denominator of the F-Ratio, changes in the model F-Ratio with an increase in the number of parameters should reflect the effect on predictive power relative to the number of fitted parameters. Thus, a correlation with a larger number of parameters might give a higher r^2 (or r^2(adj.)) but a lower F-Ratio than a correlation with fewer parameters. This would indicate that the improvement in predictive power (as indicated by a larger r^2) was not as large per parameter as for the equation with fewer parameters. Generally, the level of variability is comparable to that observed in SC permeability coefficient data (Potts and Guy, 1992). Comparing Eq. (9.5) with analogous equations for the SC permeability coefficient (e.g., Eq. (5.27)), the coefficient multiplying the MW term in Eq. (9.5) is about ½ as large that in the SC permeability correlation. This seems reasonable, since the VE permeability coefficient should decrease with molecular size, although probably less strongly than the SC permeability coefficient.

Analysis of the same database, but with $\log K_{ow}$ as the sole predictor yielded the equation:

$$\log B = -1.627(0.103) + 0.353(0.043)\log K_{ow}$$
$$(n = 63,\ r^2 = 0.528,\ r^2(\text{adj.}) = 0.521,\ \text{RMSE} = 0.66,\ \text{F-Ratio} = 68.4) \tag{9.6}$$

Thus, 52.8% of the variation in log B can be explained by variation in log K_{ow} alone.

All valid (not provisional and not excluded) B-parameters from only human skin were linearly regressed in terms of MW and logK_{ow} to obtain:

$$\log B = -0.988(0.152) + 0.368(0.061)\log K_{ow} - 0.0051(0.0009)\,\text{MW}$$
$$(n = 27,\ r^2 = 0.681,\ r^2(\text{adj.}) = 0.655,\ \text{RMSE} = 0.54,\ \text{F-Ratio} = 25.6) \tag{9.7}$$

Standard errors are reported alongside the regression coefficients in parenthesis. Both of the predictor variables logK_{ow} and MW were relevant at the 95% level of significance, and 68.1% of the variability in log B can be explained by variation in logK_{ow} and MW alone. This fit is very similar to the fit obtained to all of the valid data (Eq. (9.5)), indicating that differences (apparent in this database) between B-parameters determined in human skin and the skin of various animals are small. The MW dependence may be somewhat larger in the human skin data compared to the combined human and animal skin data, however more data is required to know conclusively.

Analysis of the same database, but with logK_{ow} as the only predictor variable yielded the equation:

$$\log B = -1.477(0.193) + 0.236(0.087)\log K_{ow}$$
$$(n = 27,\ r^2 = 0.227,\ r^2(\text{adj.}) = 0.196,\ \text{RMSE} = 0.83,\ \text{F-Ratio} = 7.4) \tag{9.8}$$

Equation (9.8) shows that very little (22.7%) of the variation in log B can be explained by variation in logK_{ow} alone in this small database of human B-parameters, indicating that the MW contribution is needed to reasonably estimate B for human skin.

Finally, the measured B-parameters listed in Table 9.1 collected from non-aqueous vehicles (i.e., neat alcohol, 40% v/v polyethylene glycol, or 25% ethanol) were excluded from the previous analysis. The database was reanalyzed without

measurements from non-aqueous vehicles but including both animal and human skin data to yield:

$$\log B = -1.301(0.118) + 0.476(0.042)\log K_{ow} - 0.0038(0.0007)\,MW$$
$$(n = 51,\ r^2 = 0.731,\ r^2(adj.) = 0.720,\ RMSE = 0.54,\ F\text{-}Ratio = 65.3) \qquad (9.9)$$

A significant level (73%) of the variation in log B can be explained by variation in $\log K_{ow}$ and MW. This is a modest improvement over Eq. (9.5) which included data from non-aqueous vehicles. Equation (9.9) probably provides the most accurate and reliable estimate of the B-parameter. A similar equation was not developed for human skin alone because only 19 datapoints were available over too limited a range of MW and $\log K_{ow}$ to be meaningful.

In Figure 9.1 the valid (not the provisional and not excluded) values of B are compared with predictions from the method of estimation proposed by Cleek and Bunge (Cleek and Bunge, 1993), Eqs. (9.3)-(9.4), plotted as a function of $\log K_{ow}$. The upper and lower horizontal dashed lines designate a deviation from a perfect prediction (i.e., $B = B_{pred}$) by an order of magnitude too large and too small respectively. The B-values which are calculated from SC and VE permeability coefficients measured using aqueous vehicles are distinguished from those B-values calculated from permeability coefficients measured using non-aqueous vehicles. This figure shows that predictions made using Eqs. (9.3) and (9.4) on average underestimate the experimental B-values by approximately an order of magnitude. The negative slope indicates that Eqs. (9.3) and (9.4) predict a larger dependence on K_{ow} than is observed experimentally. The data from experiments with nonaqueous vehicles (i.e., neat alcohol, 40% (v/v) polyethylene glycol solution, or 25% ethanol solution) appear to be underestimated by Eqs. (9.3) and (9.4) more than the measurements from aqueous vehicles. To the extent that this actually reflects a trend, rather than chance, these observations are consistent with increased SC permeability resulting from damage or alteration of the SC. As indicated in Fig. 9.1, the

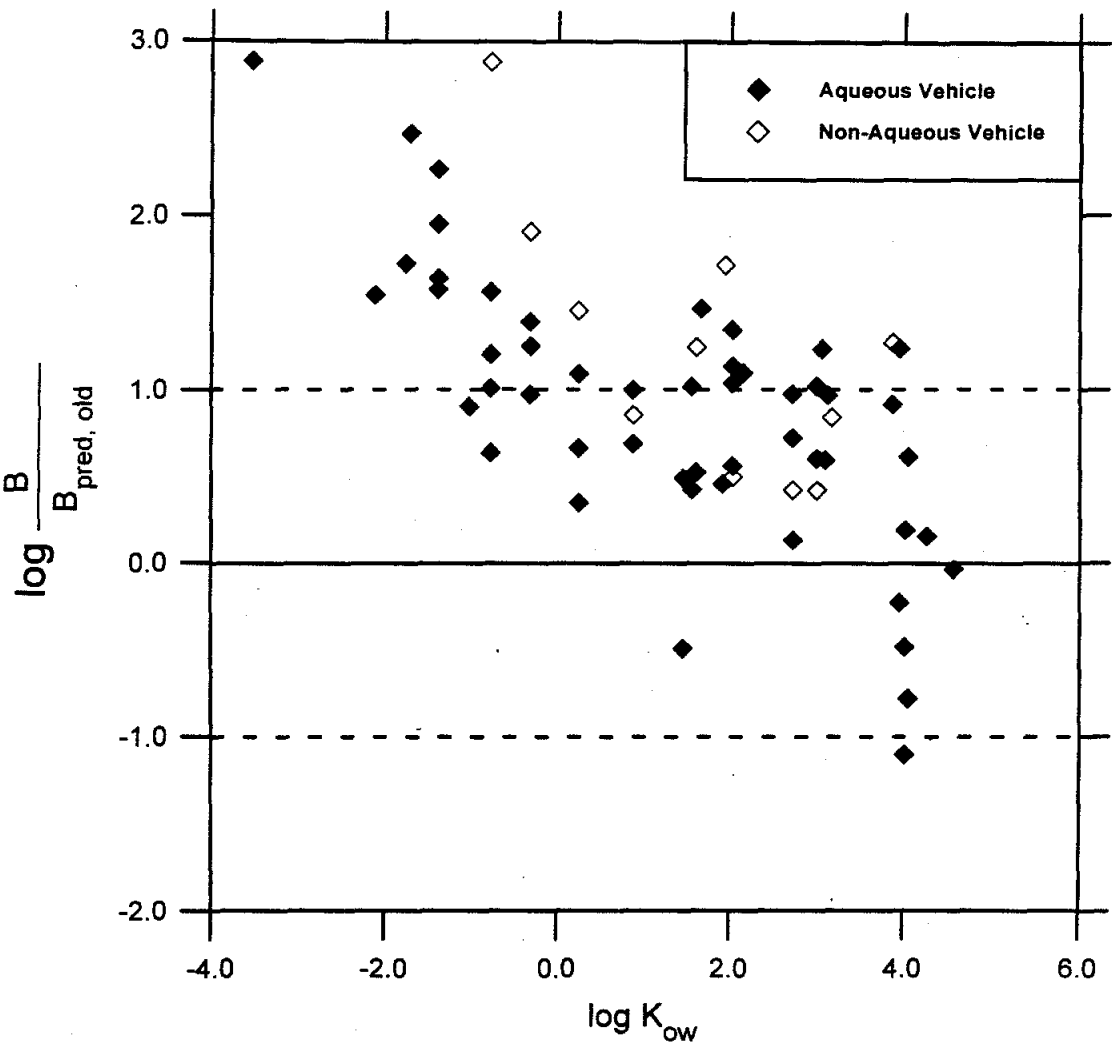

Figure 9.1 Validated B-parameters, measured using aqueous and nonaqueous vehicles and animal and human skins, compared with predictions by Eqs. (9.3) and (9.4), $B_{pred,old}$, plotted as a function of $\log K_{ow}$.

data are predominantly distributed over the range ($-2.0 > \log K_{ow} > 4.0$). B-values for more lipophilic compounds are not available and are needed.

In Figure 9.2 the valid B-database is compared with predictions from Eqs. (9.3)-(9.4) plotted as a function of MW. Aqueous-vehicle B-values measured from aqueous vehicles are distinguished from B-values measured from non-aqueous vehicles. Many of the valid B-values are for compounds with relatively low molecular weights (i.e., MW < 200). The ratio of $B/B_{pred,\ old}$ shows no obvious correlation with MW indicating that the MW dependence predicted by Eqs. (9.3) and (9.4) are consistent with the data.

In Figure 9.3 the valid B-database is compared with predictions from Eq. (9.9) plotted as a function of $\log K_{ow}$. B-values calculated from SC and VE permeability coefficients measured using human skin are distinguished from B-values calculated from SC and VE permeability coefficients measured using the skin of various animals (the type of animal is specified in Table 9.1). Also, aqueous measurements are distinguished from non-aqueous measurements. As indicated in Figure 9.3, Eq. (9.9) is an significant improvement over Eqs. (9.3) and (9.4) which underestimates the B-values in this database by approximately a factor of ten (on average). Only 4 of the 63 measurements are misestimated by at least a factor of ten using Eq. (9.9). The animal skin B-values do not appear to differ meaningfully from the human skin B-values, which favors their inclusion in the development of the empirical correlation. Given the known differences in the various animal species included in Tables 9.1 and 9.2, this is a somewhat unexpected result which warrants further investigation when more data are available.

Figure 9.4 compares the valid B-database with predictions from Eq. (9.9) plotted as a function of MW. B-values measured using human skin are distinguished from B-values measured using animal skin. Also, aqueous measurements are distinguished from non-aqueous measurements. Many of the valid B-values are for compounds with relatively low molecular weights (i.e., MW < 200). The ratio of $B/B_{pred,\ new}$ shows no

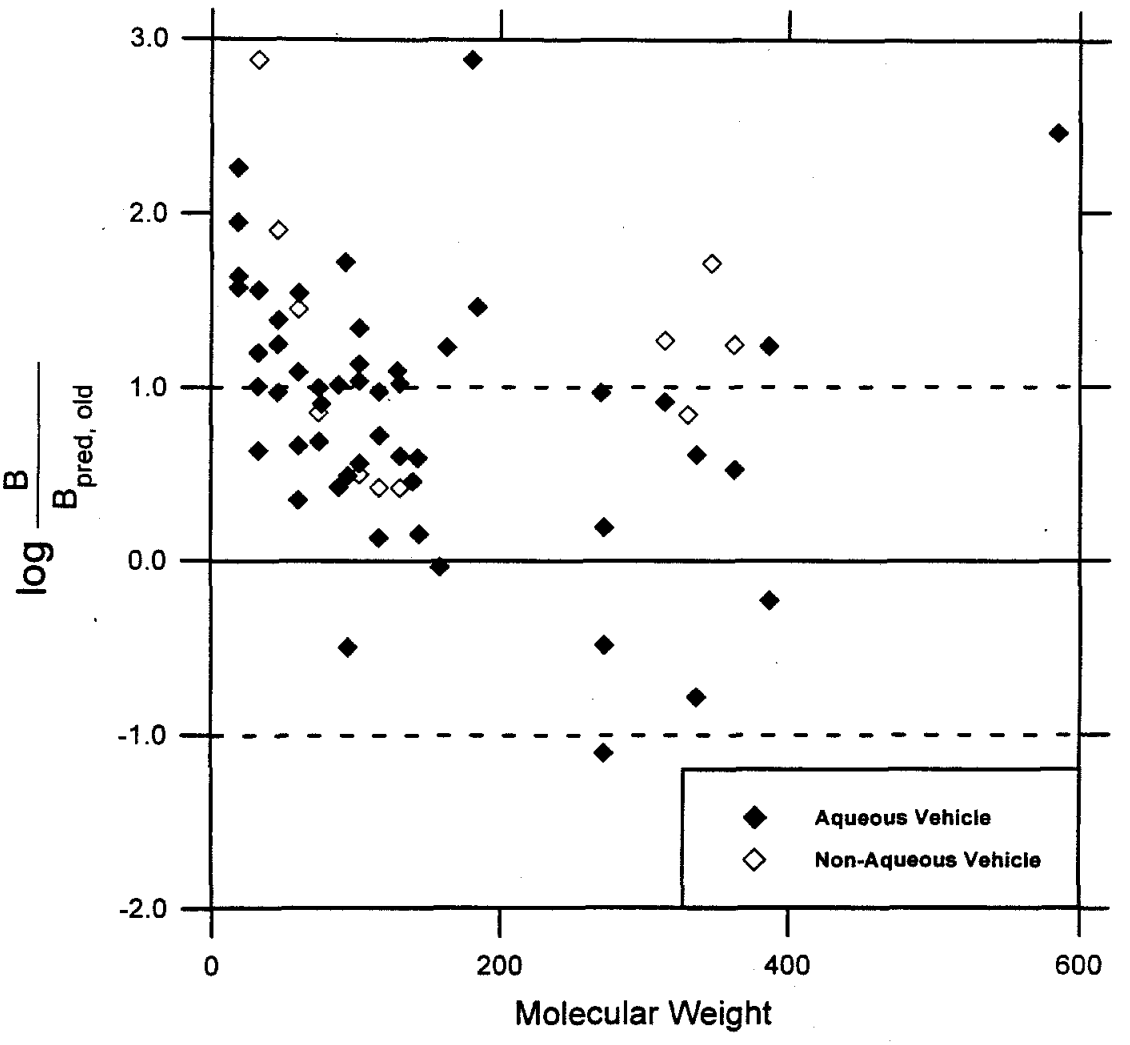

Figure 9.2 Validated B-parameters, measured using aqueous and nonaqueous vehicles and animal and human skins, compared with predictions by Eqs. (9.3) and (9.4), $B_{pred,old}$, plotted as a function of MW.

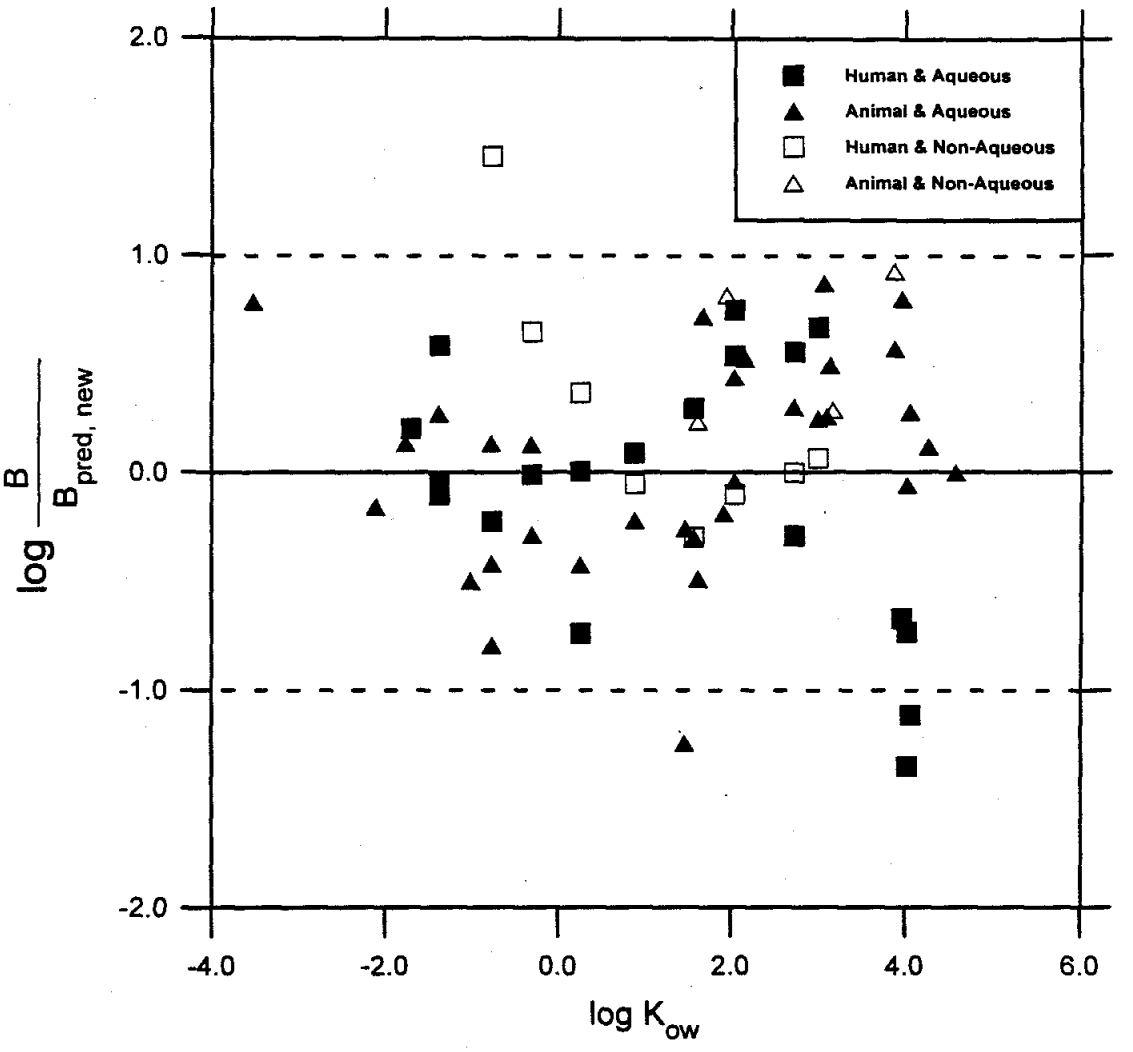

Figure 9.3 Validated B-parameters, measured with human and animal skin and using aqueous and nonaqueous vehicles, compared with predictions by Eq. (9.9), $B_{pred,new}$, plotted as a function of $\log K_{ow}$.

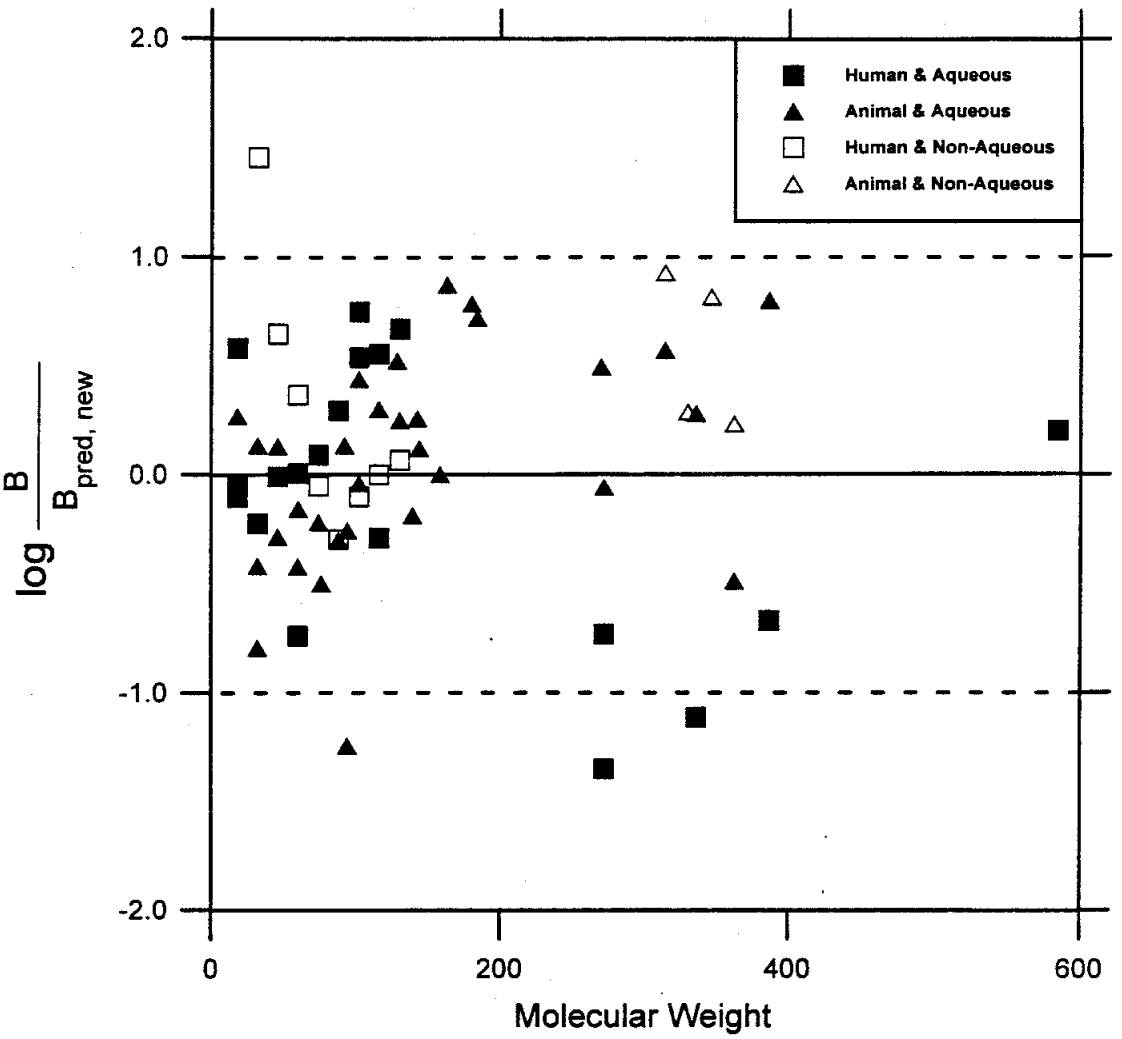

Figure 9.4 Validated B-parameters, measured using aqueous and nonaqueous vehicles and animal and human skins, compared with predictions by Eq. (9.9), $B_{pred,new}$, plotted as a function of MW.

obvious correlation with MW indicating that the MW dependence predicted by Eq. (9.9) is consistent with this set of data.

In Figure 9.5, the valid B-database, plotted as a function of $\log K_{ow}$, is compared with predictions from the old method, Eqs. (9.3) and (9.4), and two of the new correlations, Eq. (9.7) for human skin measurements from aqueous and non-aqueous vehicles and Eq. (9.9) for human and animal skin measurements from only aqueous vehicles. B-values for compounds with MW > 200 are distinguished from B-values for compounds with MW < 200. All three methods of estimation are plotted at MW equal to 100 and 300, respectively. Human skin B-values are distinguished from animal skin B-values. As expected, based on the results shown in Figures 9.1–9.4, Eq. (9.9) provides a better estimate of the B-parameter than does Eqs. (9.3) and (9.4). As illustrated in Figure 9.5, the differences in the MW dependence of the three methods are almost indistinguishable.

9.3.3. Estimation of the Viable Epidermis Permeability Coefficient

We have also examined the VE permeability coefficients presented in Table 9.2 with an equation in the form of Eq. (9.4). Unlike B-values, which are independent of the vehicle as long as the vehicle does not physicochemically alter either the SC or VE, VE-permeability coefficients are effected by the vehicle type. Assuming initially that this contribution is not significant, we analyzed all valid VE permeability coefficients, from aqueous and non-aqueous vehicles, to determine the following equation:

$$\begin{aligned} &\log P_{ew}(\text{cm / hr}) = -1.21(0.2) - 0.0001(0.001)\,\text{MW}^* - 0.016(0.07)\log K_{ow}^* \\ &(n = 63,\ r^2 = 0.001,\ r^2(\text{adj.}) = -0.032,\ \text{RMSE} = 0.98,\ \text{F - Ratio} = 0.05) \end{aligned} \tag{9.10}$$

The asterisks designate that the coefficients multiplying MW and $\log K_{ow}$ are not statistically different from zero at the 95% confidence level. As indicated by the listed goodness-of-fit parameters, Eq. (9.10) represents an extremely poor fit to the P_{ew} data. Another poor fit was obtained when an analogous equation was developed using only the

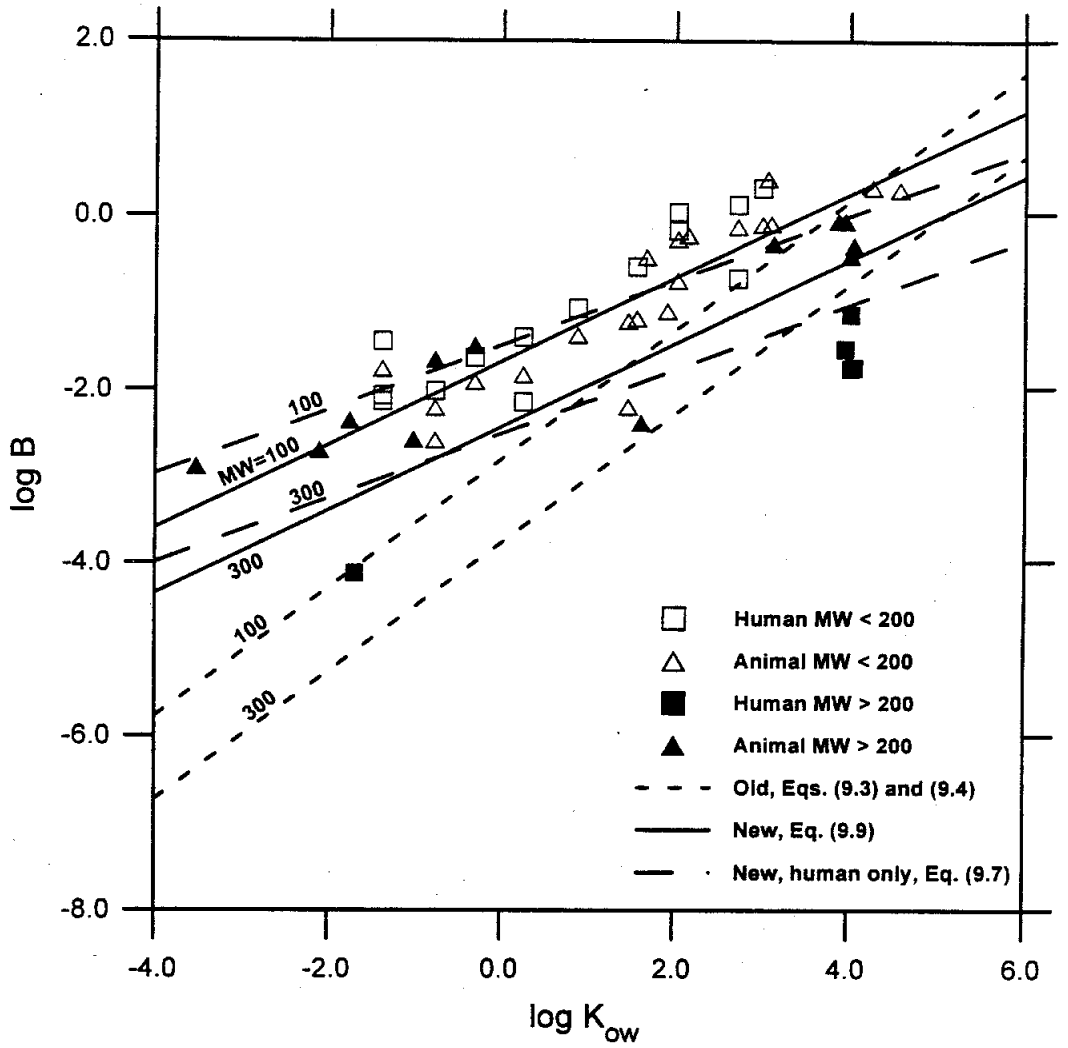

Figure 9.5 Old and new methods for predicting the B-parameter, evaluated at molecular weights of 100 and 300, compared to experimental B-parameters measured with human and animal skin from only aqueous vehicles.

P_{ew} values corresponding to the aqueous vehicle and valid B-values:

$$\log P_{ew}(\text{cm / hr}) = -0.85(0.1) - 0.0006(0.001)\,MW^{*} + 0.043(0.04)\log K_{ow}^{*} \quad (9.11)$$
$$(n = 51,\ r^2 = 0.031,\ r^2(\text{adj.}) = -0.009,\ \text{RMSE} = 0.49,\ \text{F-Ratio} = 0.77)$$

This same portion of the P_{ew} database was analyzed only in terms of $\log K_{ow}$:

$$\log P_{ew}(\text{cm / hr}) = -0.92(0.1) + 0.026(0.03)\log K_{ow}^{*} \quad (9.12)$$
$$(n = 51,\ r^2 = 0.012,\ r^2(\text{adj.}) = -0.008,\ \text{RMSE} = 0.49,\ \text{F-Ratio} = 0.62)$$

An average VE permeability coefficient may be appropriate since P_{ew} has no meaningful dependency on $\log K_{ow}$ or MW in any of these regressions. The permeability coefficient data appear to be approximately normally distributed when a histogram is generated for the $\log P_{ew}$ values (i.e., P_{ew} values appear to be lognormally distributed). The average $\log P_{ew}$ for the 51 aqueous-vehicle measurements was determined to be -0.881(±0.138). When these confidence intervals are converted into P_{ew} for the 51 measurements we calculate an average P_{ew} to be 0.1315 with an upper (95% confidence) limit of 0.1805 cm/hr and a lower limit of 0.0957 cm/hr. Larger mean permeability coefficients and larger uncertainties are calculated when the permeability coefficients are analyzed in the form of P_{ew} rather than $\log P_{ew}$ due to the existence of more extremely high P_{ew} values than expected in a normal distribution. If all 63 measurements (including the non-aqueous measurements) are included, the average P_{ew} is slightly lower, 0.057 cm/hr, but still quite uncertain (0.033-0.100 cm/hr). Although the permeability coefficient is not precisely known an average value somewhere within the interval 0.05-0.15 cm/hr seems to represent most of the data.

Alternatively, the VE permeability coefficient can be estimated by forcing consistency between the B-parameter correlation (i.e., Eq. (9.9)) and an accurately known correlation for estimating the SC permeability coefficient. That is, $\log P_{ew} = \log P_{cw} - \log B$, where $\log B$ and $\log P_{cw}$ are correlations in terms of MW and $\log K_{ow}$. Using Eq. (9.9)

to estimate the B-parameter and the suggested equation from Chapter 5:

$$\log P_{cw}\,(cm/hr) = -2.44(0.12) + 0.51(0.04)\log K_{ow} - 0.0050(0.0005)\,MW$$
$$(n = 170,\ r^2 = 0.551,\ r^2(adj.) = 0.546,\ RMSE = 0.80,\ F\text{-}Ratio = 102.6) \quad (5.27)$$

to estimate the SC permeability coefficient, we calculate the following equation for the VE permeability coefficient:

$$\log P_{ew}(cm/hr) = -1.045(0.17) + 0.081(0.06)\log K_{ow} - 0.00223(0.0009)\,MW \quad (9.13)$$

The bracketed uncertainties are calculated (e.g., for the MW term 0.0009 = $\sqrt{(0.00051)^2 + (0.0007)^2}$). The SC permeability coefficients can also be made to be consistent with the B-values by using the P_{cw} reported in Table 9.2. Analyzing the P_{cw} corresponding to the 51 valid and aqueous-vehicle B-measurements gave the correlation:

$$\log P_{cw} = -2.14(0.11) + 0.52(0.04)\log K_{ow} - 0.0045(0.0007)\,MW$$
$$(n = 51,\ r^2 = 0.785,\ r^2(adj.) = 0.776,\ RMSE = 0.51,\ F\text{-}Ratio = 87.6) \quad (9.14)$$

Using Eq. (5.27) to estimate the B-parameter and Eq. (9.14) to estimate the SC permeability coefficient, and again using the relationship $\log P_{ew} = \log P_{cw} - \log B$, we arrive at the following equation for the VE permeability coefficient:

$$\log P_{ew}(cm/hr) = -0.837(0.16) + 0.04(0.06)\log K_{ow} - 0.00072(0.001)\,MW \quad (9.15)$$

The uncertainties in brackets are calculated as described previously.

Equations (9.13) and (9.15) have very similar coefficients that are essentially not different from the fit to the P_{ew} data (i.e., Eq. (9.10)). We do not know which of these methods for estimating the VE permeability coefficient is most correct. One could speculate that certain non-random laboratory errors may cancel during the calculation of B-values and other effects (e.g., differences between species, temperature effects, natural variability in skin, skin preparation, among others) may partially be negated, making the B-parameter correlation ((9.9)) more relevant than the VE permeability coefficient

correlation (i.e., (9.10)). Clearly P_{ew} has a weaker relationship to MW and $\log K_{ow}$ than P_{cw} does and this relationship can not be well characterized with the present database.

Probably the most realistic estimate of the B-parameter that we can suggest at this time is based on P_{cw} predicted by Eq. (5.27) and an average value for P_{ew}. The evidence which we have compiled suggests that 0.10 cm/hr is a representative value for P_{ew}. Based on this estimate and Eq. (5.27) we suggest that B-values be calculated according to the equation:

$$\log B = -1.44 + 0.514 \log K_{ow} - 0.00502\ MW \qquad (9.16)$$

Uncertainties have not been calculated, since we have assumed a value for P_{ew} (= 0.10 cm/hr), but the uncertainties should be larger than for Eq. (5.27) due to uncertainties in P_{ew}.

Using Eq. (5.27), we have calculated the maximum $\log K_{ow}$ at which the VE will not effect penetration (i.e., $B \leq 0.1$) and at which the SC permeability coefficient is less permeable than the VE (i.e., $B < 1$) for compounds with different MW. Figure 9.6 summarizes these calculations for low (i.e., 0.05 cm/hr) and high (i.e., 0.15 cm/hr) limits (see discussion given above) of the VE permeability coefficient. The dashed line corresponding to P_{ew} provides a likely bracket for the most lipophilic compound (with a given MW) that can passes through the skin without being limited by the VE. The VE will be sensed at even lower $\log K_{ow}$ if the VE is less permeable (i.e., $P_{ew} = 0.05$ cm/hr). The solid lines show that the VE provides a resistance equal to that of the SC, even for molecules with $\log K_{ow}$ less than 4.0 for many light (MW < 200) compounds. Only very lipophilic ($\log K_{ow} > 4.0$) compounds are of concern when the MW is greater than 200. These results are generally in good agreement with calculations using Eq. (9.6) with which we estimate that: (1) the VE layer resistance can be neglected when $\log K_{ow}$ is less than 1.76 (which corresponds to a B-value of 0.1 or less), and (2) the SC and VE permeability coefficients are equal (i.e., $B = 1$) when $\log K_{ow}$ equals 4.56.

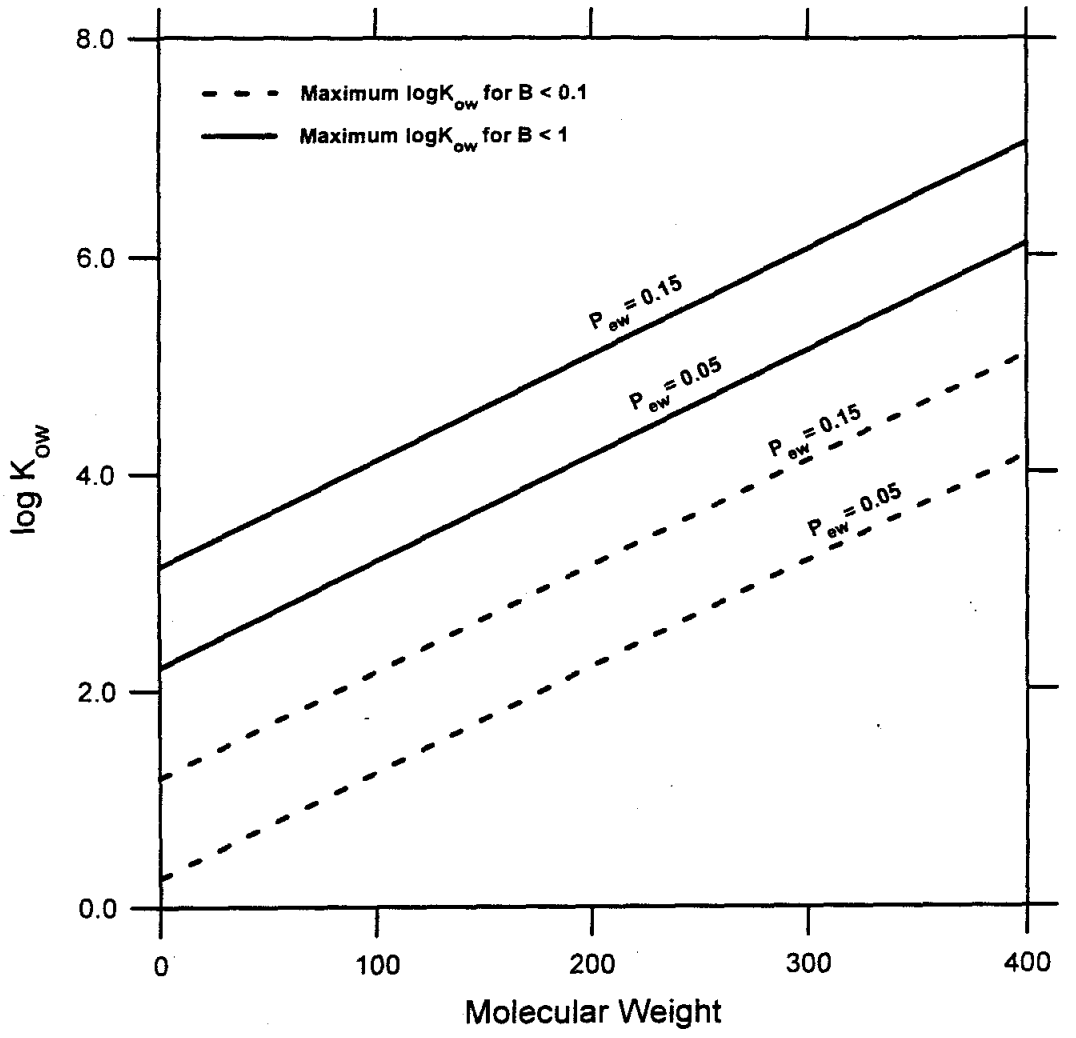

Figure 9.6 Interval of maximum $logK_{ow}$ for a VE which contributes 10% (i.e., B = 0.1) and 50% (i.e., B = 1) of the overall transport resistance for compounds with different MW. Calculations are based on a correlation for the SC permeability coefficient and observed lower (i.e., 0.05 cm/hr) and upper (i.e., 0.15 cm/hr) limits on the average VE permeability coefficient.

9.4. Conclusions

We have compiled a limited database of 70 SC and VE permeability coefficients and the ratio of these permeability coefficients (i.e., namely the B-parameter) to better explore the resistance to penetration posed by the viable epidermis (VE). Penetration of chemicals through the viable epidermis was found to depend minimally upon chemical properties such as lipophilicity and molecular weight. An average value of approximate magnitude, 0.05-0.15 cm/hr, seems appropriate for the VE permeability coefficient. Based on the analysis, the VE provides the dominant resistance for highly lipophilic ($\log K_{ow} \geq 4.5$) compounds. The database showed that an intuitive method for estimating the B-parameter has a stronger dependency upon lipophilicity than reflected in the database and provides estimates which on average underestimate the B-parameter by one order of magnitude. We propose an empirical correlation that will provide improved estimates of the B-parameter. More accurate estimation of the B-parameter will likely require the compilation of more measurements for more highly lipophilic and diverse compounds.

9.5. Notation

B	=	A parameter measuring the SC/VE permeability ratio
$B_{pred,new}$	=	Predicted B-value by Eq. (9.9)
$B_{pred,old}$	=	Predicted B-value by Eqs. (9.3) and (9.4)
D_c	=	Effective diffusivity of the absorbing chemical in the SC
D_e	=	Effective diffusivity of the absorbing chemical in the VE
f_{ui}	=	Fraction of the total chemical dose that is unionized in the vehicle
G	=	Parameter measuring the relative lag times of the damaged and undamaged SC
K_{ce}	=	Equilibrium partition coefficient between the SC and VE for the absorbing chemical
K_{cv}	=	Partition coefficient between the SC and vehicle for the absorbing chemical
K_{cw}	=	Partition coefficient between the SC and water for the absorbing chemical
K_{ev}	=	Partition coefficient between the VE and vehicle for the absorbing chemical
K_{ew}	=	Partition coefficient between the VE and water for the absorbing chemical
K_{ow}	=	Octanol-water partition coefficient of the penetrating chemical
L_c	=	Effective thickness of the SC
L_e	=	Effective thickness of the VE
MW	=	Molecular weight of the absorbing chemical
P_1	=	Permeability coefficient selected to approximate P_{cv}
P_2	=	Permeability coefficient selected to approximate P_{ev}
P_{cv}	=	Steady-state permeability of the SC from a specified vehicle
P_{cw}	=	Steady-state permeability of the SC from water
P_{ev}	=	Steady-state permeability of the VE from a specified vehicle
P_{ew}	=	Steady-state permeability of the VE from water
SC	=	Stratum corneum
$t_{lag,sc}$	=	Lag time across the SC, equals $D_c/(6L_c^2)$
$t_{lag,ve}$	=	Lag time across the VE
VE	=	Viable epidermis

9.6. References

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9.7. Appendix 9A: Documentation on B-Parameter Extraction

This appendix contains specific information about how the B-values were extracted. Details are arranged alphabetically by the last name of the lead author of the publication from which the data were taken.

When SC and VE permeability coefficients (P_{cv} and P_{ev}, respectively) were measured and reported directly, B was calculated as P_{cv}/P_{ev} unless otherwise indicated. When P_{cv} and P_{ev} were not reported, but permeability coefficients across skin containing different layers of skin were reported, P_{cv} and P_{ev} were calculated whenever possible. Calculations are based on the relationship that resistances (i.e., the reciprocal of the permeability coefficient) through different skin layers are additive. For example, when permeability coefficients through epidermis (SC and VE layers present), P_v, and completely tape-stripped epidermis (i.e., only the VE), P_{ev}, were reported, the SC permeability coefficient, P_{cv} would be calculated, $P_{cv} = P_v P_{ev}/(P_{ev}-P_v)$, and $B = P_{cv}/P_{ev} = P_1/P_2$. The calculation illustrated in this example will hereafter be known as the Type-1 calculation. For some measurements, one or more of P_{cv} and P_{ev} were not reported and could not be calculated, in which case the reported permeability coefficients were used without adjustment.

Behl et al., 1983

B-values for methanol and phenol were determined by taking the ratio of average (for dorsal and abdominal) SC and VE permeability coefficients for these compounds, through hairless mouse skin, as reported in Table IX. The SC and VE permeability coefficients are calculated from permeability coefficients measured on whole skin and dermis (Tables I - III) and skin that was stripped of SC (25 times using tape). First the VE permeability coefficient was calculated using the stripped permeability coefficient and the dermis permeability coefficient reported in Table IX, then the SC permeability coefficient was calculated using experimental permeability coefficients for whole skin and the dermis and calculated permeability coefficients for the VE, according to Eq. 2. Thus, using the average of dorsal and abdominal permeability coefficients, we calculate a B of 0.003 (= 3.2/1250) for methanol and 0.006 (= 27.8/4500) for phenol.

Blank et al., 1967

Using human abdominal skin, Blank *et al.* measured permeability coefficients for propanol and heptanol at 30°C through the epidermis (SC and VE) and delipidized epidermis as reported in Table II. The delipidized epidermis was prepared by soaking epidermis in a solution of chloroform-methanol for two days. We have calculated the

permeability coefficient of the SC according to the Type-1 procedure, by assuming that the delipidized epidermis approximately represented the permeability coefficient of the viable epidermis. Using this procedure we calculated SC permeability coefficients of 0.0016 cm/hr for propanol and 0.045 cm/hr for heptanol. B was calculated based on these SC permeability coefficients, and the permeability coefficients measured across delipidized epidermal membranes (which approximately represents P_{ew}).

Bond and Barry, 1988

Permeability coefficients through whole and stripped whole human abdominal and hairless mouse skin were digitized from Figure 3 for hexanol and water. For human skin the digitized permeability coefficient was 0.027 cm/hr (whole) and 0.053 cm/hr (stripped whole) for hexanol, and 0.0029 cm/hr (whole) and 0.087 cm/hr (stripped whole) for water. For hairless mouse the digitized permeability coefficients were 0.012 cm/hr (whole) and 0.034 cm/hr (stripped whole) for hexanol, and 0.0018 cm/hr (whole) and 0.110 cm/hr (stripped whole) for water. SC permeability coefficients were calculated from permeability coefficients measured for full-thickness and stripped full-thickness skin using the Type-1 method but replacing P_{ev} with the permeability coefficient across the combined VE-dermis layer and P_v with the permeability coefficient across intact full-thickness skin. The human SC permeability coefficients we calculated were 0.0558 cm/hr for hexanol and 0.00304 cm/hr for water, and the hairless mouse SC permeability coefficients we calculated were 0.0178 cm/hr for hexanol and 0.0018 cm/hr for water. B-values were calculated as the ratio of the calculated SC permeability coefficient to VE-dermis composite membrane permeability coefficients.

Flynn et al., 1981

Permeability coefficients for normal alcohols (C_1 through C_{10}) through hairless mouse skin were reported for full-thickness skin, heat separated epidermis, heat separated dermis, and tape-stripped full thickness skin. B-values for the normal alcohols methanol through hexanol ($C_1 - C_6$) were determined from resistances, through the SC and VE, presented in Table IV. These reported SC and VE permeability coefficients were calculated based on measurements made on other skin layers. Data for methanol through hexanol were reported in Table IV as resistances through the SC calculated from the difference of measured resistances in the full-thickness skin and heat separated dermis. SC permeability coefficients for heptanol through decanol ($C_7 - C_{10}$) were calculated from permeability coefficients measured for full-thickness skin (Table III) and 25-times stripped full-thickness skin (Table V), according to the Type-1 calculation, but replacing P_{ev} with the permeability coefficient through stripped full-thickness skin. In order of increasing carbon number, the calculated SC permeability coefficients were 0.159, 0.171,

0.267, 0.245 cm/hr, respectively. B-values for heptanol through decanol were calculated as the ratio of SC permeability coefficients to permeability coefficients measured through the stripped and full-thickness skin.

Foreman and Kelly., 1976

A B-value for nandrolone in human skin was determined from the secondarily calculated resistances, through the SC and VE-dermis layer (stripped whole skin), presented in Table 2. The SC permeability coefficient was calculated from whole skin permeability coefficients and stripped whole skin permeability coefficients, according to Eq. 4. This is consistent with the Type-1 calculation, but replacing P_{ev} with the VE-dermis permeability coefficient. Each of the separate runs (there were four) was used to calculate a B-value and then these were averaged to determine the B which is reported. The B-value for nandrolone was reserved for future analysis when an acceptable $\log K_{ow}$ can be found for this compound; it was excluded from analysis except as indicated.

Galey et al., 1976

Human skin permeability coefficients for whole skin and dermis (prepared from whole skin by heat treatment) are presented in Table II for amphetamine, β-estradiol, ouabain and water. These reported permeability coefficients were used without further calculation to obtain approximate B-values. That is, B-values for these four chemicals were calculated as the ratio of whole skin permeability coefficients to dermis permeability coefficients. Amphetamine was more than 90% ionized in the vehicle, so had to be excluded.

Huq et al., 1986

The B-values for hairless mouse skin were calculated from permeability coefficients through the SC and stripped whole thickness skin (called viable tissue) provided in Table 4. The reported SC permeability coefficients were calculated according to the Type-1 calculation, but replacing P_{ev} with the permeability coefficient across stripped whole thickness skin. The B-values which we calculate are the ratio of the calculated SC-permeability coefficients and measured permeability coefficients across stripped whole thickness skin.

Kim et al., 1992

This paper reviews data, from references 3-5, which is useful for calculating B-values. Permeability coefficients through whole skin were digitized from Figure 3, and permeability coefficients through stripped whole skin are tabulated in Table 1. B-values were calculated as the ratio of permeability coefficients through SC and stripped whole skin, where the permeability coefficients through SC were calculated according to Type-1 calculation, but replacing P_{ev} with the permeability coefficient through stripped whole skin. The SC permeability coefficients so calculated were 0.00011 cm/hr for TEAB, 0.00036 cm/hr for hydrocortisone, 0.036 cm/hr for β-estradiol, 0.057 cm/hr for estrone, and 0.132 cm/hr for progesterone.

Liu et al., 1994

A single B-value for β-estradiol in human skin was taken from the permeability coefficients, calculated separately for the SC and VE, presented in Table 4. These SC and VE permeability coefficients were calculated from measurements made with other skin layers (Tables 2 and 3), although it is not clear how the data were combined. Only four hours was used to investigate β-estradiol, so the B-value may be based on unsteady-state permeability measurements.

Roy et al., 1994

B-values for fentanyl and sufentanil, describing penetration in hairless mouse skin, were determined in three steps: (1) adjusting the full-thickness (called intact) skin permeability coefficient reported in Table 2, so that it was on a basis of unionized drug (i.e., dividing by the fraction unionized, which for fentanyl and sufentanil at pH = 7.4 are 0.73 and 0.95 respectively), (2) calculating the permeability coefficients through the SC for fentanyl and sufentanil with the Table 2 whole-skin permeability coefficients (based on the fraction of unionized drug, according to the first step), and stripped full-thickness skin permeability coefficients also presented in Table 2 according to the Type-1 calculation, and (3) calculating B as the ratio of these SC and stripped full-thickness skin permeability coefficients. No B-value was calculated for morphine, since this compound was more than 90% ionized in the pH = 5 vehicle used, and the SC permeability coefficient could therefore not be accurately adjusted for ionization.

Roy and Flynn, 1990

B-values for fentanyl and sufentanil, describing penetration in human skin, were determined using a procedure similar to that used for Roy *et al.* 1994: (1) adjusting the epidermis permeability coefficient reported in Table III, so that it was on a basis of unionized drug (i.e., dividing by the fraction unionized which for fentanyl at a pH of 8.0 is 0.92 and for sufentanil at pH of 8.0 is 0.98), (2) calculating the permeability coefficients through the SC for fentanyl and sufentanil with the epidermis permeability coefficients (based on the fraction of unionized drug, according to the first step), and VE (stripped epidermal membranes) permeability coefficients presented in Table III according to the Type-1 calculation, and (3) calculating B as the ratio of these SC and VE permeability coefficients.

Scheuplein and Blank, 1973

B-values for water and the normal alcohols (methanol through octanol) in human skin were taken from this paper. Permeability coefficients of these compounds through epidermis (SC and VE) and dermis are available in Table I (from aqueous solution) and Table II (from neat alcohol vehicles). B-values were calculated directly as the ratio of these reported epidermis and dermis permeability coefficients. For water, only the permeability coefficients reported in Table I were used to calculate B.

Tojo et al., 1987

B-values for various steroids in hairless mouse skin were determined from permeability coefficients reported in this paper. Permeation rates through whole skin and stripped whole skin, and solubilities (the vehicles were saturated solutions) are tabulated in Table II. Permeability coefficients through the whole skin and stripped whole skin were calculated by dividing the permeation rates by the solubility for each chemical. Permeability coefficients through the SC were then calculated using permeability coefficients through whole skin and stripped whole skin, according to the Type-1 calculation. B-values were calculated as the ratio of permeability coefficients through SC and stripped whole skin. Deoxycorticosterone, 17α-hydroxydesoxycorticosterone, and 11α-hydroxyprogesterone were reserved for later analysis when acceptable $\log K_{ow}$ can be found for these compounds.

Treherne, 1956

B-values for several chemicals in rabbit skin were determined from permeability coefficients, through whole skin and dermis, which are presented in Table 2. Without additional calculations B-values were calculated as the ratio of whole skin to dermis permeability coefficients.

10. A SIMPLE MODEL OF SKIN DAMAGE

10.1. Introduction

At the simplest level, skin acts as an abrasion barrier and a chemical barrier between body and the environment. It is reasonable to assume that exposure to the external world disrupts the unity of the outermost layers of the stratum corneum (SC). In fact, disruption of the integrity of the SC is a necessary and well known process, known as desquamation. In desquamation, the outer (older) layers of the SC are physically and chemically loosened from newer layers beneath, and gradually exfoliate (i.e., fall off). Desquamation occurs largely from abrasion of the skin, although certain chemical changes take place within the SC in conjunction with physical damage (Downing, 1992). Importantly, the effects of SC degradation on the barrier properties of skin have not been modeled.

The SC can be additionally compromised by irregular levels of physical abuse or chemical contact. Induction of physical damage, due to tape-stripping, abrasion, and exposure to ultraviolet light, has been quantitatively investigated (Bronaugh and Stewart, 1985). Damage from exposure to organic solvents has been investigated (Abrams *et al.*, 1993), as well as the damage resulting from penetration enhancers (Michniak, 1995; Williams and Barry, 1991). The result of either physical or chemical damage is that the integrity of the outer layers of the SC has been disrupted, and this certainly influences the rate of chemical absorption.

The effects of either natural damage, associated with desquamation, or enhanced damage, associated with extremely abrasive or chemically active substances, have not

been incorporated into models of the skin barrier. Most mathematical models treat the SC as a pseudo-homogeneous membrane with constant transport parameters: permeability (P_{cv}), diffusivity (D_c), SC-vehicle partition coefficient (K_{cv}), and thickness (L_c). Realistically, the rate of penetration through skin is likely to be positionally dependent as a result of disruption of SC integrity. We have derived a simplistic model of SC damage which offers some useful qualitative understanding of the effects damage to the external SC layers has on the rate of dermal penetration.

10.2. Method of Analysis

As a first approximation, we model damaged SC as a membrane bilaminate, consisting of a damaged outer layer of thickness L_1 with permeability coefficient P_{1v} and an unaffected interior layer of thickness L_2 with permeability coefficient P_{2v}. Specifically, we consider damage which is present initially or occurs instantaneously following exposure (i.e., by the action of a rapidly-damaging solvent), as opposed to damage which occurs gradually during a chemical exposure. As a result of damage, the outer layer is more easily penetrated by absorbing compounds.

Equations for the cumulative mass permeating passive membranes with constant diffusivities, thicknesses, and partition coefficients are given elsewhere (Cleek and Bunge, 1993; Crank, 1975). Cleek and Bunge (Cleek and Bunge, 1993) discussed equations, for the mass permeating a single membrane, developed assuming that: (1) a constant vehicle concentration (C_v^o) instantaneously equilibrates with the outer layer of the SC, (2) the initial chemical concentration in the skin is zero, and (3) the chemical concentration in the body system remains zero (i.e., the infinite-sink condition) during the entire exposure event. Using the same assumptions, Cleek (Cleek, 1993) presented equations for the mass permeating a two-membrane composite. With minor alterations, these equations can be made to model damaged skin.

When skin has not been damaged, the total resistance is approximately represented by the entire SC ($L_c = L_1 + L_2$), and the dimensionless mass penetrating into deeper tissues is represented by:

$$\frac{M_{out}}{A\,L_c\,K_{cv}\,C_v^o} = \tau - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n \exp\left(-n^2\pi^2\tau\right)}{n^2} \tag{10.1}$$

where A is the exposed area, K_{cv} is the equilibrium partition coefficient between the SC and the vehicle, and τ is defined as

$$\tau = \frac{D_c\,t_{exp}}{L_c^2} \tag{10.2}$$

where D_c is the diffusivity of the chemical in the SC and t_{exp} is the exposure time. When steady state has been attained, ($t_{exp} > t^*$), the mass penetrating into deeper tissues and blood is constant in time:

$$\frac{M_{out}^{\infty}}{A\,L_c\,K_{cv}\,C_v^o} = \tau - \frac{1}{6} \tag{10.3}$$

The lag time for penetration across an undamaged layer of SC with homogeneous properties is

$$t_{lag}^{U} = \frac{L_c^2}{6D_c} \tag{10.4}$$

For a bilaminate membrane the dimensionless mass penetrating into deeper tissues and the blood is given by:

$$\frac{M_{out}}{A(L_1+L_2)K_{cv}\,C_v^o} = \frac{L_1}{(L_1+L_2)}\left[\frac{\tau}{1+B} - \frac{3GB+B+3+G}{6G(1+B)^2} - \frac{2}{B}\sum_{n=1}^{\infty}\frac{\exp\left(-\lambda_n^2\tau\right)}{\lambda_n^2\sqrt{G}\,\sigma_n}\right] \tag{10.5}$$

where σ_n is defined as,

$$\sigma_n = \frac{1}{BG}\left[\sqrt{G}\,(1+B)\cdot\cos\left(\lambda_n/\sqrt{G}\right)\cdot\cos(\lambda_n) - (1+GB)\cdot\sin\left(\lambda_n/\sqrt{G}\right)\cdot\sin(\lambda_n)\right] \tag{10.6}$$

in terms of eigenvalues, λ_n, which are defined by:

$$\sqrt{G}\,B\tan\left(\lambda_n/\sqrt{G}\right)+\tan(\lambda_n)=0 \tag{10.7}$$

The other parameters are:

$$B = \frac{D_1 L_2 K_{1v}}{D_2 L_1 K_{2v}} \tag{10.8}$$

$$G = \frac{D_2 L_1^2}{D_1 L_2^2} \tag{10.9}$$

where D_1 and D_2 are the diffusion coefficients in the exterior and interior layers of the SC and K_{1v} and K_{2v} are the SC-vehicle partition coefficients for the exterior and interior layers of the SC. The dimensionless time, τ, is still expressed by Eq. (10.2). When steady state has been attained (i.e., $t_{exp} > t^*$) the rate of penetration is constant:

$$\frac{M_{out}^{\infty}}{A(L_1+L_2)K_{cv}C_v^o} = \frac{L_1}{L_1+L_2}\left[\frac{\tau}{1+B} - \frac{3GB+B+3+G}{6G(1+B)^2}\right] \tag{10.10}$$

The general lag-time expression for penetration across a two-layer damaged SC composite is:

$$t_{lag}^{D} = \frac{L_1^2}{6D_1}\left[\frac{3GB+B+3+G}{G(1+B)}\right] \tag{10.11}$$

Next, we develop equations for comparing time lags for damaged and undamaged skin. Two comparisons will be made: (1) treatment-damaged skin with untreated skin, and (2) skin that does not have positionally constant transport parameters with hypothetical skin with constant transport parameters and the same overall permeability.

10.2.1. Treatment-Damaged Stratum Corneum

First, we compare time lags for untreated (control) SC with SC that has been damaged through treatment. This finds application in the interpretation of time lags measured with and without the prior application of a chemical that enhances penetration ((Michniak, 1995; Williams and Barry, 1991), among others). In this scenario, a layer of external SC is damaged in the treatment, but the remaining interior SC is unaffected. The treated SC is modeled as a bilaminant consisting of a damaged outer layer, and an undamaged interior layer with the same diffusivity and SC-vehicle partition coefficient as the control SC. We will refer to this as Type-A skin damage.

The model and notation, for Type-A skin damage is depicted in Figure 10.1. The damaging treatment is assumed not to alter the total SC thickness. The unaffected transport parameters of the interior layer (i.e., diffusion coefficient and partition coefficient) are assumed to equal those of the untreated SC. For Type-A skin damage, the ratio of lag times across the damaged skin (t_{lag}^{D}) and undamaged skin (t_{lag}^{U}) becomes:

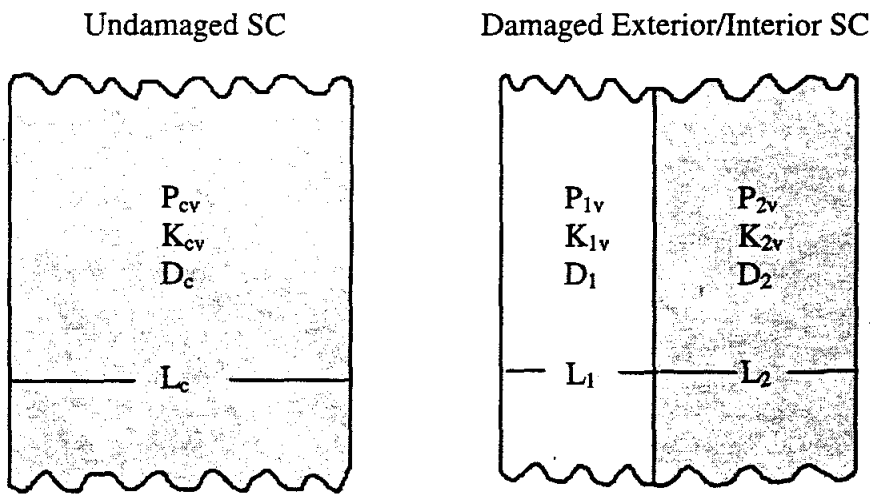
$$\frac{t_{lag}^{D}}{t_{lag}^{U}} = \frac{D_2}{D_1}\left(\frac{L_1}{L_1+L_2}\right)^2\left(\frac{3GB+B+3+G}{G(1+B)}\right) \tag{10.12}$$

In Eq. (10.12) we have not made any assumptions about the ratio of SC-vehicle partition coefficients for damaged and undamaged layers of SC (i.e., the ratio K_{1v}/K_{2v}). In a subsequent analysis, we assume that damage only effects the ratio of diffusion coefficients (i.e., D_1/D_2) and that $K_{1v} = K_{2v}$.

10.2.2. Positionally-Dependent Transport Parameter Comparison

This analysis determines the consequences of assuming that skin transport parameters do not vary with position on interpretation of measured lag times. Specifically, we quantify the bias introduced when penetration data, collected on skin with positionally dependent transport parameters, is analyzed as if the transport

Figure 10.1 Assumptions and notation for two comparisons of undamaged and damaged SC membranes: (1) Type-A - comparison of undamaged (control) SC with SC damaged by a treatment which alters the diffusivity to a thickness L_1 but does not change the SC thickness or partitioning, and (2) Type-B - comparison of a hypothetically undamaged SC with damaged SC having the same thickness, equilibrium partitioning, and overall permeability.



Damage Type	Undamaged SC	Exterior SC (Damaged)	Interior SC (Damaged)
Type-A	$P_{cv} = K_{cv}D_c/L_c$ $= K_{2v}D_2/(L_1+L_2)$	K_{1v} D_1 $L_c = L_1+L_2$	$K_{2v} = K_{cv}$ $D_2=D_c$ $L_c = L_1+L_2$
Type-B	$P_{cv} = P_v\ (1+P_{1v}/P_{2v})$ $= K_{cv}D_c/(L_1+L_2)$	$K_{1v} = K_{cv}$ D_1 $L_c = L_1+L_2$	$K_{2v} = K_{cv}$ D_2 $L_c = L_1+L_2$

parameters were constant and positionally independent. This is a relevant concern, since physicochemical changes accompanying desquamation are likely correlated with transport properties. We will refer to this as Type-B skin damage.

The model and notation, for this type of skin damage is depicted in Figure 10.1. In this analysis a two-layer SC membrane with an outer-layer permeability P_{1v} and an inner-layer permeability P_{2v} is compared with a hypothetical homogeneous (in transport parameters) SC membrane having the same overall permeability:

$$P_{cv} = P_v = \left[\frac{1}{P_{1v}} + \frac{1}{P_{2v}}\right]^{-1} \tag{10.13}$$

The thickness of the composite membrane and the single (hypothetical) membrane are the same. For this type of skin damage the time lag expression (Eq. (10.12)) becomes:

$$\frac{t_{lag}^{D}}{t_{lag}^{U}} = \frac{K_{1v}}{K_{cv}}\left(\frac{L_1}{L_1 + L_2}\right)\left(\frac{3GB + B + 3 + G}{G(1+B)^2}\right) \tag{10.14}$$

If we also assume that damage is only diffusional (i.e., that $K_{1v} = K_{2v} = K_{cv}$), Eq. (10.14) further reduces to:

$$\frac{t_{lag}^{D}}{t_{lag}^{U}} = \frac{L_1}{L_1 + L_2}\left(\frac{3GB + B + 3 + G}{G(1+B)^2}\right) \tag{10.15}$$

10.2.3. Results

Figure 10.2 illustrates the effect of Type A or B damage on the cumulative mass released (Eq. 10.5) compared to undamaged stratum corneum when $D_1/D_2 = 10$ and $L_1/L_2 = 1$. Penetration through the treatment (Type A) damaged skin is greater than through naturally (Type B) damaged or undamaged skin. The lag times through damaged skin are shorter than through undamaged skin.

Figure 10.3 compares calculated lag times through damaged SC and undamaged SC, as a function of the diffusivity ratio (D_1/D_2) and depth effected ($L_1/(L_1 + L_2)$). The

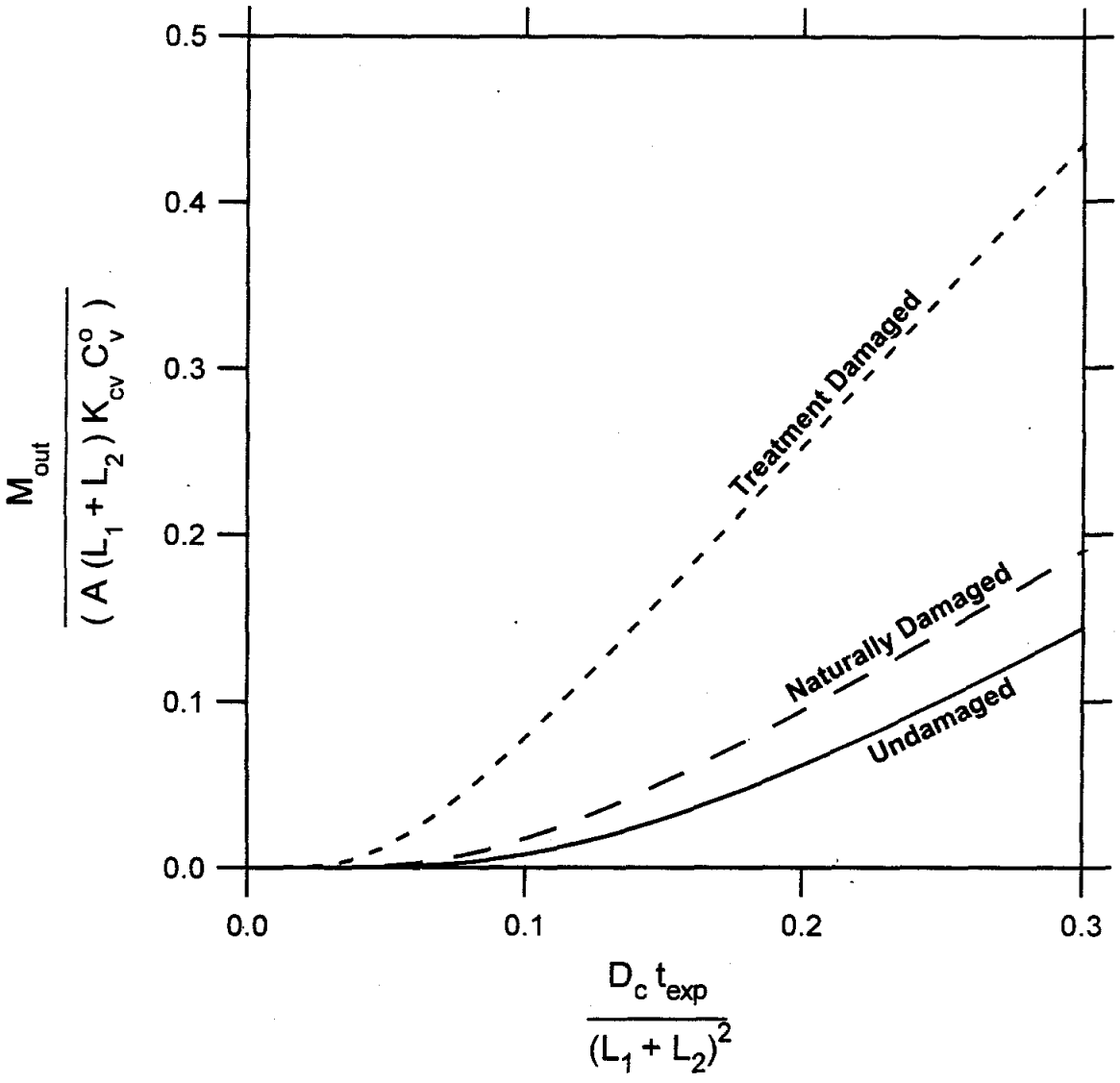


Figure 10.2 Comparison of cumulative mass released for penetration through damaged and undamaged stratum corneum as a function of dimensionless time for $D_1/D_2 = 10$ and $L_1/L_2 = 1$.

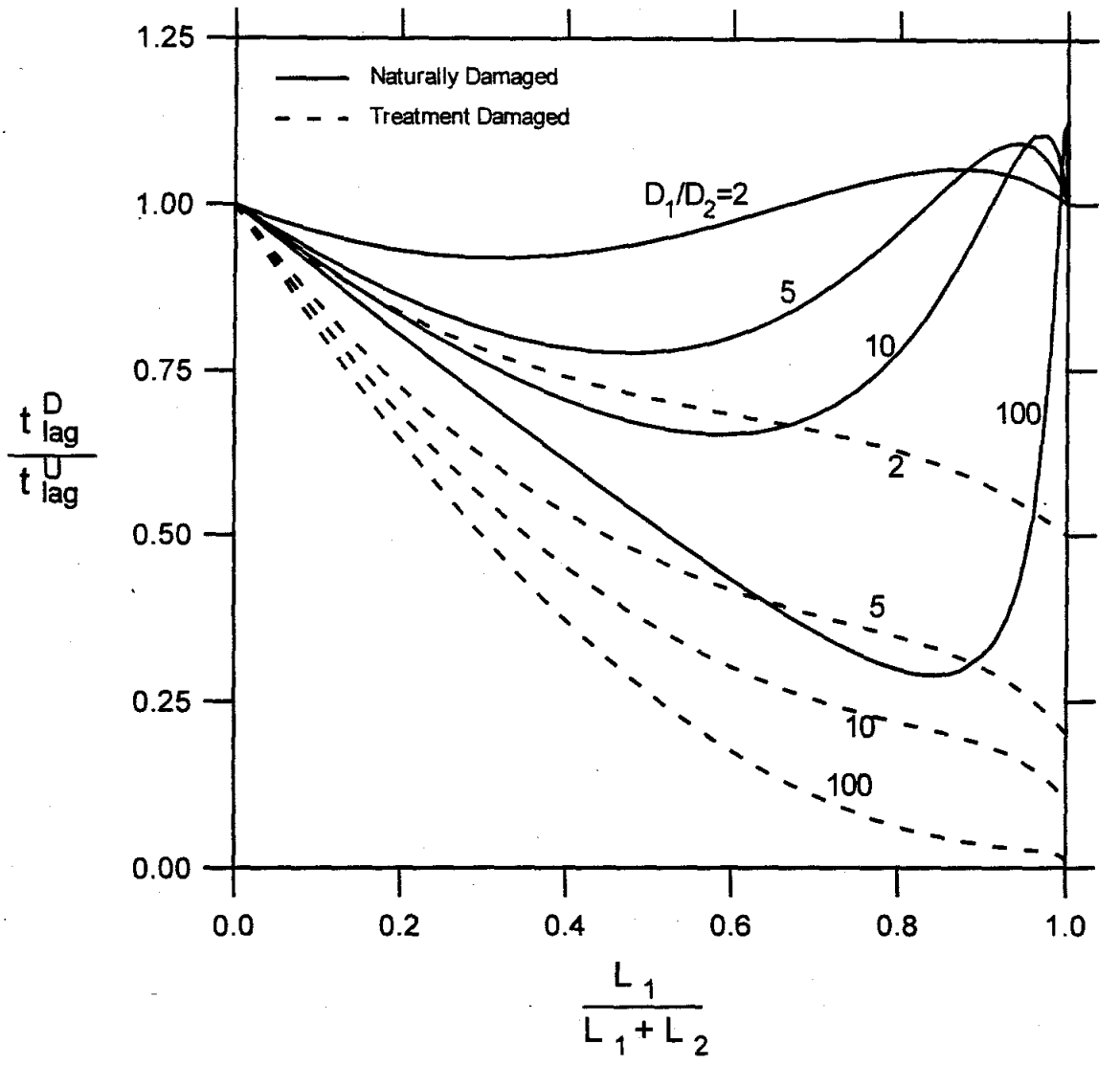


Figure 10.3 Comparison of lag times for penetration through damaged and undamaged stratum corneum as a function of the ratio of diffusion coefficients in the damaged stratum corneum two-membrane composite (i.e., D_1/D_2) and fractional thickness of the damaged layer of stratum corneum (i.e., $L_1/(L_1+L_2)$).

lag times predicted for damaged SC are generally shorter than those for undamaged SC. For Type A damage (dashed curves), the observed lag time could be considerably smaller than the lag time if the SC was undamaged. For Type B damage (solid lines), the misestimation of lag times is much less severe except at high diffusivity ratios and moderate fractions of SC damage (i.e., $0.3 < L_1/(L_1 + L_2) < 0.8$). Specifically, if the outer half of the SC is one order of magnitude less resistant to penetration than the interior SC (i.e., $D_1/D_2 = 10$), then the estimated lag time is only 66% of what would be estimated assuming the SC was undamaged. If the exterior half of the SC was only 5-times more permeable, than the interior half, the lag time would be 77% of what would be estimated assuming the SC was undamaged. There is no reason to account for damage introduced by Type-B experiments unless penetration through the outer SC is more than 5-fold higher than through interior SC.

10.3. Notation

A	=	Area of chemical exposure
B	=	A parameter measuring the SC/VE permeability ratio
C_v^0	=	Concentration of the absorbing chemical in the vehicle; assumed to remain constant during the exposure period, t_{exp}
D_1	=	Diffusion coefficient of damaged outer layer
D_2	=	Diffusion coefficient of unaffected interior layer
D_c	=	Effective diffusivity of the absorbing chemical in the SC
D_e	=	Effective diffusivity of the absorbing chemical in the VE
G	=	Ratio of lag times in the damaged and undamaged SC
K_{1v}	=	Equilibrium partition coefficient between the damaged outer layer of the SC and the vehicle for the absorbing chemical
K_{2v}	=	Partition coefficient between the undamaged interior layer of the SC and the vehicle for the absorbing chemical
K_{ce}	=	Partition coefficient between the SC and VE for the absorbing chemical
K_{cv}	=	Partition coefficient between the SC and vehicle for the absorbing chemical
K_{ev}	=	Partition coefficient between the VE and vehicle for the absorbing chemical
L_1	=	Thickness of damaged outer layer
L_2	=	Thickness of unaffected interior layer
L_c	=	Effective thickness of the SC
L_e	=	Effective thickness of the VE
M_{out}	=	Cumulative mass leaving the SC during an exposure period, t_{exp}
P_{1v}	=	Permeability coefficient of damaged outer layer
P_{2v}	=	Permeability coefficient of unaffected interior layer
P_{cv}	=	Steady-state permeability of the SC from vehicle v
P_{ev}	=	Steady-state permeability of the VE from vehicle v
t_{exp}	=	Time of exposure to absorbing compound
t^*	=	Time to approximately reach steady state
t_{lag}^D	=	Lag time across damaged SC
t_{lag}^U	=	Lag time across undamaged SC

Greeks

τ	=	Dimensionless exposure time ($= D_c\, t_{exp} / L_c^2$)

Superscripts

∞	=	Steady-state period of absorption

10.4. References

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