10301

DEMONSTRATION RESULTS OF PHYTOREMEDIATION OF EXPLOSIVES-CONTAMINATED GROUNDWATER USING CONSTRUCTED WETLANDS AT THE MILAN ARMY AMMUNITION PLANT, MILAN, TENNESSEE

Volume IV of IV

Prepared for
U.S. ARMY ENVIRONMENTAL CENTER
Aberdeen Proving Ground, Maryland 21010-5401

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Prepared by
Tennessee Valley Authority
Resource Management
Muscle Shoals, Alabama 35662-1010

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Demonstration Results of Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands At The Milan Army Ammunition Plant, Milan, Tennessee

Volume IV of IV

Prepared for
U.S. Army Environmental Center
Pollution Prevention and Environmental Technology Division
Aberdeen Proving Ground, MD 21010-5401
POC: Ms. Darlene F. Bader

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14. ABSTRACT
This report describes a demonstration of phytoremediation methods to remediate explosives-contaminated groundwater. The demonstration was conducted at the Milan Army Ammunition Plant near Milan, Tennessee, using a gravel-based subsurface flow wetland and a lagoon-based surface flow wetland. The report provides demonstration results as they are related to the effectiveness of the wetlands; and provides cost and other information necessary for the transfer of the technology to the user community. The report concludes that gravel-based wetlands could be used to remediate groundwater contaminated with a variety of explosives including TNT and RDX and that, based on the demonstration results, the use of lagoon-based wetlands could not be recommended.

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Phytoremediation of Explosives-Contaminated Groundwater in Constructed Wetlands

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APPENDIX B

TOXICITY RESULTS

APPENDIX B-1

MAAP Wetlands Project, Winter Effluent Testing, 1997
(January 15-22, 1997 Test)

April 16, 1997

Joseph J Hoagland, CTR 1K-M

MAAP WETLANDS PROJECT, WINTER EFFLUENT TESTING, 1997

Attached is the subject report for the winter toxicity study, conducted January 15-22, 1997.

Toxicity was demonstrated in samples from the well (IC_{25}=19.6% for fathead minnows and 13.6% for daphnids) and the discharge and duplicate samples from both the gravel and lagoon wetlands (based on T-Test comparisons).

Please call me at (205) 729-3342 if you have questions or comments.

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STANDARD REPORT FORM

TOXICITY BIOMONITORING USING *PIMEPHALES PROMELAS* (FATHEAD MINNOW), AND *CERIODAPHNIA DUBIA* (DAPHNIDS)

Study: MAAP Wetlands Project, Winter Testing 1997

Principal Investigator: Damien J. Simbeck

Starting Date: January 15, 1997

Ending Date: January 22. 1997

1.0 EXECUTIVE SUMMARY

During Phase I of the Milan Army Ammunitions Plant (MAAP) constructed wetlands project, simulated constructed wetlands (20-L aquaria) were used to test the efficiency of TNT breakdown by gravel-bed and lagoon type wetlands. Toxicity tests using the daphnid, *Ceriodaphnia dubia*, indicated toxicity in water samples from several treatment regimes, including all lagoon type aquaria. Some treatment regimes, all with gravel substrate, demonstrated no toxicity. [1] Phase II of this project involved the construction of full sized wetlands at the MAAP site. Since no information pertaining to the toxicity in the site well water was obtained during Phase I of this study, site water was tested during Phase II, using both fish, *Pimephales promelas*, and the daphnid. Seasonal studies are to be conducted from this site to determine if seasonal differences in plant growth would change toxicity. This report summarizes results of the first seasonal study, conducted during the winter, 1996-97.

Toxicity was demonstrated in water samples from the site well (Fish IC_{25}=19.6%; Daphnid IC_{25}=13.6%). Toxicity was reduced after the site water was passed through both type treatment cells, however toxicity was more greatly reduced after treatment in the gravel wetlands (Fish survival 18%, Daphnid reproduction 33.3 young [no toxicity]) versus the lagoon system (Fish survival 0%; Daphnid reproduction 17.5 young).

Because testing demonstrated toxicity in both the lagoon and gravel discharges, definitive testing will be conducted from these sites. IC_{25} values will be calculated to determine to what extent toxicity is reduced after each treatment. Comparisons will be made of water analyses (metal and TNT by-products) to attempt to define a cause for the toxicity.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): Test samples were whole water samples collected from the Milan Army Ammunition Plant (MAAP) site. Samples were collected from the inflow pipe (well) and from the discharge pipes of both the gravel and lagoon wetlands. Duplicate (QA) samples were collected from both discharge pipes.

2.2 Control and/or Dilution Water: Moderately Hard Synthetic Water (MHSW) was used as control and dilution water for all tests (modified for daphnids; see Section 3.2.2).

2.3 Test Treatments: The well sample was tested in a serial dilution test with dilutions of 12.5, 25, 50, 75 and 100 percent. Since the initial pH of this sample was below the EPA required limits (6.0-9.0 S.U.), a second 100 percent sample was tested after the pH was raised into the acceptable range. A pH adjusted control (adjusted to same pH as the adjusted well water) was included in the test to determine possible effects of the pH adjustment. The gravel and lagoon discharge samples and the duplicate samples were tested at 100 percent only. The fish test was a single test with unadjusted and pH adjusted controls. The daphnid tests were conducted as two separate tests with separate controls. The well water test included the pH adjusted sample and adjusted control. The gravel and lagoon discharges and duplicate samples were tested together with a single control.

3.0 TEST ORGANISMS/CULTURE CONDITIONS

3.1 Species: Pimephales promelas, Fathead minnow

3.1.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.1.2 Culture Water: Culture medium consisted of MHSW. Reagents for MHSW were added to Milli-Q UF product water. Water was passed through a packed column degasser to bring dissolved oxygen gasses to near saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C $\pm$ 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow-through recirculating system. Flow rate to aquaria used for spawning is approximately one aquarium (5-gal) per hour. Approximately 20 percent of the system water is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for testing.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked, and sexually spent individuals are replaced as necessary. Every 3-4 months, a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Dates: January 10-11, 1997

3.1.6 Hatch Dates: January 14, 1997/1520 CST to January 15, 1997/1250 CST

3.1.8 Diseases and Treatment: none

3.1.9 Food and Feeding: Larvae are fed brine shrimp (*Artemia* sp.) nauplii <24-h old beginning after hatching to ensure food availability if larvae begin feeding prior to test initiation.

3.2 Species: *Ceriodaphnia dubia*, daphnid

3.2.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.2.2 Culture Water: Culture medium is MHSW containing trace elements, macronutrients, and vitamins (modified from Elendt and Bias, 1990). [2] Water used for culture contains EDTA, while water used for test control and dilution does not.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates from broods as described below on 2 or 3 consecutive days, 6-10 days prior to test initiation. Adults up to 14 days old may be used for neonate production. These animals are raised individually, and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

3.2.5 Food and Feeding: *Ceriodaphnia* are fed food made according to methods modified from EPA/600/4-89/ [3]

In addition to the yeast/alfalfa fish food recipe, the alga *Selenastrum capricornutum* concentrated to 30 x 10^6 cells/mL is also fed as part of the regular diet. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [3]

4.1.1 Modifications/Amendments to Method 1000.0:

Temperatures on Days 2 and 5 exceeded the EPA recommended temperature range of 25°C±1°C. The variations occurred randomly among the test treatments, and did not appear to have any adverse effects on the test results.

4.1.2 Date/Time Test Initiated: January 15, 1997/1330 CST

4.1.3 Date/Time Test Terminated: January 22, 1997/1245-1305 CST

4.1.4 Test Chamber: 400 mL plastic cups
Volume in Chamber: 250 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Control/Dilution Water: Moderately Hard Synthetic Water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (*Artemia*) nauplii <24-h old three times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, and conductivity. Alkalinity and hardness were measured on the control, and 100 percent samples each time a new sample was used. Final measurements taken daily before renewal were temperature, DO, pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.3.

4.1.12 Statistics: Statistical analyses consisted of IC_{25} calculations using the EPA Bootstrap statistical program for the well sample. T-tests were run using Toxis® and SAS software to determine the significance of effects in the gravel and lagoon discharge and duplicate samples since samples verses controls.

4.2 *Ceriodaphnia* Survival and Reproduction Test, EPA Test Method 1002.0 [3]

4.2.1 Modifications/Deviations to Method 1002.0:

4.2.1.1 Modified culture medium (see Section 3.2.2)

4.2.1.2 Feeding regime (see Section 4.2.10)

4.2.1.3 Temperatures on Day 6 exceeded the EPA recommended temperature range of 25°C±1°C. The variations occurred randomly among the test treatments, and did not appear to have any adverse effects on the test results.

4.2.2 Date/Time Test Initiated:

Well Sample	January 15, 1997/1120-1130 CST
Discharge/Duplicate Samples	January 15, 1997/1040-1050 CST

4.2.3 Date/Time Test Terminated:

Well Sample	January 21, 1997/1115-1135 CST
Discharge/Duplicate Samples	January 21, 1997/1030-1050 CST

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of Organisms per Chamber: 1

4.2.6 Number of Replicates per Treatment: 10

4.2.7 Control/Dilution Water: Modified MHSW

4.2.8 Renewal period: 24-h

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL prepared food and 0.2 mL algae concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, and conductivity. Alkalinity and hardness were measured in the control, and 100 percent samples each time a new sample was used. Final measurements of temperature were taken daily in 10 randomly selected cups when tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.3.

4.2.12 Statistics: Statistical analyses consisted of IC_{25} calculations using the EPA Bootstrap statistical program for the well sample. T-tests were run using Toxis® and SAS software to determine the significance of effects in the gravel and lagoon discharge and duplicate samples since samples verses controls.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, the TTL Quality Assurance Plan and SOP Manual, and EPA/600/4-91/002. [3] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study folder.

5.2.2 Instruments: All identification, service and calibration information retaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study folder.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [4]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-42.4, respectively. [4]

5.2.5 The pH was measured using an Orion Model 250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedure ES-43.7 and ES-42.8, respectively. [4]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were made according to TVA procedures ES-43.3 and ES-42.3, respectively. [4]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an endpoint of 4.5 according to TVA procedure ES-42.1. [4]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine was determined using the DPD Titrimetric Method according to TVA procedure ES-42.9, Rev. 0. [4]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish -- 7-day chronic (IC_{25})
Daphnids -- 7-day chronic (IC_{25})

5.3.2 Standard Toxicant Used: Sodium Chloride
Source/Brand: Fisher

5.3.3 Dilution Water Used: MHSW - fish chronic and modified MHSW - daphnid chronic

5.3.4 Statistics: Chronic Test, IC25 - EPA Bootstrap Procedure, Toxis®

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

6.1.1 Summary of Results:

Toxicity was demonstrated in the well sample, resulting in an IC_{25} of 19.6%. Survival in both the gravel and lagoon discharges, as well as the duplicate samples, was significantly reduced.

6.1.2 Results, Survival and Growth Data:

	Survival (%)							Dry Weight (g)				
	Day							Replicate				
Treatment	1	2	3	4	5	6	7	1	2	3	4	Avg
Unadjusted Control	100	100	100	100	100	100	98	0.30	0.28	0.31	0.27	0.29
Adjusted Control	100	100	100	100	100	98	98	0.31	0.32	0.28	0.33	0.31
Well 12.5%	100	100	100	100	100	98	98	0.29	0.28	0.26	0.25	0.27
Well 25%	98	98	98	98	95	88	88	0.19	0.21	0.12	0.18	0.18
Well 50%	80	70	60	18	13	3	3	0.00	0.00	0.00	0.00	0.00
Well 75%	33	13	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00
Well 100%	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00
Well 100% adjusted	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00
Gravel Discharge	100	75	38	23	20	18	18*	0.31	0.19	0.30	0.00	0.20
Lagoon Discharge	88	48	45	15	3	3	0*	0.00	0.00	0.00	0.00	0.00
Gravel Duplicate	100	75	43	28	23	20	15*	0.42	0.00	0.22	0.11	0.19
Lagoon Duplicate	63	48	30	15	5	3	0*	0.00	0.00	0.00	0.00	0.00

Well IC_{25}=19.6%

*Gravel Discharge and Duplicate, Lagoon Discharge and Duplicate-all significantly reduced based on T-Test.

6.2 Daphnid Survival and Reproduction Test

6.2.1 Summary of Results

Toxicity was demonstrated in the well sample, resulting in an IC_{25} of 13.6%. Reproduction in the lagoon discharge and duplicate samples was significantly reduced, while no toxicity was demonstrated in either the gravel discharge or duplicate samples.

6.2.2 Results, Survival and Reproduction Data:

	Survival (%)						Reproduction (young/female)										
	Day						Replicate										
Treatment	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	Avg
Unadjusted Control	100	100	100	100	100	100	29	36	35	30	32	35	28	34	36	37	33.2
Adjusted Control	100	100	100	100	100	100	34	33	34	33	34	·34	33	36	37	41	34.9
Well 12.5%	100	100	100	100	100	100	27	29	24	25	26	25	22	24	28	27	25.7
Well 25%	100	100	100	100	100	100	5	16	18	18	20	19	12	19	23	14	16.4
Well 50%	100	100	100	100	100	100	0	3	0	2	1	1	1	3	3	3	1.7
Well 75%	100	100	100	100	100	90	0	0	0	0	0	0	0	0	0	0	0.0
Well 100%	40	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0.0
Well 100% adjusted	100	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0.0

Well IC_{25}=13.6%

Survival and Reproduction Data, continued.

	Survival (%)						Reproduction (young/female)										
	Day						Replicate										
Treatment	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	Avg
Control	100	100	100	100	100	100	34	33	33	35	36	35	33	33	33	36	34.1
Gravel Discharge	100	100	100	100	100	100	28	35	32	36	37	33	33	36	31	32	33.3
Lagoon Discharge	100	100	100	100	100	100	19	13	20	21	16	19	21	19	14	13	17.5*
Gravel Duplicate	100	100	100	100	100	100	25	36	32	33	34	29	23	34	33	34	31.3
Lagoon Duplicate	100	100	100	100	100	100	18	14	15	18	19	18	21	21	15	20	17.9*

*Lagoon Discharge and Duplicate-all significantly reduced based on T-Test.

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature:

6.3.1.1 Fathead Minnow: 25.3°C (24.5°C-26.3°C)

6.3.1.2 *Ceriodaphnia*: Well: 25.2°C (24.1°C-26.2°C)
Discharges 25.2°C (24.6°C-26.2°C)

6.3.2 Results: Water chemistry summary for MAAP Wetlands Project, Winter Testing.

See Appendix A, Water Chemistry Mean Values and Ranges for Milan Army Ammunition Plant Winter Toxicity Test, January 15-22, 1997

6.4 Reference Toxicant Tests

6.4.1 Summary of Results:

The most recent monthly reference toxicant tests conducted prior to the period of this study were within control chart limits for both test species.

Species	Date	Time	Duration	Toxicant	Results (IC_{25})	Control Chart Mean	Control Chart Range
P. Promelas	01-09-97	0900	7-days	NaCl	1996 mg/L	2157 mg/L	1613-2700 mg/L
C. dubia	01-15-97	0800	6-days	NaCl	1336 mg/L	979 mg/L	580-1378 mg/L

7.0 CONCLUSIONS

Toxicity was demonstrated in water samples from the site well (Fish IC_{25}=19.6%; Daphnid IC_{25}=13.6%). Toxicity was reduced after the site water was passed through both type treatment cells, however toxicity was more greatly reduced after treatment in the gravel wetlands (Fish survival 18%, Daphnid reproduction 33.3 young [no toxicity]) versus the lagoon system (Fish survival 0%; Daphnid reproduction 17.5 young).

Because fish testing demonstrated toxicity in both the lagoon and discharges, definitive testing will be conducted from these sites. IC_{25} values will be calculated to determine to what extent toxicity is reduced after each treatment. Comparisons will be made of water analyses (metal and TNT by-products) to attempt to define a cause for the toxicity.

8.0 REFERENCES

1. Simbeck, D. J. ERC-Wetlands TNT/RDX Degradation, Fourth Quarter, 1995. Toxicity Test Standard Report, November 2, 1995.

2. Elendt, B. P., and W. R. Bias. "Trace Nutrient Deficiency in Daphnia magna Cultured in Standard Medium for Toxicity Testing. Effects of the Optimization of Culture Conditions on Life History Parameters of D. magna." Water Resources, Great Britain, Vol. 24, No. 9, (1990) pp 1157-1167.

3. Lewis, P. A., D. J. Klemm, J. M. Lazorchak, T. J. Norberg-King, W. H. Peltier, M. A. Heber. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA/600/4-91/002 (July 1994).

4. Field Operations Natural Resources Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

Appendix A
Water Chemistry Mean Values and Ranges for Milan Army Ammunition Plant
Winter Toxicity Test, January 15-22, 1997
Well Test

	Temperature		Dissolved Oxygen			pH			Conductivity		Alkalinity	Hardness	TRC†
	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
Sample ID	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	S.U.	S.U.	S.U.	(µmhos)	(µmhos)	*	*	*
Unadj. Control Fish	25.3 (24.7-26.1)	-	8.2 (8.0-8.4)	6.3 (5.9-6.8)	-	8.1 (8.0-8.2)	7.8 (7.7-7.9)	-	334 (331-337)	341 (335-347)	68.5 (67-70)	96.0 (94-98)	-
Unadj. Control Cerio	-	25.2 (24.1-26.2)	7.9 (7.7-8.3)	-	7.5 (7.3-7.6)	8.1 (8.0-8.2)	-	8.0 (7.9-8.1)	325 (319-330)	-	61.5 (60-63)	98.0 (96-100)	-
Adjusted Control Fish	25.6 (24.9-26.3)	-	8.2 (8.0-8.4)	6.3 (5.8-7.1)	-	6.1 (6.0-6.2)	7.1 (6.9-7.2)	-	373 (361-418)	381 (368-422)	25.0 (23-27)	98.0 (96-100)	-
Adjusted Control Cerio	-	25.2 (24.1-26.2)	7.9 (7.7-8.3)	-	7.4 (7.2-7.6)	6.0 (6.0-6.1)	-	7.4 (7.2-7.6)	359 (346-377)	-	20.5 (16-25)	98.0 (96-100)	-
Well-12.5% Fish	25.2 (24.5-26.1)	-	8.2 (8.1-8.3)	6.5 (6.0-6.8)	-	7.8 (7.6-7.9)	7.7 (7.6-7.8)	-	327 (323-330)	337 (332-343)	-	-	-
Well-12.5% Cerio	-	25.2 (24.1-26.2)	7.9 (7.7-8.1)	-	7.5 (7.3-7.6)	7.8 (7.6-7.9)	-	8.0 (7.9-8.1)	317 (310-324)	-	-	-	-
Well-25% Fish	25.2 (24.6-25.7)	-	8.1 (8.0-8.3)	6.4 (6.2-6.9)	-	7.5 (7.2-7.6)	7.7 (7.6-7.8)	-	319 (316-320)	327 (323-332)	-	-	-
Well-25% Cerio	-	25.2 (24.1-26.2)	7.9 (7.7-8.1)	-	7.5 (7.3-7.6)	7.5 (7.3-7.6)	-	7.9 (7.8-8.0)	311 (307-317)	-	-	-	-
Well-50% Fish	25.2 (24.8-25.6)	-	8.2 (8.0-8.3)	6.7 (6.4-7.1)	-	7.1 (6.7-7.3)	7.5 (7.4-7.6)	-	303 (301-304)	310 (308-316)	-	-	-
Well-50% Cerio	-	25.2 (24.1-26.2)	7.9 (7.8-8.1)	-	7.5 (7.3-7.6)	7.2 (6.9-7.3)	-	7.8 (7.7-7.8)	297 (293-302)	-	-	-	-
Well-75% Fish	25.2 (24.8-25.6)	-	8.2 (8.1-8.2)	6.9 (6.8-7.0)	-	6.6 (6.2-6.8)	7.3 (7.1-7.4)	-	288 (288-288)	294 (291-296)	-	-	-
Well-75% Cerio	-	25.2 (24.1-26.2)	8.0 (7.9-8.1)	-	7.5 (7.3-7.6)	6.8 (6.4-6.9)	-	7.5 (7.4-7.5)	283 (277-287)	-	-	-	-
Well-100% Unadjusted	25.3 (25.3-25.3)	25.2 (24.1-26.2)	8.4 (8.2-8.4)	7.1 (7.1-7.1)	7.4 (7.3-7.6)	5.3 (5.1-5.5)	6.6 (6.6-6.6)	7.0 (6.8-7.1)	274 (272-275)	281 (281-281)	3.0 (2-4)	101.3 (100-104)	<0.1 (<0.1-<0.1)
Well-100% Adjusted	24.5 (24.5-24.5)	25.2 (24.1-26.2)	8.1 (7.9-8.3)	7.1 (7.1-7.1)	7.4 (7.1-7.6)	6.1 (6.0-6.2)	7.0 (7.0-7.0)	7.1 (7.0-7.1)	284 (275-301)	293 (293-293)	4.3 (2-6)	102.7 (100-106)	-

* mg/L $CaCO_3$
†Total Residual Chlorine

Appendix A (continued)
Water Chemistry Mean Values and Ranges for Milan Army Ammunition Plant
Winter Toxicity Test, January 15-22, 1997
Discharge Test

	Temperature		Dissolved Oxygen			pH			Conductivity		Alkalinity	Hardness	TRC†
	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
Sample ID	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	S.U.	S.U.	S.U.	(µmhos)	(µmhos)	*	*	*
Unadj. Control Fish	25.3 (24.7-26.1)	-	8.2 (8.0-8.4)	6.3 (5.9-6.8)	-	8.1 (8.0-8.2)	7.8 (7.7-7.9)	-	334 (331-337)	341 (335-347)	68.5 (67-70)	96.0 (94-98)	-
Unadj. Control Cerio	-	25.2 (24.6-26.2)	7.9 (7.7-8.3)	-	7.4 (7.2-7.7)	8.1 (8.0-8.2)	-	8.0 (8.0-8.0)	325 (319-330)	-	61.5 (60-63)	98.0 (96-100)	-
Gravel Discharge	25.3 (24.7-25.8)	25.2 (24.6-26.2)	8.3 (8.0-8.4)	6.6 (6.2-6.8)	7.4 (7.3-7.7)	8.0 (7.9-8.1)	8.1 (8.0-8.2)	8.4 (8.4-8.5)	433 (407-481)	400 (374-450)	206.0 (190-227)	203.3 (194-222)	<0.1 (<0.1-<0.1)
Lagoon Discharge	25.2 (24.6-25.9)	25.2 (24.6-26.2)	8.3 (8.3-8.4)	7.0 (6.8-7.1)	7.4 (7.3-7.7)	6.6 (6.5-6.8)	6.8 (6.7-7.1)	7.2 (7.1-7.3)	284 (271-298)	289 (275-306)	6.3 (6-7)	110.0 (106-114)	<0.1 (<0.1-<0.1)
Gravel Duplicate	25.6 (25.3-26.0)	25.2 (24.6-26.2)	8.3 (8.2-8.4)	6.5 (6.0-6.7)	7.5 (7.3-7.7)	8.0 (7.9-8.0)	8.1 (8.0-8.2)	8.4 (8.4-8.5)	433 (408-481)	403 (382-458)	205.7 (190-227)	204.0 (194-222)	<0.1 (<0.1-<0.1)
Lagoon Duplicate	25.6 (25.0-26.1)	25.2 (24.6-26.2)	8.3 (8.2-8.4)	6.7 (6.4-7.0)	7.4 (7.3-7.7)	6.6 (6.5-6.9)	6.8 (6.7-6.9)	7.2 (7.1-7.2)	283 (269-296)	289 (276-302)	6.7 (6-7)	108.0 (106-110)	<0.1 (<0.1-<0.1)

* mg/L $CaCO_3$
†Total Residual Chlorine

APPENDIX B-2

MAAP Wetlands Project, Winter Effluent Definitive Testing, 1997
(February 26-March 5, 1997 Test)

May 8, 1997

Joseph J Hoagland, CTR 1K-M

MAAP WETLANDS PROJECT, WINTER EFFLUENT DEFIINITVE TESTING, 1997

Attached is the subject report for the winter toxicity definitive study, conducted February 26-March 5, 1997.

Toxicity was not demonstrated in samples from either the gravel or lagoon wetlands discharge samples during the definitive study (IC_{25} >100%). Possible causative agents for the original study are discussed in the attached report.

Please call me at (205) 729-3342 if you have questions or comments.

Damien J. Simbeck
Biologist
Toxicity Testing Laboratory
TTL 1A-BFN

DJS
Attachment
cc (Attachment):
R. A. Almond, CEB 4C-M
H. S. Coonrod, WET 1A-M
F. J. Sikora, CEB 1C-M
J. R. Trimm, CEB 1E-M
Files, WM, CST 17B-C

Memo0297.doc

STANDARD REPORT FORM

TOXICITY BIOMONITORING USING *PIMEPHALES PROMELAS* (FATHEAD MINNOW), AND *CERIODAPHNIA DUBIA* (DAPHNIDS)

Study: MAAP Wetlands Project, Winter Definitive Testing 1997

Principal Investigator: Damien J. Simbeck

Starting Date: February 26, 1997

Ending Date: March 5, 1997

1.0 EXECUTIVE SUMMARY

Toxicity to both fathead minnows and daphnids was demonstrated in samples from the well at MAAP, as well as the gravel (minnow only) and lagoon discharge pipes during the first study period of Phase II [1]. Since the original study involved only screening toxicity tests for the gravel and lagoon discharges, the severity of the toxicity could not be determined. Definitive testing of these discharges was conducted February 26-March 5, 1997 to determine the toxicity endpoints (toxic concentrations). This report summarizes results of the follow-up study, conducted during the winter, 1996-97.

Although toxicity was demonstrated in water samples from the gravel and lagoon discharges during the original study, no toxicity (IC_{25}>100%) was demonstrated during the definitive test. Basic water chemistry parameters (DO, pH, conductivity, alkalinity and hardness) were not significantly different from the original samples.

A comparison of the results from chemical analyses of water samples split from the first day's toxicity samples for each study period was made to attempt to determine the causative agent(s) of the toxicity. Comparisons revealed six chemicals which might be responsible for the toxicity. These were manganese, TNT, Trinitroso-RDX, HMX, RDX and TNB. Only manganese was found in highest concentrations in the well sample, less in the original gravel and lagoon samples and least in the follow-up samples. The concentrations, however, were all below known toxic levels of manganese to fathead minnows and daphnids (No Effect Concentrations (NOEC) of 8 and 4 mg/L, respectively). [2]

TNT and its by-products were found in detectable amounts in all these samples (see Appendix A). TNT and TNB were present in highest levels in the well sample, and in lower levels in the gravel discharge during both studies. They were absent (<Detection Limits) in the lagoon samples from both studies. Trinitroso-RDX was present in the lagoon samples from both studies, though greatly reduced in the definitve study. It was absent from all well and gravel samples. TNT and/or TNB may have caused the toxicity in the well and gravel samples and Trinitroso-RDX in the lagoon sample. Toxic effects of these chemicals are not known, however, so further testing of these substances would help explain the results of these studies.

The other potential toxic compounds were HMX and RDX. HMX and RDX were present in highest concentrations in the gravel discharges during the first study, suggesting that they probably were not the causative agents. Also present in detectable levels were mononitroso-RDX, 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotuluene, 2,4-dintrotoluene and 2,4-diamino-6-nitrotoluene. Mononitroso-RDX was present only in lagoon samples during both studies, and was higher in the definitive study. 4-Amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotuluene were present only in the original gravel sample and in higher levels in both definitve samples. These three were probably not causative agents. 2,4-Dintrotoluene was present in higher levels in the well sample, lower in the original gravel sample and absent from the original lagoon and both follow-up samples, however the levels present in the well and original gravel samples were not significantly different (0.06-0.07 mg/L vs. 0.05 mg/L), so it is doubtful it was a causative agent. 2,4-Diamino-6-nitrotoluene was detectable only in the follow-up lagoon sample. Synergistic effects may have been involved during both studies, so it is impossible to eliminate these chemicals as potential toxic agents. Other chemicals analyzed during these studies were not detected (<Detection Limit).

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): Test samples were whole water samples collected from the Milan Army Ammunition Plant (MAAP) site. Samples were collected from the discharge pipes of both the gravel and lagoon wetlands.

2.2 Control and/or Dilution Water: Moderately Hard Synthetic Water (MHSW) were used as control and dilution water for all tests (modified for daphnids; see Section 3.2.2).

2.3 Test Treatments: The lagoon and gravel discharge samples were each tested in a serial dilution test with dilutions of 12.5, 25, 50, 75 and 100 percent. The lagoon sample was tested using both the fathead minnow and the daphnid. The gravel sample was tested using only the fathead minnow, since no toxicity to daphnids was demonstrated during the original study [1]. The fathead minnow test was a single test using both dilution series and a single control. The daphnid test was a single test using the lagoon discharge dilution series and a single control.

3.0 TEST ORGANISMS/CULTURE CONDITIONS

3.1 Species: Pimephales promelas, Fathead minnow

3.1.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.1.2 Culture Water: Culture medium consisted of MHSW. Reagents for MHSW were added to Milli-Q UF product water. Water was passed through a packed column degasser to bring dissolved oxygen gasses to near saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C $\pm$ 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow-through recirculating system. Flow rate to aquaria used for spawning is approximately one aquarium (5-gal) per hour. Approximately 20 percent of the system water is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for testing.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked, and sexually spent individuals are replaced as necessary. Every 3-4 months, a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Dates: February 21-23, 1997

3.1.6 Hatch Dates: February 25, 1997/1310 CST to February 26, 1997/0930 CST

3.1.8 Diseases and Treatment: none

3.1.9 Food and Feeding: Larvae are fed brine shrimp (*Artemia* sp.) nauplii <24-h old beginning after hatching to ensure food availability if larvae begin feeding prior to test initiation.

3.2 Species: *Ceriodaphnia dubia*, daphnid

3.2.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.2.2 Culture Water: Culture medium is MHSW containing trace elements, macronutrients, and vitamins (modified from Elendt and Bias, 1990). [3] Water used for culture contains EDTA, while water used for test control and dilution does not.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates from broods as described below on 2 or 3 consecutive days, 6-10 days prior to test initiation. Adults up to 14 days old may be used for neonate production. These animals are raised individually, and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

3.2.5 Food and Feeding: *Ceriodaphnia* are fed food made according to methods modified from EPA/600/4-89/ [4]

In addition to the yeast/alfalfa fish food recipe, the alga *Selenastrum capricornutum* concentrated to 30 x 10^6 cells/mL is also fed as part of the regular diet. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [4]

4.1.1 Modifications/Amendments to Method 1000.0:

None

4.1.2 Date/Time Test Initiated: February 26, 1997/1010 CST

4.1.3 Date/Time Test Terminated: March 5, 1997/0945-1025 CST

4.1.4 Test Chamber: 400 mL plastic cups
Volume in Chamber: 250 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Control/Dilution Water: Moderately Hard Synthetic Water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (*Artemia*) nauplii <24-h old three times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, and conductivity. Alkalinity and hardness were measured on the control, and 100 percent samples each time a new sample was used. Final measurements taken daily before renewal were temperature, DO, pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.3.

4.1.12 Statistics: Statistical analyses consisted of IC_{25} calculations using the EPA Bootstrap statistical program.

4.2 *Ceriodaphnia* Survival and Reproduction Test, EPA Test Method 1002.0 [4]

4.2.1 Modifications/Deviations to Method 1002.0:

4.2.1.1 Modified culture medium (see Section 3.2.2)

4.2.1.2 Feeding regime (see Section 4.2.9)

4.2.2 Date/Time Test Initiated: February 26, 1997/0900-0910 CST

Date/Time Test Terminated: March 4, 1997/0905-0945 CST

4.2.3 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.4 Number of Organisms per Chamber: 1

4.2.5 Number of Replicates per Treatment: 10

4.2.6 Control/Dilution Water: Modified MHSW

4.2.7 Renewal period: 24-h

4.2.8 Test Temperature: 25°C ± 1°C

4.2.9 Feeding Regime During Test: Each organism was fed 0.1 mL prepared food and 0.2 mL algae concentrate daily (added to renewal water before introduction of test organism).

4.2.10 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, and conductivity. Alkalinity and hardness were measured in the control, and 100 percent samples each time a new sample was used. Final measurements of temperature were taken daily in 10 randomly selected cups when tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.3.

4.2.11 Statistics: Statistical analyses consisted of IC_{25} calculations using the EPA Bootstrap statistical program.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, the TTL Quality Assurance Plan and SOP Manual, and EPA/600/4-91/002. [4] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study folder.

5.2.2 Instruments: All identification, service and calibration information retaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study folder.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [5]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-42.4, respectively. [5]

5.2.5 The pH was measured using an Orion Model 250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedure ES-43.7 and ES-42.8, respectively. [5]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were made according to TVA procedures ES-43.3 and ES-42.3, respectively. [5]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an endpoint of 4.5 according to TVA procedure ES-42.1. [5]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine was determined using the DPD Titrimetric Method according to TVA procedure ES-42.9, Rev. 0. [5]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish -- 7-day chronic (IC_{25})
Daphnids -- 7-day chronic (IC_{25})

5.3.2 Standard Toxicant Used: Sodium Chloride
Source/Brand: Fisher

5.3.3 Dilution Water Used: MHSW - fish chronic and modified MHSW - daphnid chronic

5.3.4 Statistics: IC25 - EPA Bootstrap Procedure

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

6.1.1 Summary of Results:

No significant toxicity was demonstrated from either sample, based on IC_{25} caluclations (IC_{25}'s >100%).

6.1.2 Results, Survival and Growth Data:

	Survival (%)							Dry Weight (mg)				
	Day							Replicate				
Treatment	1	2	3	4	5	6	7	1	2	3	4	Avg
Control	100	100	100	100	100	100	100	0.34	0.31	0.31	0.38	0.34
Lagoon 12.5%	100	100	100	98	98	98	98	0.36	0.46	0.36	0.37	0.39
Lagoon 25%	100	100	100	100	100	100	100	0.38	0.47	0.44	0.46	0.44
Lagoon 50%	100	100	100	100	100	100	100	0.36	0.43	0.43	0.38	0.40
Lagoon 75%	100	100	100	100	100	100	98	0.36	0.45	0.37	0.40	0.40
Lagoon 100%	100	100	100	98	98	98	98	0.37	0.47	0.34	0.34	0.38
Gravel 12.5%	100	100	100	100	100	100	100	0.35	0.36	0.42	0.35	0.37
Gravel 25%	100	100	100	100	100	100	100	0.31	0.33	0.39	0.39	0.36
Gravel 50%	100	100	100	98	98	98	98	0.32	0.35	0.40	0.40	0.37
Gravel 75%	100	100	95	85	85	85	85	0.30	0.33	0.40	0.37	0.35
Gravel 100%	100	100	100	75	73	73	73	0.29	0.48	0.39	0.02	0.30

IC_{25}>100% for both samples

6.2 Daphnid Survival and Reproduction Test

6.2.1 Summary of Results

No significant toxicity was demonstrated from the lagoon sample, based on IC_{25} caluclations (IC_{25}>100%).

6.2.2 Results, Survival and Reproduction Data:

	Survival (%)							Reproduction (young/female)										
	Day							Replicate										
Treatment	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	9	10	Avg
Control	100	100	100	100	100	100	-	35	38	36	34	35	37	35	29	34	39	35.2
Lagoon 12.5%	100	100	100	100	100	100	-	35	32	37	31	34	38	34	29	37	36	34.3
Lagoon 25%	100	100	100	100	100	90	-	36	33	34	36	36	37	38	33	34	39	35.6
Lagoon 50%	100	100	100	100	100	100	-	39	36	31	31	25	*	32	32	35	37	33.1
Lagoon 75%	100	100	100	100	100	100	-	35	34	27	29	28	34	33	25	35	37	31.7
Lagoon 100%	100	100	100	100	100	100	-	29	28	28	29	23	31	26	29	30	34	28.7

IC_{25}>100%

*Animal lost, probably missed during previous day renewal

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature:

Fathead Minnow: 25.1°C (24.2°C-25.6°C)

Ceriodaphnia: 25.3°C (24.4°C-26.0°C)

6.3.2 Results: Water chemistry summary for MAAP Wetlands Project, Winter Definitive Testing.

See Appendix A, TNT/By-Products Analyses Summary for Milan Army Ammunition Plant Winter Toxicity Tests, 1997

See Appendix B, Water Chemistry Mean Values and Ranges for Milan Army Ammunition Plant Winter Toxicity Definitive Test, February 26-March 5, 1997

6.3 Reference Toxicant Tests

Summary of Results:

The most recent monthly reference toxicant tests conducted prior to the period of this study were within control chart limits for both test species.

Species	Date	Time	Duration	Toxicant	Results (IC_{25})	Control Chart Mean	Control Chart Range
P. Promelas	01-28-97	1300	7-days	NaCl	1869 mg/L	2134 mg/L	1590-2679 mg/L
C. dubia	02-26-97	0735	6-days	NaCl	1325 mg/L	1004 mg/L	571-1438 mg/L

7.0 CONCLUSIONS

Although toxicity was demonstrated in water samples from the gravel and lagoon discharges during the original study, no toxicity (IC_{25}>100%) was demonstrated during the definitive test. Basic water chemistry parameters (DO, pH, conductivity, alkalinity and hardness) were not significantly different from the original samples.

A comparison of the results from chemical analyses of water samples split from the first day's toxicity samples for each study period was made to attempt to determine the causative agent(s) of the toxicity. Comparisons revealed six chemicals which might be responsible for the toxicity. These were manganese, TNT, Trinitroso-RDX, HMX, RDX and TNB. Only manganese was found in highest concentrations in the well sample, less in the original gravel and lagoon samples and least in the follow-up samples. The concentrations, however, were all below known toxic levels of manganese to fathead minnows and daphnids (No Effect Concentrations (NOEC) of 8 and 4 mg/L, respectively). [2]

TNT and its by-products were found in detectable amounts in all these samples (see Appendix A). TNT and TNB were present in highest levels in the well sample, and in lower levels in the gravel discharge during both studies. They were absent (<Detection Limits) in the lagoon samples from both studies. Trinitroso-RDX was present in the lagoon samples from both studies, though greatly reduced in the definitve study. It was absent from all well and gravel samples. TNT and/or TNB may have caused the toxicity in the well and gravel samples and Trinitroso-RDX in the lagoon sample. Toxic effects of these chemicals are not known, however, so further testing of these substances would help explain the results of these studies.

The other potential toxic compounds were HMX and RDX. HMX and RDX were present in highest concentrations in the gravel discharges during the first study, suggesting that they probably were not the causative agents. Also present in detectable levels were mononitroso-RDX, 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotuluene, 2,4-dintrotoluene and 2,4-diamino-6-nitrotoluene. Mononitroso-RDX was present only in lagoon samples during both studies, and was higher in the definitive study. 4-Amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotuluene were present only in the original gravel sample and in higher levels in both definitve samples. These three were probably not causative agents. 2,4-Dintrotoluene was present in higher levels in the well sample, lower in the original gravel sample and absent from the original lagoon and both follow-up samples, however the levels present in the well and original gravel samples were not significantly different (0.06-0.07 mg/L vs. 0.05 mg/L), so it is doubtful it was a causative agent. 2,4-Diamino-6-nitrotoluene was detectable only in the follow-up lagoon sample. Synergistic effects may have been involved during both studies, so it is impossible to eliminate these chemicals as potential toxic agents. Other chemicals analyzed during these studies were not detected (<Detection Limit).

8.0 REFERENCES

1. Simbeck, D. J. MAAP Wetlands Project, Winter Effluent Testing, 1997. TVA Toxicity Test Standard Report, April 16, 1997.

2. Schweinforth, R. L.. Manganese Short-Term Chronic Exposure. TVA Toxicity Test Standard Report, January, 1990.

3. Elendt, B. P., and W. R. Bias. "Trace Nutrient Deficiency in Daphnia magna Cultured in Standard Medium for Toxicity Testing. Effects of the Optimization of Culture Conditions on Life History Parameters of D. magna." Water Resources, Great Britain, Vol. 24, No. 9, (1990) pp 1157-1167.

4. Lewis, P. A., D. J. Klemm, J. M. Lazorchak, T. J. Norberg-King, W. H. Peltier, M. A. Heber. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA/600/4-91/002 (July 1994).

5. Field Operations Natural Resources Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

Appendix A
TNT/By-Products Analyses Summary for Milan Army
Ammunition Plant Winter Toxicity Tests. 1997

	INFLOW-1	INFLOW-2	LAGOON	GRAVEL	LAGOON	GRAVEL
Ammonia	0.49	0.41	2.28	0.29	0.10	0.16
Unionoized	<0.001	<0.001	0.006	0.015	0.001	0.011
Chloride	2.55	2.52	5.94	3.49	3.50	2.59
Cadmium	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Calcium	23.9	22.2	72.9	26.9	59.6	23.7
Copper	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Iron	0.07	<0.02	0.20	0.02	<0.02	<0.02
Lead	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Magnesium	9.18	8.68	7.37	10.3	6.77	9.31
Manganese	0.23	0.21	0.04	0.13	<0.008	0.02
Nitrate	29.38	29.36	3.34	30.52	12.49	25.84
Nickel	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
PO_4	0.01	<0.01	0.01	0.01	0.01	<0.01
Zinc	<0.009	<0.009	<0.009	<0.009	<0.009	<0.009
TKN	0.50	0.78	3.48	0.94	0.67	1.28
TOC	3.85	3.96	2.59	4.17	2.01	4.41
2,6-Diamino-4-nitrotoluene	<0.00550	<0.00550	<0.00550	<0.00550	<0.00500	<0.00500
Trinitroso-RDX	<0.00220	<0.00220	1.2	<0.00220	0.2939	<0.00500
HMX	0.11	0.10	<0.00350	0.18	0.07338	0.1017
2,4-Diamino-6-nitrotoluene	<0.00500	<0.00500	<0.00500	<0.00500	0.03259	<0.00500
Mononitroso-RDX	<0.00320	<0.00320	0.02	<0.00320	0.2982	<0.00500
RDX	4.0	4.1	<0.00080	4.2	1.876	3.707
1,3,5-Trinitrobenzene	0.35	0.35	<0.00220	0.12	<0.00500	0.03064
2,4,6-Trinitrotoluene	4.2	4.3	<0.00210	0.70	<0.00500	0.1186
4-Amino-2,6-dinitrotoluene	<0.00390	<0.00390	<0.00390	0.04	0.03061	0.03003
2-Amino-4,6-dinitrotoluene	<0.00210	<0.00210	<0.00210	0.08	0.01183	0.04475
2,6-Dinitrotoluene	<0.00490	<0.00490	<0.00490	<0.00490	<0.00600	<0.00600
2,4-Dinitrotoluene	0.06	0.07	<0.00230	0.05	<0.00500	<0.00500
Tetranitro-2,2'-azoxytoluene	<0.00450	<0.00450	<0.00450	<0.00450	<0.00500	<0.00500
Tetranitro-2',4-azoxytoluene	<0.00470	<0.00470	<0.00470	<0.00470	<0.00500	<0.00500
Tetranitro-4,4'-azoxytoluene	<0.00470	<0.00470	<0.00470	<0.00470	<0.00500	<0.00500
Dinitro-4,4'-azoxytoluene	<0.00150	0<0.00150	<0.00150	<0.00150	<0.00500	<0.00500
TSS	<2	<2	3	<2	<2	3.4

All samples <Detection Limit

Appendix B

Water Chemistry Mean Values and Ranges for Milan Army Ammunition Plant

Winter Toxicity Definitive Test, February 26-March 5, 1997

	Temperature		Dissolved Oxygen			pH			Conductivity		Alkalinity	Hardness	TRC†
	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
Sample ID	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	S.U.	S.U.	S.U.	(µmhos)	(µmhos)	*	*	*
Control Fish	25.2 (24.8-25.5)	-	8.1 (8.0-8.3)	6.3 (6.0-6.6)	-	8.1 (8.0-8.2)	7.7 (7.5-7.9)	-	324 (322-327)	333 (327-341)	66.0 (66-66)	90.0 (90-90)	-
Control Cerio	-	25.3 (24.4-26.0)	7.9 (7.7-8.0)	-	7.5 (7.4-7.6)	8.2 (8.1-8.3)	-	8.1 (8.0-8.2)	324 (322-327)	-	68.0 (68-68)	94.0 (94-94)	-
Lagoon-12.5% Fish	25.0 (24.2-25.4)	-	8.0 (8.0-8.1)	6.4 (6.1-6.9)	-	8.1 (8.0-8.2)	7.7 (7.6-7.8)	-	315 (313-319)	328 (320-352)	-	-	-
Lagoon-25% Fish	25.1 (24.8-25.6)	-	8.0 (8.0-8.1)	6.1 (5.4-6.5)	-	8.0 (7.9-8.1)	7.6 (7.5-7.7)	-	305 (302-307)	310 (306-315)	-	-	-
Lagoon-50% Fish	25.0 (24.8-25.3)	-	8.0 (7.9-8.1)	6.4 (6.1-6.6)	-	7.8 (7.7-8.0)	7.5 (7.5-7.6)	-	286 (282-289)	290 (285-296)	-	-	-
Lagoon-75% Fish	25.0 (24.3-25.6)	-	8.0 (7.9-8.1)	6.4 (5.7-6.9)	-	7.6 (7.5-7.7)	7.3 (7.2-7.4)	-	268 (262-273)	274 (265-283)	-	-	-
Lagoon-12.5% Cerio	-	25.3 (24.4-26.0)	7.9 (7.8-8.0)	-	7.5 (7.3-7.7)	8.1 (8.0-8.2)	-	8.1 (8.0-8.2)	314 (312-315)	-	-	-	-
Lagoon-25% Cerio	-	25.3 (24.4-26.0)	7.9 (7.8-8.0)	-	7.5 (7.3-7.6)	8.0 (8.0-8.1)	-	8.0 (7.9-8.1)	306 (304-307)	-	-	-	-
Lagoon-50% Cerio	-	25.3 (24.4-26.0)	8.0 (7.9-8.0)	-	7.5 (7.3-7.5)	7.9 (7.8-8.0)	-	7.9 (7.7-8.0)	287 (282-290)	-	-	-	-
Lagoon-75% Cerio	-	25.3 (24.4-26.0)	8.0 (7.9-8.0)	-	7.5 (7.3-7.6)	7.6 (7.5-7.7)	-	7.6 (7.5-7.8)	269 (262-272)	-	-	-	-
Lagoon-100%	25.2 (25.0-25.5)	25.3 (24.4-26.0)	8.3 (8.1-8.4)	6.6 (6.1-6.9)	7.5 (7.3-7.6)	7.0 (6.8-7.1)	6.9 (6.8-6.9)	7.3 (7.1-7.6)	249 (240-257)	254 (245-266)	7.0 (6-8)	90.0 (82-94)	<0.1 (<0.1-<0.1)
Gravel-12.5% Fish	25.1 (24.8-25.4)	-	8.0 (8.0-8.1)	6.1 (5.2-6.6)	-	8.2 (8.1-8.2)	7.8 (7.6-7.9)	-	331 (328-334)	340 (333-351)	-	-	-
Gravel-25% Fish	25.0 (24.7-25.3)	-	8.0 (8.0-8.1)	6.1 (5.3-6.7)	-	8.1 (8.1-8.2)	7.9 (7.9-8.0)	-	339 (335-342)	346 (339-353)	-	-	-
Gravel-50% Fish	24.9 (24.6-25.5)	-	8.0 (8.0-8.1)	6.1 (5.6-6.7)	-	8.1 (8.1-8.2)	8.0 (7.9-8.1)	-	354 (348-362)	362 (354-375)	-	-	-
Gravel-75% Fish	25.0 (24.5-25.5)	-	8.0 (8.0-8.1)	6.0 (5.4-6.9)	-	8.1 (8.1-8.1)	8.1 (8.0-8.2)	-	373 (364-384)	377 (367-400)	-	-	-
Gravel-100%	25.1 (24.8-25.5)	-	8.3 (8.1-8.4)	6.1 (5.7-6.8)	-	8.1 (8.0-8.1)	8.2 (8.1-8.2)	-	393 (382-409)	390 (378-412)	168.3 (146-190)	186.7 (178-192)	<0.1 (<0.1-<0.1)

* mg/L $CaCO_3$

†Total Residual Chlorine

APPENDIX B-3

MAAP Wetlands Project, Summer Effluent Testing, 1997
(August 6-13, 1997 Test)

October 3, 1997

Joseph J Hoagland, CTR 1K-M

MAAP WETLANDS PROJECT, SUMMER EFFLUENT TESTING, 1997

Attached is the subject report for the summer toxicity screening study, conducted August 6-13, 1997.

Toxicity was not demonstrated in samples from either the gravel or lagoon wetlands discharge samples during this screening study. Toxicity to both daphnids and fathead minnows was demonstrated from well samples.

Please call me at (205) 729-3342 if you have questions or comments.

Damien J. Simbeck
Biologist
Toxicity Testing Laboratory
TTL 1A-BFN

DJS
Attachment
cc (Attachment):
R. A. Almond, CEB 4C-M
H. S. Coonrod, WET 1A-M
F. J. Sikora, CEB 1C-M
J. R. Trimm, CEB 1E-M
Files, WM, CST 17B-C

STANDARD REPORT FORM

TOXICITY BIOMONITORING USING *PIMEPHALES PROMELAS* (FATHEAD MINNOW), AND *CERIODAPHNIA DUBIA* (DAPHNIDS)

Study: MAAP Wetlands Project, Summer Testing 1997

Principal Investigator: Damien J. Simbeck

Starting Date: August 6, 1997

Ending Date: August 13, 1997

1.0 EXECUTIVE SUMMARY

During Phase I of the Milan Army Ammunitions Plant (MAAP) constructed wetlands project, simulated constructed wetlands (20-L aquaria) were used to test the efficiency of TNT breakdown by gravel-bed and lagoon type wetlands. Toxicity tests using the daphnid, *Ceriodaphnia dubia*, indicated toxicity in water samples from several treatment regimes, including all lagoon type aquaria. Some treatment regimes, all with gravel substrate, demonstrated no toxicity. [1] Phase II of this project involved the construction of full sized wetlands at the MAAP site. Since no information pertaining to the toxicity in the site well water was obtained during Phase I of this study, site water was tested during Phase II, using both fish, *Pimephales promelas*, and the daphnid. Seasonal studies were conducted from this site to determine if seasonal differences in plant growth would change toxicity. During the winter testing period, January 15-22, 1997, toxicity to daphnids and fathead minnows was demonstrated in all samples tested. Repeat testing February 26-March 5, 1997 indicated no toxicity. [2][3] This report summarizes results of the second seasonal study, conducted during the summer, 1997.

Toxicity was again demonstrated in water samples from the site well (Fish survival=0 percent; Daphnid survival=90.0 percent, no reproduction). Toxicity was eliminated after the well water was passed through either type treatment cells. Levels of TNT and TNT by-products were all below potentially toxic levels in all samples except the well. Un-ionized ammonia levels were below toxic levels in all samples.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): Test samples were whole water samples collected from the Milan Army Ammunition Plant (MAAP) site. Samples were collected from the inflow pipe (well) and from the discharge pipes of both the gravel and lagoon wetlands. Duplicate (QA) samples were collected from both discharge pipes.

2.2 Control and/or Dilution Water: Moderately Hard Synthetic Water (MHSW) was used as control and dilution water for all tests (modified for daphnids; see Section 3.2.2).

2.3 Test Treatments: All samples were tested at 100 percent only. Although the initial pH of the well sample was below the EPA required limits (6.0-9.0 S.U.), the pH was not adjusted. Testing during the winter study indicated that pH adjustment did not alter the toxicity of the sample.

3.0 TEST ORGANISMS/CULTURE CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: Aquatic BioSystems, Inc., Fort Collins, Colorado.

3.1.2 Hatch Dates: August 5, 1997/1230-1530 CDT

3.2 Species: *Ceriodaphnia dubia*, daphnid

3.2.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.2.2 Culture Water: Culture medium is MHSW containing trace elements, macronutrients, and vitamins (modified from Elendt and Bias, 1990). [4] Water used for culture contains EDTA, while water used for test control and dilution does not.

3.2.3 Temperature of Culture: 25°C $\pm$ 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates from broods as described below on 2 or 3 consecutive days, 6-10 days prior to test initiation. Adults up to 14 days old may be used for neonate production. These animals are raised individually, and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

3.2.5 Food and Feeding: *Ceriodaphnia* are fed food made according to methods modified from EPA/600/4-89/ [5]

In addition to the yeast/alfalfa fish food recipe, the alga *Selenastrum capricornutum* concentrated to 30 x 10^6 cells/mL is also fed as part of the regular diet. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [5]

4.1.1 Modifications/Amendments to Method 1000.0:

4.1.2 Date/Time Test Initiated: August 6, 1997/1305 CDT

4.1.3 Date/Time Test Terminated: August 13, 1997/1315 CDT

4.1.4 Test Chamber: 400 mL plastic cups
Volume in Chamber: 250 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Control/Dilution Water: Moderately Hard Synthetic Water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (*Artemia*) nauplii <24-h old three times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, and conductivity. Alkalinity and hardness were measured on the control, and 100 percent samples each time a new sample was used. Final measurements taken daily before renewal were temperature, DO, pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.3.

4.1.12 Statistics: T-tests were run using Toxis® and SAS software to determine the significance of effects in all samples.

4.2 *Ceriodaphnia* Survival and Reproduction Test, EPA Test Method 1002.0 [5]

4.2.1 Modifications/Deviations to Method 1002.0:

4.2.1.1 Modified culture medium (see Section 3.2.2)

4.2.1.2 Feeding regime (see Section 4.2.10)

4.2.1.3 Temperatures in two cups, both 23.7°C, on Day 1 were below the EPA recommended temperature range of 25°C±1°C. The variations occurred randomly among the test treatments, and did not appear to have any adverse effects on the test results.

4.2.2 Date/Time Test Initiated: August 6, 1997/1205-1220 CDT

4.2.3

4.2.4 Date/Time Test Terminated: August 12, 1997/1200-1235 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of Organisms per Chamber: 1

4.2.6 Number of Replicates per Treatment: 10

4.2.7 Control/Dilution Water: Modified MHSW

4.2.8 Renewal period: 24-h

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL prepared food and 0.2 mL algae concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, and conductivity. Alkalinity and hardness were measured in the control, and 100 percent samples each time a new sample was used. Final measurements of temperature were taken daily in 10 randomly selected cups when tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.3.

4.2.12 Statistics: T-tests were run using Toxis® and SAS software to determine the significance of effects in all samples.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, the TTL Quality Assurance Plan and SOP Manual, and EPA/600/4-91/002. [5] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study folder.

5.2.2 Instruments: All identification, service and calibration information retaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study folder.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [6]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-42.4, respectively. [6]

5.2.5 The pH was measured using an Orion Model 250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedure ES-43.7 and ES-42.8, respectively. [6]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were made according to TVA procedures ES-43.3 and ES-42.3, respectively. [6]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an endpoint of 4.5 according to TVA procedure ES-42.1. [6]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine was determined using the DPD Titrimetric Method according to TVA procedure ES-42.9, Rev. 0. [6]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish -- 7-day chronic (IC_{25})
Daphnids -- 7-day chronic (IC_{25})

5.3.2 Standard Toxicant Used: Sodium Chloride
Source/Brand: Fisher

5.3.3 Dilution Water Used: MHSW - fish chronic and modified MHSW - daphnid chronic

5.3.4 Statistics: Chronic Test, IC25 - EPA Bootstrap Procedure, Toxis®

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

6.1.1 Summary of Results:

Toxicity was demonstrated in the well sample. No toxicity was demonstrated in any gravel or lagoon sample based on T-Test comparisons.

6.1.2 Results, Survival and Growth Data:

	Survival (%)							Dry Weight (g)				
	Day							Replicate				
Treatment	1	2	3	4	5	6	7	1	2	3	4	Avg
Control	100	100	100	100	98	98	98	0.31	0.38	0.24	0.29	0.31
Well 100%	0*	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00
Gravel Discharge	100	100	100	100	100	100	100	0.36	0.30	0.34	0.35	0.34
Lagoon Discharge	100	100	100	100	100	100	100	0.39	0.37	0.34	0.38	0.37
Gravel Duplicate	100	100	100	100	100	100	98	0.35	0.36	0.36	0.28	0.34
Lagoon Duplicate	100	100	100	100	100	100	100	0.33	0.34	0.40	0.35	0.36

*Well significantly reduced based on T-Test.

6.2 Daphnid Survival and Reproduction Test

6.2.1 Summary of Results

Toxicity was demonstrated in the well sample. No toxicity was demonstrated in any gravel or lagoon sample based on T-Test comparisons.

6.2.2 Results, Survival and Reproduction Data:

	Survival (%)						Reproduction (young/female)										
	Day						Replicate										
Treatment	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	Avg
Control	100	100	100	100	100	100	32	28	26	31	32	33	32	32	28	35	30.9
Well 100%	100	100	100	100	100	90	0	0	0	0	0	0	0	0	0	0	0.0*
Gravel Discharge	100	100	100	100	100	100	16	29	26	26	14	29	31	32	29	33	26.5
Lagoon Discharge	100	100	100	100	100	90	30	27	30	36	31	33	33	25	29	15	28.9
Gravel Duplicate	100	100	100	100	100	100	26	26	30	22	30	21	34	33	31	19	27.2
Lagoon Duplicate	100	100	100	100	100	100	29	26	28	29	30	29	32	25	23	29	28.0

*Well significantly reduced based on T-Test.

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature:

6.3.1.1 Fathead Minnow: 24.4°C (24.0°C-25.0°C)

6.3.1.2 *Ceriodaphnia*: 24.9°C (23.7°C-25.6°C)

6.3.2 Results: Water chemistry summary for MAAP Wetlands Project, Summer Testing.

See Appendix A, Water Chemistry Mean Values and Ranges for Milan Army Ammunition Plant Summer Toxicity Test, August 6-13, 1997

6.4.1 Summary of Results:

The most recent monthly reference toxicant tests conducted prior to the period of this study were within control chart limits for both test species.

Species	Date	Time	Duration	Toxicant	Results (IC_{25})	Control Chart Mean	Control Chart Range
P. promelas	08-06-97	1305	7-days	NaCl	1832 mg/L	1941 mg/L	1708-2174 mg/L
C. dubia	07-22-97	0800	6-days	NaCl	1239 mg/L	1031 mg/L	592-1471 mg/L

7.0 CONCLUSIONS

Toxicity was again demonstrated in water samples from the site well (Fish survival=0 percent; Daphnid survival=90.0 percent, no reproduction). Toxicity was eliminated after the well water was passed through either type treatment cells. Levels of TNT and TNT by-products were all below potentially toxic levels in all samples except the well. Un-ionized ammonia levels were below toxic levels in all samples.

8.0 REFERENCES

1. Simbeck, D. J. ERC-Wetlands TNT/RDX Degradation, Fourth Quarter, 1995. Toxicity Test Standard Report, November 2, 1995.

2. Simbeck, D. J. MAAP Wetlands Project, Winter Effluent Testing, 1997. Toxicity Test Standard Report, April, 1997

3. Simbeck, D. J. MAAP Wetlands Project, Winter Effluent Definitive Testing, 1997. Toxicity Test Standard Report, May, 1997

4. Elendt, B. P., and W. R. Bias. "Trace Nutrient Deficiency in Daphnia magna Cultured in Standard Medium for Toxicity Testing. Effects of the Optimization of Culture Conditions on Life History Parameters of D. magna." Water Resources, Great Britain, Vol. 24, No. 9, (1990) pp 1157-1167.

5. Lewis, P. A., D. J. Klemm, J. M. Lazorchak, T. J. Norberg-King, W. H. Peltier, M. A. Heber. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA/600/4-91/002 (July 1994).

6. Field Operations Natural Resources Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

App[illegible]ix A

Water Chemistry Mean Values an[illegible]ges ( ) for Fathead Minnow Test,
MAAP Summer Toxicity Test, August 6-13, 1997

Test/ Sample ID	Temp.	Dissolved Oxygen Initial	Dissolved Oxygen Final	pH Initial	pH Final	Conductivity Initial	Conductivity Final	Alkalinity	Hardness	TRC†
	(°C)	(mg/L)	(mg/L)	S.U.	S.U.	(µmhos)	(µmhos)	*	*	mg/L
Fathead/ Control	24.5 (24.2-24.8)	8.1 (8.0-8.3)	6.2 (5.5-7.1)	8.2 (8.2-8.3)	7.6 (7.5-7.9)	331 (330-332)	339 (337-340)	68.0 (68-68)	86.0 (86-86)	-
Fathead/ Well-100%	24.3 (24.3-24.3)	8.4 (8.4-8.4)	6.7 (6.7-6.7)	4.9 (4.9-4.9)	6.7 (6.7-6.7)	250 (250-250)	253 (253-253)	3.0 (3-3)	86.0 (86-86)	<0.1 (<0.1-<0.1)
Fathead / Gravel-Discharge	24.5 (24.2-24.7)	7.3 (6.7-7.7)	6.1 (5.4-7.0)	7.6 (7.5-7.7)	8.1 (8.0-8.2)	506 (487-516)	492 (466-504)	226.3 (215-235)	218.6 (212-226)	<0.1 (<0.1-<0.1)
Fathead / Lagoon-Discharge	24.5 (24.1-25.0)	8.4 (8.3-8.4)	6.1 (5.6-6.7)	7.4 (7.1-7.6)	7.3 (7.0-7.5)	247 (244-252)	251 (247-254)	26.9 (25-29)	87.1 (86-90)	<0.1 (<0.1-<0.1)
Fathead / Gravel-Duplicate	24.3 (24.0-24.7)	7.8 (7.2-8.4)	6.2 (5.8-7.1)	7.7 (7.5-7.9)	8.1 (8.1-8.2)	503 (490-518)	491 (470-508)	225.1 (215-235)	216.9 (206-226)	<0.1 (<0.1-<0.1)
Fathead / Lagoon-Dupilcate	24.5 (24.3-24.7)	8.4 (8.3-8.4)	6.1 (5.7-6.6)	7.5 (7.2-7.6)	7.3 (7.0-7.5)	247 (243-250)	251 (248-254)	26.4 (25-29)	87.4 (86-92)	<0.1 (<0.1-<0.1)

Water Chemistry Mean Values and Ranges ( ) for Daphnid Test,
MAAP Summer Toxicity Test, August 6-12, 1997

Test/ Sample ID	Temperature	Dissolved Oxygen Initial	Dissolved Oxygen Final	pH Initial	pH Final	Conductivity Initial	Alkalinity	Hardness	TRC†
	(°C)	(mg/L)	(mg/L)	S.U.	S.U.	(µmhos)	*	*	mg/L
Daphnid/ Control	24.9 (23.7-25.6)	7.7 (7.5-8.1)	7.7 (7.5-8.0)	8.1 (8.0-8.2)	8.1 (7.8-8.2)	332 (331-335)	69.0 (69-69)	84.0 (84-84)	-
Daphnid/ Well-100%	24.9 (23.7-25.6)	7.1 (6.2-8.4)	7.5 (7.5-7.7)	5.1 (4.9-5.6)	6.8 (6.6-6.9)	250 (248-252)	3.2 (3-4)	82.3 (80-86)	<0.1 (<0.1-<0.1)
Daphnid / Gravel-Discharge	24.9 (23.7-25.6)	7.2 (6.7-7.7)	8.3 (8.0-8.6)	7.6 (7.5-7.7)	8.4 (8.3-8.4)	504 (487-516)	225.0 (215-235)	217.7 (212-226)	<0.1 (<0.1-<0.1)
Daphnid / Lagoon-Discharge	24.9 (23.7-25.6)	8.4 (8.3-8.4)	8.1 (8.0-8.2)	7.4 (7.1-7.6)	7.9 (7.6-8.1)	247 (244-252)	26.8 (25-29)	87.3 (86-90)	<0.1 (<0.1-<0.1)
Daphnid / Gravel-Duplicate	24.9 (23.7-25.6)	7.7 (7.2-8.4)	8.4 (8.1-8.8)	7.7 (7.5-7.9)	8.4 (8.3-8.4)	501 (490-518)	223.7 (215-235)	215.3 (206-226)	<0.1 (<0.1-<0.1)
Daphnid / Lagoon-Dupilcate	24.9 (23.7-25.6)	8.4 (8.3-8.4)	8.0 (7.8-8.1)	7.5 (7.4-7.6)	7.8 (7.6-8.0)	247 (243-250)	26.3 (25-29)	87.7 (86-92)	<0.1 (<0.1-<0.1)

* mg/L $CaCO_3$

†Total Residual Chlorine

Appendix A (continued)
Chemical Analysis of MAAP samples collected 08/06/97

SITE		NH_4	Un NH_4 Init	Un NH_4 Fish	Un NH_4 Cerio	TN-RDX	MN-RDX	RDX	TNT	HMX	Cd	Cu	Fe	Pb	Mn	Ni	Zn
1	WELL	0.66	<0.001	0.003	0.003	<	<	4.17	4.41	0.09	<0.03	<0.02	0.11	<0.3	0.28	<0.07	<0.009
4	WELL	0.59	<0.001	0.003	0.003	<	<	4.15	4.44	0.08	<0.03	<0.02	0.09	<0.3	0.23	<0.07	<0.009
3	GRAVEL	0.17	0.005	0.015	0.027	0.002	<	<	<	<	<0.03	<0.02	0.13	<0.3	0.01	<0.07	<0.009
6	LAGOON	0.23	0.004	0.004	0.013	<	<	0.203	0.007	0.006	<0.03	<0.02	0.04	<0.3	0.12	<0.07	<0.009

all in mg/L

APPENDIX B-4

MAAP Wetlands Project, Summer Gravel/Sediment Toxicity Testing, 1997
(August 15-25, 1997 Test)

October 20, 1997

Joseph J Hoagland, CTR 1K-M

MAAP WETLANDS PROJECT, SUMMER GRAVEL/SEDIMENT TOXICITY TESTING, 1997

Attached is the subject report for the summer gravel/sediment toxicity study, conducted August 15-25, 1997.

Toxicity to the amphipods was demonstrated in samples from gravel cell 21 and both lagoon cells during this study. Toxicity to the midge was demonstrated in sediments from both lagoon cells. Gravel was not tested with the midge.

Please call me at (205) 729-3342 if you have questions or comments.

Damien J. Simbeck
Biologist
Toxicity Testing Laboratory
TTL 1A-BFN

DJS
Attachment
cc (Attachment):
R. A. Almond, CEB 4C-M
H. S. Coonrod, WET 1A-M
F. J. Sikora, CEB 1C-M
J. R. Trimm, CEB 1E-M
Files, WM, CST 17B-C

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STANDARD REPORT FORM

TOXICITY BIOMONITORING USING *HYALELLA AZTECA* (AMPHIPOD) AND *CHIRONOMUS TENTANS* (MIDGE)

Test Title: MAAP Wetlands Project, Summer Sediment Testing Report, 1997

Principal Investigator: Damien J. Simbeck

Starting Date: August 15, 1997

Ending Date: August 25, 1997

1.0 EXECUTIVE SUMMARY

During Phase I of the Milan Army Ammunitions Plant (MAAP) wetlands project, gravel from various aquaria wetlands was tested for toxicity using the amphipod, *Hyalella azteca.* No toxicity to the amphipods was noted during Phase I. [1] During Phase II of this project, onsite wetlands at MAAP were used to treat contaminated groundwater. Two wetland types, gravel bed and lagoon, were utilized. Substrates from various cells in these wetlands were tested for toxicity using the amphipod, as well as a midge, *Chironomus tentans.* The midge was used to test only the sediments from the lagoon wetlands, since it normally burrows into the substrate, and could not do so in the gravel. Amphipods were used to test both substrate types.

During the winter testing period, toxicity (significantly reduced survival) was demonstrated to the amphipod in gravels from cells 16 and 17, and to both species in sediments from the lagoon cells. [2]

During the summer testing period, toxicity (significantly reduced survival) was demonstrated to amphipods in gravel from cell 21 and to both species in sediments from the lagoon cells. Detectable concentrations of potentially toxic RDX, HMX, and explosive by-products were found in both sediment samples (see Appendix A). Concentrations in the gravel samples from cell 21, however, were all below detection limits. Un-ionized ammonia concentrations in overlying water samples were all below potentially toxic levels (200 μg/L). Possible causitive agents could not be identified in the gravel sample.

2.0 SAMPLE COLLECTION/TREATMENT

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test substrates used for biomonitoring were whole sediments or surface gravels collected from various cells of the constructed wetlands at Milan Army Ammunition Plant, Milan, TN. Gravel samples were tested with amphipods only.

2.2 Bioassay Tests:

2.2.1 Amphipod: The amphipod tests was a single test conducted with formulated sediment (negative) control and gravel (substrate) control.

2.2.2 Midge: The midge tests was a single test conducted with formulated sediment (negative) control.

2.3 Overlying Water: Moderately hard synthetic water plus dechlorinated tap water (1:1 v:v) was used for overlying water.

2.4 Control Sediment: Formulated sediment (80% clay, 20% sand; 2% TOC) was used for the negative control sediment. Gravel collected from TVA's Wetlands facility, Muscle Shoals, AL was used as the substrate control for the gravel samples.

2.5 Sample Date and Time: August 9, 1997/1030-1400 CDT

2.6 Sampling Method: Samples were grab samples collected by TVA Wetlands personnel. Two (gravel) or three (sediment)1-L samples from each location were collected in amber bottles.

2.7 Sample Storage/Handling: Samples were placed on ice in ice coolers, and shipped to TTL. Samples remained on ice in coolers at TTL until used.

2.8 Sample Transport: Samples were shipped to TTL via TVA mail courier.

2.9 Sample Pretreatment: All sub-samples were homogenized prior to use in the toxicity test. Samples were placed into test beakers (100 mL/replicate) on the day prior to test initiation.

2.10 Test Treatments: Gravel samples tested (amphipod only) were collected from cells 16, 17, 18, 19, 20, and 21. Sediment samples were collected from cells 24 and 28. Each sample was tested at 100% (undiluted) only.

3.0 CULTURE CONDITIONS

3.1 *Hyalella azteca*, amphipod

3.1.1 Source: Environmental Consulting and Testing, Inc., Superior, Wisconson.

3.1.5 Test Organism Hatched:. August 4-5, 1997

3.1.6 Age at test initiation: 10-11 days old

3.2 *Chironomus tentans*, midge

3.2.1 Source: In-house culture, TVA, Toxicity Testing Laboratory

3.2.2 Culture Water: Culture water consists of moderately hard synthetic water mixed with dechlorinated tap water (1:1 v:v). Reagents for synthetic water were added to Milli-Q UF product water. Culture water was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 110 mg/L as $CaCO_3$

3.2.3 Temperature of Culture: 23°C±2°C

3.2.4 General Maintenance: Larval midges (0-14 days old) were reared in 1.5-L glass aquaria with 1 L water and 30 mL sand. Water was renewed three times/week. When larvae were 7-14 days old, they were transferred to a 20-L glass aquaria containing 15 L water and 100 mL sand. Aquaria were placed into a flow-through recirculating system. Flow rate to aquaria was approximately one-half aquaria (10 L) per hour. Approximately 50 percent of the system water was replaced weekly. All tanks were fed either a fish flake food or *Chlorella* suspension daily. Adults that emerged from the aquaria were placed into 8-L glass aquaria with screened top and approximately 500 mL water for egg deposition. A 5x20-cm piece of screen was placed into aquaria to provide a resting place for the adults. Egg cases were collected daily and placed into 1.5-L aquaria with 1 L water and 30 mL sand.

3.2.5 Egg Masses Collected: July 31-August 4,1997

3.2.6 Age at test initiation: 2nd or 3rd instar

3.2.7 Average Head Capsule Width: 0.40 mm (Range = 0.38-0.44 mm)

4.0 TEST METHODS

4.1 Amphipod, *Hyalella azteca*, Survival test, EPA Test Method 100.1 [3]
Midge, *Chironomus tentans*, Survival test, EPA Test Method 100.2 [3]

4.1.1 Modifications/Deviations to 100.1 (Amphipod): None

4.1.2 Modifications/Deviations to 100.2 (Midge): None

4.2 Date/Time Test Initiated:
Amphipod: August 15, 1997/0930 CDT
Midge: August 15, 1997/0920 CDT

4.3 Date/Time Test Terminated:
Amphipod: August 25, 1997/0730-1300 CDT
Midge: August 25, 1997/0700-0930 CDT

4.4 Age of Test Organism:
Amphipod: 10-11 days old
Midge: 2nd-3rd instar (>50% 3rd instar)

4.5 Test Chamber: 400 mL beaker with 350 µm Nitex® mesh covering a notch at the top.

4.6 Volume in Chamber: 100 mL gravel, 175 mL water

4.7 Number of Organisms per Replicate: 10

4.8 Number of Replicates per Treatment: 8

4.9 Test Control Substrate: Negative Control (Formulated Sediment) and Substrate Control (Gravel) for amphipods only

4.10 Dilution Substrate: NA

4.11 Overlying Water: Moderately hard synthetic water plus dechlorinated tap water, mixed 1:1 (v:v).

4.12 Dilution water: NA

4.13 Test Temperature: 23°C ± 1°C (Instantaneous temperature 23°C ± 3°C)

4.14 Photoperiod: 16 L:8 D

4.15 Renewal Period: Overlying water renewed twice daily by slow flow delivery system.

4.16 Renewal Method: Water was fed from a head tank with eight 10-mL syringes to supply slow flow (about 200 mL/10 minutes) into each test chamber.

4.17 Feeding Regime During Test:

Amphipod: 1.5 mL/replicate YCT once daily. YCT was prepared according to EPA/600/4-89/001. [3]

Midge: 1.5 mL/replicate fish flake food suspension once daily. Suspension was prepared by adding 2.67 g fish flake food to 1 L water and blending for 15 minutes.

Feeding was suspended if DO levels were $\leq$5.5 mg/L in any treatment on two consecutive days or $\leq$5.0 in any treatment on a single day.

4.18 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions were temperature (adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness.

"Final" measurements of temperature, was taken in one replicate per treatment before renewal. "Final" measurements of DO, pH and conductivity were taken daily and alkalinity and hardness were taken on Days 1, 5 and 10 in a composite of $\approx$75 mL samples removed daily from all replicates per treatment before renewal. On Days 1, 5 and 10, test solutions (100 mL) were preserved with 1:4 H_2SO_4 and refrigerated until sent to TVA's Environmental Chemistry Laboratory in Chattanooga Tennessee, for ammonia analysis using the automated alkaline phenate methodology.

4.19 Test Endpoint Determination:

4.19.1 Survival: Test animals were counted as dead if they could not be found at test termination.

4.19.2 Growth: Growth was determined by dry weight measurements after test termination. Ash-free dry weight measurements were made for the midge test.

4.20 Statistics: Statistical analyses were made using the Toxis® or SAS statistical programs.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, EPA/600/4-89/001 and the TTL Quality Assurance Plan and SOP Manual. [4][5] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study folder.

5.2.2 Instruments: All identification, service and calibration information pertaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study folder.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [6]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-42.4, respectively. [6]

5.2.5 The pH was measured using an Orion Model 250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedure ES-43.7 and ES-42.8, respectively. [6]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were made according to TVA procedures ES-43.3 and ES-42.3, respectively. [6]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an endpoint of 4.5 according to TVA procedure ES-42.1. [6]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colormetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine was determined using the DPD Titrimetric Method according to TVA procedure ES-42.9. [6]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Amphipod or Midge-- 96-hr acute, water only (LC_{50})

5.3.2 Dilution Water Used: Moderately hard synthetic water plus dechlorinated tap water (1:1 v:v).

5.3.3 Statistics: Probit, Spearman-Karber, etc.

6.0 RESULTS

6.1 Summary of Bioassay Results:

6.2 Amphipod Test

Summary of Results: Ten day exposure of amphipods to the MAAP gravels and sediments resulted in significant reduction in survival in gravels from cell 21 and both sediment cells. No significant reductions in survival or growth were observed in gravels from cells 16-20.

6.2.1 Survival Data

Amphipod Survival Data (Percent Surviving)

	Replicate								
	1	2	3	4	5	6	7	8	Mean
Formulated Sediment	80	90	80	80	90	100	80	80	85.0
Gravel Control	100	90	90	100	90	90	90	90	92.5
Gravel 16	100	100	90	70	80	70	90	90	86.4
Gravel 17	90	90	100	90	100	100	100	100	96.3
Gravel 18	100	80	80	90	90	100	90	90	90.0
Gravel 19	90	90	80	90	90	100	90	100	91.3
Gravel 20	60	100	90	90	90	90	70	90	85.0
Gravel 21	50	70	40	40	50	80	70	90	61.3*
Sediment 24	30	70	60	30	100	70	70	50	60.0†
Sediment 28	60	80	50	90	50	40	80	60	63.8†

*Statistically significant reduction in survival compared to the Gravel Control
† Statistically significant reduction in survival compared to the Form. Sediment Control

6.2.2 Growth Data

Amphipod Growth Data (mg Dry Weight)

Replicate

	1	2	3	4	5	6	7	8	Mean
Formulated Sediment	0.053	0.042	0.038	0.059	0.058	0.050	0.049	0.058	0.051
Gravel Control	0.035	0.034	0.029	0.044	0.052	0.056	0.058	0.064	0.047
Gravel 16	0.077	0.096	0.108	0.129	0.125	0.146	0.097	0.106	0.110
Gravel 17	0.081	0.103	0.100	0.101	0.088	0.104	0.099	0.074	0.094
Gravel 18	0.068	0.063	0.049	0.068	0.073	0.074	0.090	0.069	0.069
Gravel 19	0.078	0.056	0.061	0.086	0.083	0.054	0.060	0.054	0.066
Gravel 20	0.052	0.055	0.079	0.063	0.063	0.064	0.047	0.074	0.062
Gravel 21	0.052	0.026	0.038	0.045	0.042	0.045	0.027	0.053	0.041*
Sediment 24	0.020	0.037	0.023	0.047	0.034	0.029	0.039	0.026	0.032*
Sediment 28	0.017	0.019	0.030	0.031	0.026	0.018	0.024	0.023	0.023*

*No statistical comparison made due to significantly reduced survival

6.3 Midge Test

Summary of Results: Ten day exposure of midges to the MAAP sediments resulted in significant decrease in survival to midges in sediments from cells 24 and 28. Growth comparisons were not determined, since survival was significantly reduced.

6.3.1 Survival Data

Midge Survival Data (Percent Surviving)

Replicate

	1	2	3	4	5	6	7	8	Mean
Formulated Sediment	80	70	100	90	50	90	90	80	81.3
Sediment 24	40	80	60	70	60	80	40	80	63.8*
Sediment 28	60	60	60	60	70	90	60	60	65.0*

*Statistically significant reduction in survival compared to Formulated Sediment Control

6.3.2 Growth (Dry Weight Data)

Midge Growth Data (mg Dry Weight)

	Replicate								
	1	2	3	4	5	6	7	8	Mean
Formulated Sediment	1.113	1.083	0.981	1.072	1.814	0.981	1.093	1.257	1.174
Sediment 24	0.868	0.569	0.685	0.553	0.505	0.524	0.620	0.523	0.606
Sediment 28	1.037	0.897	0.338	0.757	0.590	0.696	0.915	0.725	0.744

6.3.3 Growth Data (Ash-free Dry Weight)

Midge Growth Data (mg Ash-free Dry Weight)

	Replicate								
	1	2	3	4	5	6	7	8	Mean
Formulated Sediment	0.480	0.513	0.422	0.477	0.844	0.463	0.509	0.535	0.530
Sediment 24	0.407	0.261	0.282	0.286	0.273	0.238	0.330	0.288	0.296
Sediment 28	0.372	0.368	0.210	0.305	0.231	0.309	0.257	0.325	0.297

6.4 Physical/Chemical Parameters

6.4.1 Overall Test Temperature:
Amphipod Test: 22.5°C (20.4°C-25.2°C)
Midge Test: 22.5°C (21.0°C-25.0°C)

6.4.2 Results: Water chemistry and sediment analysis summaries for MAAP Summer Sediment Test, August 15-25, 1997.

See: Appendix A TNT/By-Products Analyses Summary for Milan Army Ammunition Plant Summer Sediment Toxicity Test, August 15-25, 1997.

See: Appendix B Water Chemistry Mean Values and Ranges for MAAP Summer Sediment Toxicity Test, August 15-25, 1997.

6.5 Reference Toxicant Tests

6.5.1 Summary of Results:

Amphipod and midge reference toxicant tests conducted prior to each MAAP Summer Sediment Test showed acute results within control chart.

6.5.2 Summary of Results

Species	Date	Time	Duration	Toxicant	Results (LC_{50})	Control Chart Mean	Control Chart Range
H. azteca	08-13-97	1255	96-h	KCl	291	324 mg/L	171-476 mg/L
C. tentans	07-21-97	0900	96-h	KCl	5223	5874 mg/L	4634-7114 mg/L

7.0 CONCLUSIONS

During the summer testing period, toxicity (significantly reduced survival) was demonstrated to amphipods in gravel from cell 21 and to both species in sediments from the lagoon cells. Detectable concentrations of potentially toxic RDX, HMX and explosive by-products were found in both sediment samples (see Appendix A). Concentrations in the gravel samples from cell 21, however, were all below detection limits. Un-ionized ammonia concentrations in overlying water samples were all below potentially toxic levels (200 µg/L). Possible causitive agents could not be identified in the gravel sample.

8.0 REFERENCES

1. Simbeck, D. J. ERC Wetlands TNT/RDX Degradation. TVA Toxicity Testing Laboratory Standard Report, December, 1995.

2. Simbeck, D. J. MAAP Wetlands Project, Winter Gravel/Sediment Toxicity Testing, 1997. TVA Toxicity Testing Laboratory Standard Report, May, 1997.

2. U.S. EPA. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, EPA 600/R-94/024 (August 1994)

4. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Mendick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (August 1989).

5. Toxicity Testing Laboratory Quality Assurance Program and Standard Operating Procedures Manual, Division of Water Resources, Tennessee Valley Authority (August 1992).

6. Field Operations Natural Resources Engineering Procedure Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

Appendix A
TNT/By-Products Analyses Summary for Milan Army Ammunition
Plant Summer Sediment Toxicity Test, August 15-25, 1997

	Gravel 16	Gravel 17	Gravel 18	Gravel 19	Gravel 20	Gravel 21	Sediment 24	Sediment 28
2,6-Diamino-4-nitrotoluene	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253
Trinitroso-RDX	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253
HMX	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	0.106	0.0752
2,4-Diamino-6-nitrotoluene	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253
Mononitroso-RDX	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	0.0477	0.0390
RDX	<0.0023	<0.0023	<0.0024	<0.0024	<0.0024	<0.0023	1.23	1.02
1,3,5-Trinitrobenzene	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253
2,4,6-Trinitrotoluene	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253
4-Amino-2,6-dinitrotoluene	0.0033	0.0033	<0.0020	<0.0020	<0.0020	<0.0020	0.122	<0.0253
2-Amino-4,6-dinitrotoluene	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	0.133	0.0262
2,6-Dinitrotoluene	<0.0023	<0.0023	<0.0024	<0.0024	<0.0024	<0.0023	<0.0302	<0.0303
2,4-Dinitrotoluene	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253
Tetranitro-2,2'-azoxytoluene	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253
Tetranitro-2',4-azoxytoluene	<0.0042	<0.0042	<0.0044	<0.0044	<0.0044	<0.0043	<0.0553	<0.0556
Tetranitro-4,4'-azoxytoluene	<0.0030	<0.0030	<0.0032	<0.0032	<0.0032	<0.0031	<0.0402	<0.0404
Dinitro-4,4'-azoxytoluene	<0.0053	<0.0053	<0.0056	<0.0056	<0.0056	<0.0055	<0.0704	<0.0707
1,3-Dinitrobenzene	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253
3,5-Dinitroaniline	<0.0019	<0.0019	<0.0020	<0.0020	<0.0020	<0.0020	<0.0252	<0.0253

All samples <Detection Limit

All in mg/kg

Appendix B

Water Chemistry Mean Values and Ranges for MAAP Summer Sediment Toxicity Test, August 15-25, 1997

Initial Chemistry

Source	Temperature	Dissolved Oxygen	pH	Conductivity	Hardness	Alkalinity
	(°C)	(mg/L)	(S.U.)	(µmhos)	(mg/L as $CaCO_3$)	(mg/L as $CaCO_3$)
Overlying Water	23.3 (22.7-24.0)	8.0 (7.7-8.2)	8.3 (8.2-8.3)	309 (301-313)	108 (106-112)	83 (81-87)

Final Chemistry -Midge

Source	Temperature	Dissolved Oxygen	pH	Conductivity	Hardness	Alkalinity	Unionized Ammonia
	(°C)	(mg/L)	(S.U.)	(µmhos)	(mg/L as $CaCO_3$)	(mg/L as $CaCO_3$)	(µg/L)
Formulated Sediment	22.4 (21.2-24.9)	6.0 (4.7-7.3)	7.6 (7.5-7.7)	359 (335-397)	121 (118-124)	94 (91-97)	<2 (<1-3)
Sediment 24	22.6 (21.3-25.0)	6.1 (5.5-6.6)	7.6 (7.4-7.7)	309 (296-313)	105 (102-106)	81 (80-82)	4 (1-8)
Sediment 28	22.3 (21.0-24.9)	6.3 (5.8-6.6)	7.5 (7.4-7.6)	295 (269-304)	97 (90-102)	71 (65-75)	3 (1-7)

Appendix B (continued)

Water Chemistry Mean Values and Ranges for MAAP Summer Sediment Toxicity, August 15-25, 1997

Final Chemistry-Amphipod

Source	Temperature	Dissolved Oxygen	pH	Conductivity	Hardness	Alkalinity	Unionized Ammonia
	(°C)	(mg/L)	(S.U.)	(µmhos)	(mg/L as $CaCO_3$)	(mg/L as $CaCO_3$)	(µg/L)
Formulated Sediment	22.8 (21.4-25.2)	6.5 (5.6-7.3)	7.7 (7.5-7.8)	355 (331-396)	119 (116-120)	93 (90-95)	<1 (<1-1)
Gravel Control	22.7 (21.3-25.2)	7.2 (6.6-7.8)	8.0 (7.8-8.1)	315 (300-321)	107 (102-110)	80 (75-84)	11 (4-15)
Gravel 16	22.1 (20.4-24.8)	6.1 (5.5-6.7)	7.7 (7.6-7.8)	325 (319-332)	112 (110-114)	89 (84-93)	6 (1-14)
Gravel 17	22.4 (21.2-25.0)	6.3 (5.3-6.8)	7.8 (7.6-7.8)	321 (317-325)	113 (112-114)	85 (83-87)	2 (1-5)
Gravel 18	22.5 (21.3-25.1)	6.3 (5.7-6.8)	7.8 (7.7-7.8)	324 (317-333)	112 (108-114)	88 (82-93)	5 (1-12)
Gravel 19	22.5 (21.1-25.0)	6.2 (5.2-6.7)	7.8 (7.7-7.9)	323 (316-332)	114 (110-118)	89 (83-94)	<3 (<1-7)
Gravel 20	22.9 (21.2-25.2)	5.4 (5.1-5.8)	7.9 (7.8-8.0)	352 (346-367)	129 (128-132)	105 (100-110)	4 (2-6)
Gravel 21	22.6 (21.3-25.0)	6.0 (5.6-6.3)	8.0 (7.8-8.0)	354 (340-375)	130 (124-136)	105 (96-116)	2 (1-3)
Sediment 24	22.4 (21.0-24.8)	6.6 (6.1-7.1)	7.6 (7.5-7.8)	306 (295-309)	105 (100-108)	80 (79-81)	<4 (<1-9)
Sediment 28	22.1 (20.4-24.7)	6.7 (6.2-7.2)	7.6 (7.4-7.7)	301 (278-308)	105 (104-106)	74 (70-77)	<3 (<1-8)

APPENDIX C

TEST PLAN FOR THE ALTERNATE CARBON SOURCE AND HIGHER FLOWRATE STUDY

U.S. Army Environmental Center

TEST PLAN FOR ALTERNATE CARBON SOURCE AND HIGHER FLOWRATE STUDY AT MAAP

Prepared for
U.S. ARMY ENVIRONMENTAL CENTER
Aberdeen Proving Ground, Maryland 21010-5401

Prepared by
Tennessee Valley Authority
Environmental Research Center
Muscle Shoals, Alabama 35662-1010

July 1997

TVA Contract No. TV-88826V

ISO Form 45, 1 Feb 93 replaces THAMA Form 45 which is obsolete

Test Plan for Alternate Carbon Source and Higher Flowrate Study at MAAP

1.0 Introduction

The Wetlands Demonstration program at the Milan Army Ammunition Plant shows that TVA's subsurface flow (SSF) constructed wetland is remediating explosive contaminated groundwater. The SSF wetland is a two-bed system consisting of an anaerobic gravel bed (17,000 ft^3) and an aerobic reciprocating gravel bed (5,800 ft^3). Explosive contaminated groundwater is fed to the system at a rate of 5 gallons per minute (gpm) and powdered milk is fed to the system, every two weeks, as a carbon source for system microorganisms. Currently, the treatment system is reducing the total nitrobody concentration to below the desired level (2 ppb).

2.0 Problem Statement

Even though facility operations have been successful, it has become apparent that additional data would be helpful to improve the design, operation, and economic success of scaled-up systems. Areas of interest include:

- Establishing the effect of long term plant growth on explosive remediation.
- Examining nitrobody remediation at cold temperature.
- Examining the use of alternate carbon sources in the anaerobic cell (cell A1).
- Establishing the anaerobic cell's maximum flow rate.

The issues of long-term plan growth and cold temperature remediation are being addressed in a separate proposal for extending the operating period of the existing demonstration program. This test plan examines the use of alternate carbon sources and the impact of increasing system flow rates.

Small scale test cells are being used in this test, rather than the large scale demonstration cells, because steady state conditions are being maintained at the demonstration site and time constraints limit opportunities to vary system conditions. The smaller system's operating conditions can be easily manipulated without

impacting the demonstration site. Their use assures the timely production of information. The test cells proposed are similar to those used by TVA in the pre-demonstration feasibility study.

3.0 Proposal

3.1 Overview of Experimental Setup and Operation

The proposed system will consist of four gravel-filled 40 ft^3 containers or test cells. The test cells will be located on the gravel bed just inside demonstration cell A1 above the inlet header, Figure 3-1. Placing the test cells within demonstration cell A1 allows effluent from the test cells to flow directly into the demonstration cell, eliminating concerns about post-treatment of the test cell effluent. Placement at this location also significantly reduces the complexity of test cell design by reducing the need for sub-system support.

Contaminated groundwater from the inlet piping to demonstration cell A1 will be fed to each test cell at a rate of 38 ml/min. This results in a retention time of 7.5 days per cell. The total flow rate to all four test cells, 152 ml/min, will be approximately 0.8% of the 5 gpm of flow entering demonstration cell A1.

Two of the containers, test cells 1 and 2, will be maintained at conditions similar to those in demonstration cell A1. Conditions in the other two test cells, cells 3 and 4, will be manipulated. All of the test cells will be planted with a mixture of wool grass, canary grass, and sweet flag. The gravel in each container will be inoculated with a small charge of gravel from the existing wetlands. Each test cell (Figure 3-2) will hold 108 gallons of water as estimated by a gravel porosity of 45%. Construction and operational details are provided in Appendix A.

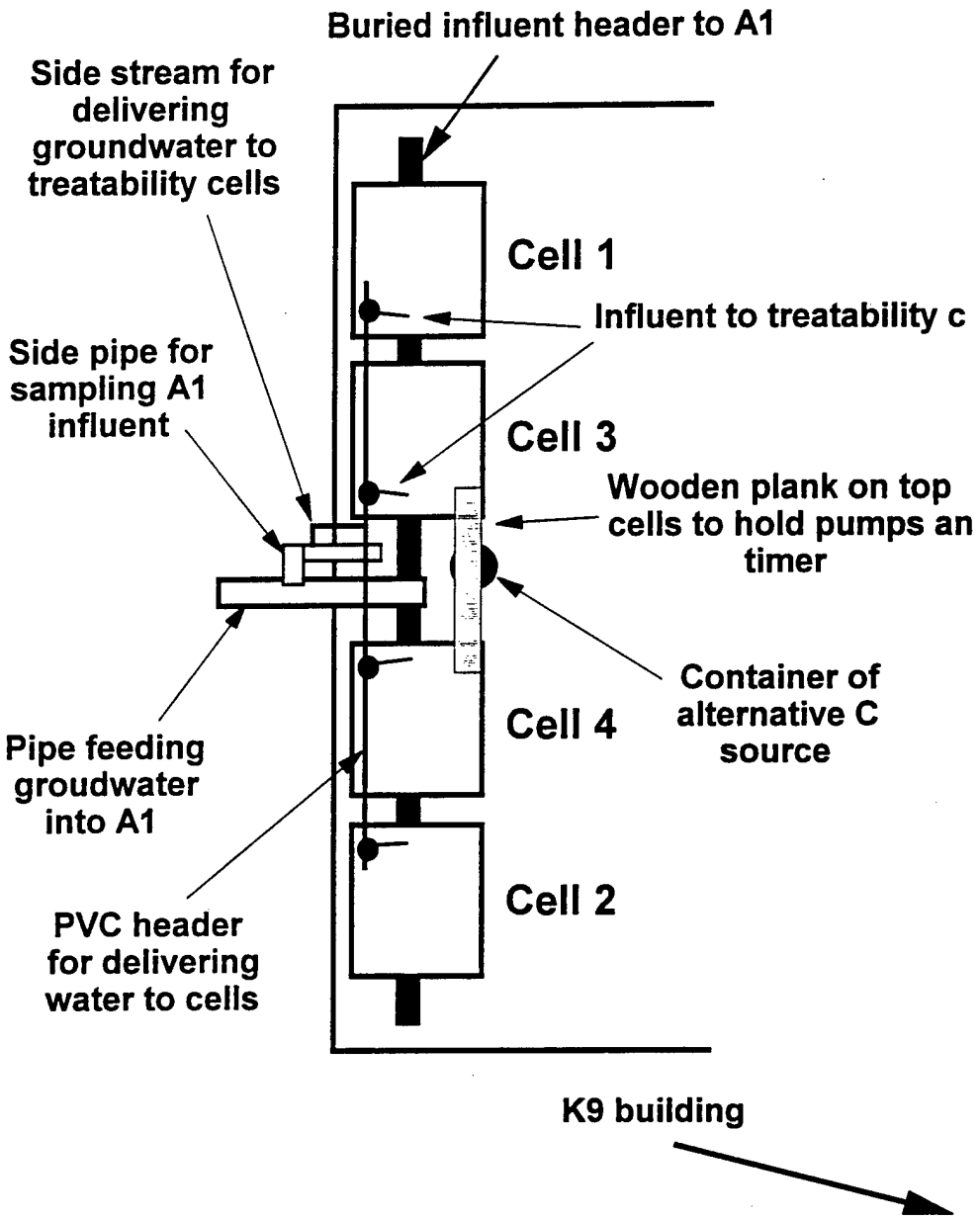


Figure 3-1

Placement of Test Cells in Demonstration Cell A1

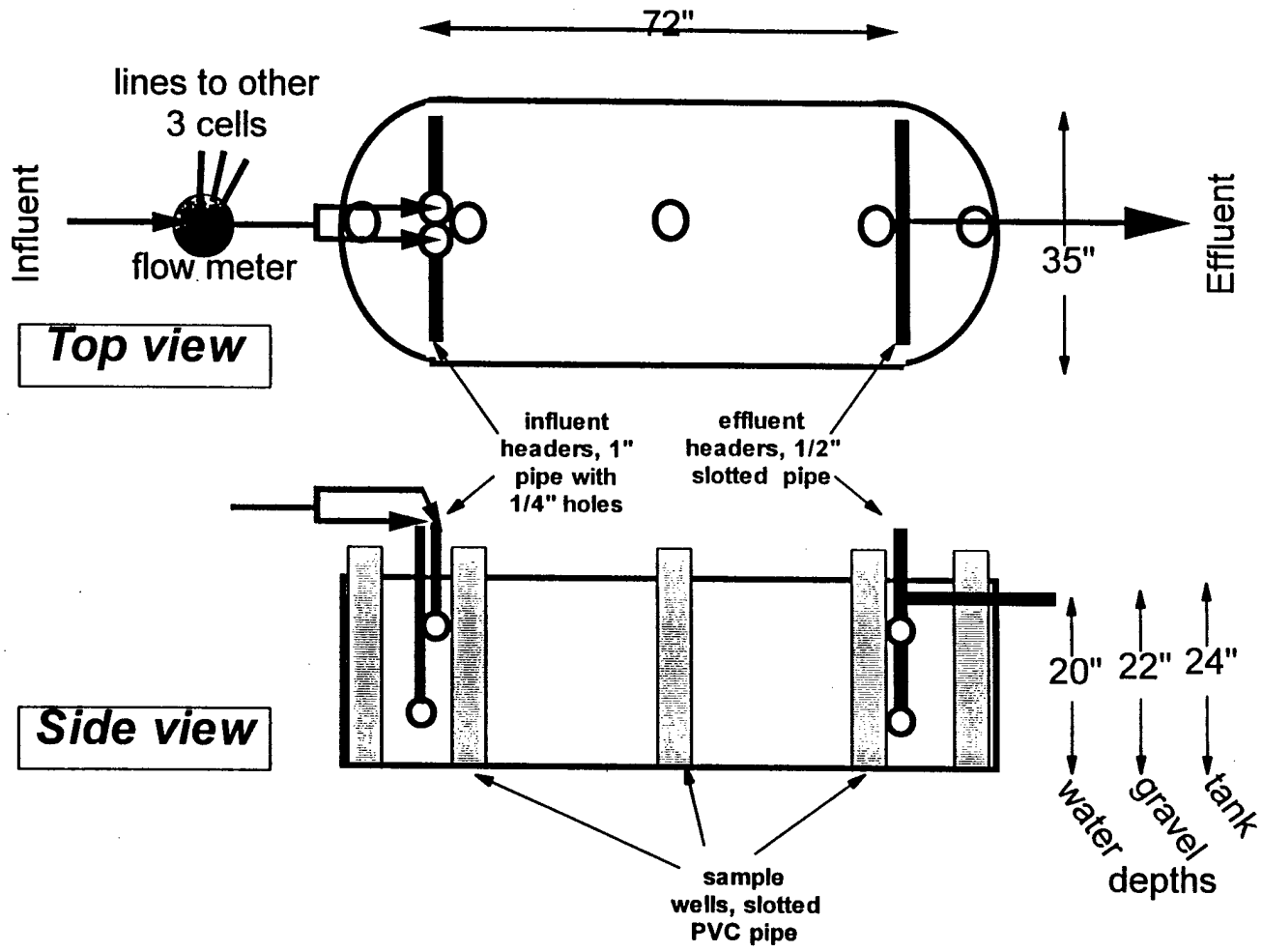


Figure 3-2

Diagram of Typical Test Cell

Three tests are envisioned.

- Use of syrup (sucrose) as a carbon source
- Use of sewage as a carbon source (sewage sludge inoculated with sewage wastewater)
- System response to a doubling of the inlet flow rate

Experimental parameters for the three tests are summarized in Table 3-1. During the first test, the use of syrup (50% sucrose) as a carbon source will be examined. During the syrup test, test cells 1 and 2 will be operated using powdered milk while syrup is used in test cells 3 and 4. Throughout the syrup test, all of the test cell's other operating parameters will be maintained as in the demonstration unit with one exception: the carbon dose rate for the syrup will be increased. The carbon dose rate is presently once every two weeks in the demonstration unit and will be increased to once every 12 hours in the test cells. The dose rate for the powdered milk will remain at once every two weeks as in the demonstration test plan. The dose rate for the powdered milk will not be altered because milk powder's low solubility makes it a poor candidate for unattended addition. The syrup with be added to the test cells via a piston pump.

The syrup test will be conducted over a four-week period. During this time, two sets of water samples will be obtained from selected points within each test cell. One sample set will be collected after the second week of operation. The second sample set will be collected at the end of week four. Sample point identification and information about the analysis to be obtained are provided in Section 3.2 "Overview of Sampling Operations."

The use of sewage wastewater as a carbon source will be examined during the second test. The sewage test will begin immediately after the syrup test is complete with no transition time. Prior to conducting the sewage test, the water in each test cell will be removed and replaced with fresh water from well MI-051. The sewage wastewater's components, treated wastewater and sewage sludge, will be obtained just downstream of the primary treatment unit from a Milan Army Ammunition Plant (MAAP) or TVA

Table 3-1
Experimental Operating Parameters

Test	Test Cell	Water Treatment Conditions			Carbon Source		
		Retention Time (Days)	Flow Rate (ml/min)	Test Length (Weeks)	Source Type	Feed Rate (g/day)	Dose Rate[1]
Carbon Source - Syrup	1	7.5	38	4	Powdered Milk	20	Every 2 weeks
	2	7.5	38	4	Powdered Milk	20	Every 2 weeks
	3	7.5	38	4	Syrup	20	Every 12 hours
	4	7.5	38	4	Syrup	20	Every 12 hours
Carbon Source - Sewage	1	7.5	38	4	Powdered Milk	20	Every 2 weeks
	2	7.5	38	4	Powdered Milk	20	Every 2 weeks
	3	7.5	38	4	Sewage	20	Every 12 hours
	4	7.5	38	4	Sewage	20	Every 12 hours
High Flow Rate	1	7.5	38	2	Powdered Milk	20	Every 2 weeks
	2	3.75	76	2	Powdered Milk	20	Every 2 weeks
	3	7.5	38	2	Syrup	20	Every 12 hours
	4	3.75	76	2	Syrup	20	Every 12 hours

1.) See Appendix A.

wastewater treatment unit. Of the two components, sewage sludge is the primary carbon source. The sewage sludge and treated wastewater will be mixed into a slurry similar to a milk powder slurry and be pumped into test cells 3 and 4 every two weeks. The procedures to be used are the same as those indicated above for the syrup test; except the sewage dose rate will be once every two weeks rather than the syrup dose rate of once every twelve hours. Prior to initiating this test, the sewage slurry will be sampled and analyzed for metals, total organic carbon (TOC), total nitrogen, total phosphorus, chemical oxygen demand (COD), volatile and semi-volatile organics (optional). Total volatile and semi-volatile organics will be analyzed only if the sewage wastewater's origin suggests the presence of organic compounds (solvents, oils, etc.) is possible. The sewage dosage to be used in the test cells will provide a total carbon dose equal to the sucrose and will be based on the sewage slurry's carbon content. Sample point identification and information about the analyses to be obtained are provided in Section 3.2 "Overview of Sampling Operations."

During the third test, an increase in the flow rate of the contaminated groundwater will be studied. Two carbon sources, milk powder and syrup, will be used during the high flow rate test. Test cells 1 and 2 will be operated with milk powder. The flow rate in cell 1 will be maintained at 38 ml/min while the flow rate in cell 2 will be increased to 76 ml/min. Test cells 3 and 4 will be operated with syrup. The flow rate in test cell 3 will be maintained at 38 ml/min while the flow rate in test cell 4 will be increased to 76 ml/min. Each test cell will be subject to different operating conditions, thus no replication will be present during this test.

The flow rate test will be conducted over a two-week period. A single set of samples will be obtained from the system at the end of the test period. Sample point identification and information about the analyses to be obtained are provided in Section 3.2 "Overview of Sampling Operations."

As indicated in the attached GANTT chart, the project will be completed by 28 August 1997 (Figure 3-3).

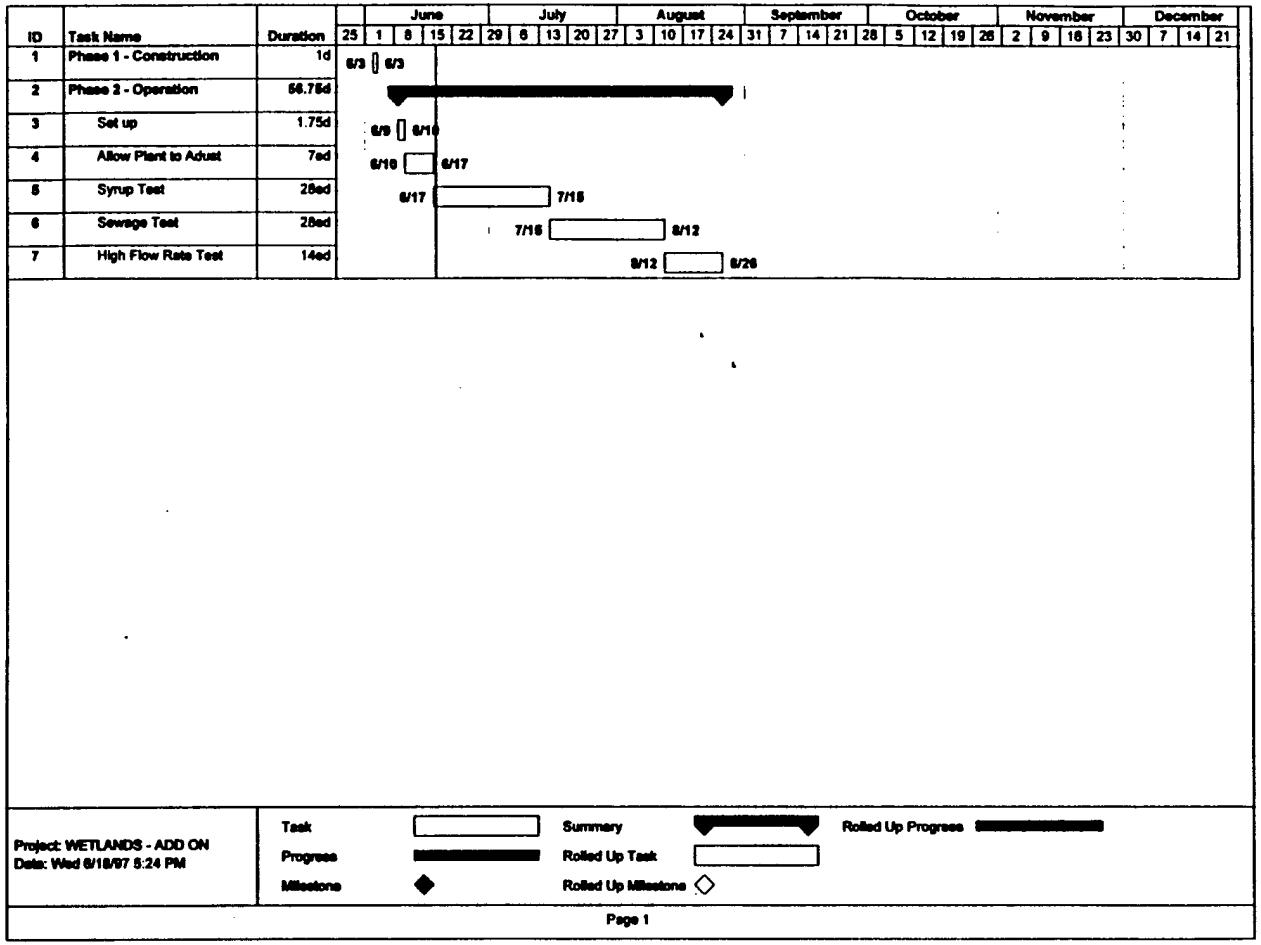


Figure 3-3

GANTT Chart for Project Operations

3.2 Overview of Sampling Operations

Sampling will be performed with three objectives in mind:

- Monitoring of ambient system conditions.
- Assessing the efficiency of the treatment systems.
- Assessing sewage wastewater characteristics.

Ambient conditions to be monitored include: water flow rate, dissolved oxygen content, pH, temperature, oxidation-reduction potential, and electrical conductivity (EC). Treatment efficiency is to be assessed by analysis for explosive content, explosive byproduct content, chemical oxygen demand (COD), total organic carbon (TOC), and plant nutrient levels. Sewage wastewater characteristics are to be assessed by analysis for metals, volatile and semi-volatile organics, total organic carbon, chemical oxygen demand, Total Kjeldahl Nitrogen (TKN), and total phosphorus.

The number of water sample sets to be collected from each test cell will vary with the type of test to be conducted. During the syrup and sewage tests, two sets of water samples will be obtained from each test cell. One set will be collected after two weeks of operations, the second at the end of week four. During the high flow rate test, only a single set of water samples will be obtained from the test cells. In addition, just prior to the sewage treatment test, a sample of the sewage will be obtained and analyzed for the parameters listed in Table 3-2.

The sample collected from each test cell will be identical. A summary outlining the analytical parameters is provided in Table 3-3. The locations of the sampling points are illustrated in Figure 3-4. Sample positions 1 through 9 refer to:

- A composite sample of the influents to each test cell (sample point 1)
- The sampling well located at the mid-point of each test cell (sample points 2, 4, 6, 8)
- The effluents of each test cell (sample points 3, 5, 7, 9)

Table 3-2

Sample Parameters for Determining Sewage Wastewater Characteristics

Parameters	Frequency	Method
Explosives		
TNT	Once	Modified 8330
RDX	Once	Modified 8330
TNB	Once	Modified 8330
HMX	Once	Modified 8330
2,4 DNT	Once	Modified 8330
2,6 DNT	Once	Modified 8330
Explosives Byproducts		
2A-DNT (TNT byproduct)	Once	Modified 8330
4A-DNT (TNT byproduct)	Once	Modified 8330
2,6 DANT (TNT byproduct)	Once	Modified 8330
2,4 DANT (TNT byproduct)	Once	Modified 8330
Mono-nitroso RDX (RDX byproduct)	Once	Modified 8330
Tri-nitroso RDX (RDX byproduct)	Once	Modified 8330
Other		
Metals (As, Ca, Cd, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Se, Zn)	Once	200 Series
Total Volatile and Semi-volatile Organics [1]	Once	8260A & 8270B
Total Organic Carbon (TOC)	Once	415 Series
Chemical Oxygen Demand (COD)	Once	410 Series
Plant Nutrients		
Total Kjeldahl Nitrogen (TKN)	Once	351 Series
Total Phosphorus	Once	Lachat 10-115-10-1-C

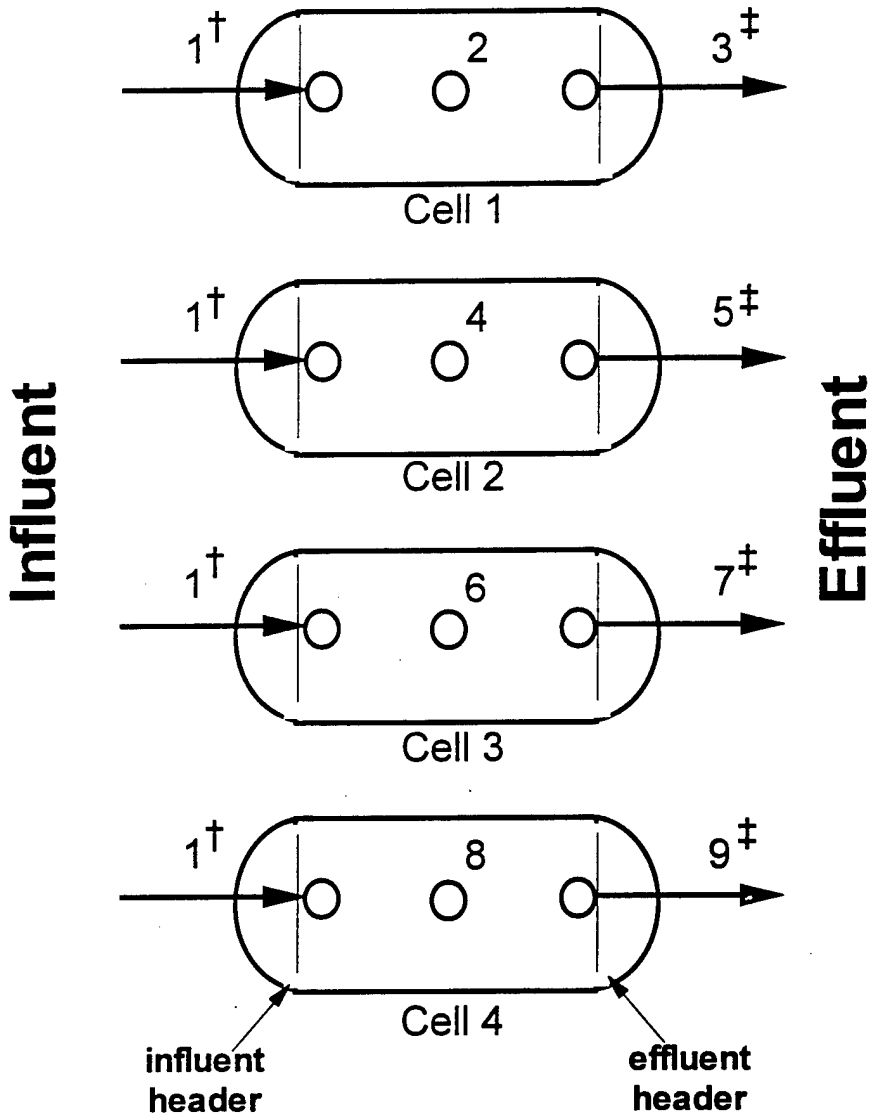
[1]Will be done only if the nature of the wastewater source indicates the possible presence of organics (solvents, oils, etc.)

Table 3-3

Sampling Parameters for Determining Treatment System Efficiency

Water Quality Parameters	Frequency	Method[1]	Position Number[2]
Explosives (Total Nitrobodies)			
TNT	At end of week 2 & week 4	Modified 8330	1-9
RDX	At end of week 2 & week 4	Modified 8330	1-9
TNB	At end of week 2 & week 4	Modified 8330	1-9
HMX	At end of week 2 & week 4	Modified 8330	1-9
2,4 DNT	At end of week 2 & week 4	Modified 8330	1-9
2,6 DNT	At end of week 2 & week 4	Modified 8330	1-9
Explosives Byproducts			
2A-DNT (TNT byproduct)	At end of week 2 & week 4	Modified 8330	1-9
4A-DNT (TNT byproduct)	At end of week 2 & week 4	Modified 8330	1-9
2,6 DANT (TNT byproduct)	At end of week 2 & week 4	Modified 8330	1-9
2,4 DANT (TNT byproduct)	At end of week 2 & week 4	Modified 8330	1-9
Mono-nitroso RDX (RDX byproduct)	At end of week 2 & week 4	Modified 8330	1-9
Tri-nitroso RDX (RDX byproduct)	At end of week 2 & week 4	Modified 8330	1-9
Test Cell Monitoring			
pH, DO, Temperature, and EC	At end of week 2 & week 4	Meter[3] (YSI 600 sonde)	1-9 at mid-depth
Oxidation-reduction potential	At end of week 2 & week 4	2580	1-9 at mid-depth
Other			
Total Organic Carbon (TOC)	At end of week 2 & week 4	415 Series	1-9
Total Chemical Demand (COD)	At end of week 2 & week 4	410 Series	1-9
Plant Nutrients			
NH_4-N	At end of week 2 & week 4	350 Series	1-9
TKN	At end of week 2 & week 4	351 Series	1-9
(NO_3+NO_2)-N	At end of week 2 & week 4	353 Series	1-9
$(PO_4$-P)	At end of week 2 & week 4	365 Series	1-9

(1) See Appendix B of test plan for details on methods and procedures.
(2) See location of sampling positions in Figure 3-2.
(3) Meter methods: pH method 150.1; DO method 360.1; Temperature method 170.1; EC method 120.1 Oxidation-reduction potential, or redox potential, is measured by method 2580.



† A composite influent water sample will be taken for laboratory analysis. In-situ measurements with a hand-held sonde will be taken in the well next to the header.

‡ Effluent water sample leaving the system will be taken for laboratory analysis. In-situ measurements with a hand-held sonde will be taken in the well next to the header.

Figure 3-4

Location of Sample Points

The water samples at points 1-9 will be analyzed for explosive content, explosive byproduct content, TOC, COD, nutrient content (i.e., ammonium, nitrate, and phosphate levels), pH, temperature, and electrical conductivity. The explosive analytes include:

- 2,4,6 Trinitrotoluene (TNT)
- Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)
- Trinitrobenzene (TNB)
- Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)
- 2,4 Dinitrotoluene (2,4-DNT)
- and 2,6 Dinitrotoluene (2,6-DNT)

The total nitrobody count can be found by adding up the total concentration of the explosives listed above.

By-products analytes included the TNT by-products:

- 2-amino dinitrotoluene (2A-DNT)
- 4-amino dinitrotoluene (4A-DNT)
- 2,6 diamino nitrotoluene (2,6-DANT)
- 2,4 diamino nitrotoluene (2,4-DANT)

And the RDX by-products:

- Mononitroso RDX
- Trinitroso RDX

Analysis for COD and TOC are included to determine how much residual organic carbon is released from the systems.

Ambient conditions in the cell will also be measured at sample positions 1 through 9 for:

- Dissolved oxygen (DO)
- pH
- Water temperature
- Electrical conductivity (EC)
- Oxidation-Reduction potential

The dissolved oxygen, pH, water temperature, and electrical conductivity measurements will be collected using a hand-held sonde placed in the sampling wells. Oxidation-reduction potential will be taken with in situ platinum tipped copper wire and a portable mV meter and calomel reference electrode. The location of the *in situ* measurements are described in Figure 3-4.

3.3 Water Sampling Procedures

During each sampling period, water samples from sampling points 1 through 9 (Figure 3-4) will be collected from sampling points located in the test cells. Water from sampling point 1 will be collected as a composite from the four inlets to each cell. The water from sampling position 1 will be collected directly from the tubing delivering groundwater to the test cells. Effluent water (sampling positions 3, 5, 7, and 9) will be collected from the effluent pipe from each test cell. The water samples collected from interior wells (sampling positions 2, 4, 6, and 8) will be taken as whole water column samples with a coliwasa tube.

In all cases where water samples are obtained, approximately 300 ml of water will be collected and placed in a stainless steel beaker. Part of the solution will be transferred to a 60 mL amber glass bottle wrapped in aluminum foil. This sample will undergo analysis for explosives and explosive byproducts. A second part of the sample will be transferred to a 60 mL plastic bottle. This sample will undergo COD analysis (to be conducted within 24 hours of collection). A third part of the sample will be transferred to a 120 mL plastic bottle. This sample will undergo nutrient analyses. The third sample will be preserved with 1.1 mL of 1 N H_2SO_4. All collection containers will be placed in an ice chest containing ice or a commercial ice substitute and will be

transported to TVA's laboratory in Muscle Shoals, AL in the custody of a TVA employee. The samples will be refrigerated upon arrival at the lab. All samples received from the test site will be handled in accordance with TVA's chain of custody procedures.

After collecting the water samples, the pH, DO, water temperature, EC, and oxidation-reduction potential of the system's water will be determined at all sampling positions with portable probes in each sampling well at mid-depth. The pH, DO, temperature, EC, and oxidation-reduction potential will be monitored and recorded on a data collection sheet in the field.

Prior to conducting the test with sewage wastewater, a wastewater sample will be collected and analyzed as in Table 3-2. In this case, approximately 300 ml of water will be collected and placed in a stainless steel beaker. Part of the solution will be transferred to a 60 mL amber glass bottle wrapped in aluminum foil for explosives and explosive byproduct analyses. A second part of the sample will be transferred to a 60 mL plastic bottle for COD analysis (to be determined within 24 h after collection). A third part of the sample will be transferred to a 120 mL plastic bottle for nutrient and metal analyses. The third sample will be preserved with 1.1 mL of 1 N H_2SO_4. The stainless steel beaker will then be used to collect additional wastewater samples. 80 ml will be transferred to two 40 ml glass containers with hole top type caps. These samples will be submitted for volatile organic analysis. Two liters of the sewage wastewater will be transferred to two 1-liter glass containers. These samples will be submitted for analysis of semi-volatile organics. All collection containers will be placed in an ice chest containing ice or a commercial ice substitute and will be transported to TVA's laboratory in Muscle Shoals, AL, in the custody of a TVA employee. The samples will be refrigerated upon arrival at the lab. All samples received from the test site will be handled in accordance with TVA's chain of custody procedures.

3.4 Sample Identification

Example sample identification codes for one sampling period are provided in Table 3-4. Each identification code consists of a series of six (6) numbers, dates, or letters. These items are listed according to the following code formula:

A . B . C . D . E . F

Where:

A = Serial count of the sample.

B = TR for treatability study (as distinguished from the demonstration study).

C = A code identifying the treatment cell number or a test of the sewage wastewater sample. Codes for the test cells are C1, C2, C3, and C4. Composite influent samples are designated as CC. The sewage wastewater is designated as SW.

D = Sample location numbers (1, 2, 3, 4, 5, 6, 7, 8, or 9).

E = Date sample was collected.

F = The initials of the individual collecting the sample.

For example, the fifth example identification code listed in Table 3-4 is:

5 . TR . C2 . 5 . 6/24/97 . FJS

Where:

- The first code item, 5, is the serial count of that sample.
- The second code item, TR, identifies the sample as belonging to the treatability study.
- The third code item, C2, indicates test cell 2.
- The fourth code item, 5, indicates sample point five.
- The fifth code item, 6/24/97, indicates the date the sample was taken.
- The letters of the sixth code item, FJS, provide the initials of the individual taking the sample.

Table 3-4

Example Sample ID Numbers

Test Cell	Example Sample ID
Composite influent into each test cell	1.TR.CC.1.6/24/97.FJS
1	2.TR.C1.2.6/24/97.FJS
1	3.TR.C1.3.6/24/97.FJS
2	4.TR.C2.4.6/24/97.FJS
2	5.TR.C2.5.6/24/97.FJS
3	6.TR.C3.6.6/24/97.FJS
3	7.TR.C3.7.6/24/97.FJS
4	8.TR.C4.8.6/24/97.FJS
4	9.TR.C4.9.6/24/97.FJS

CC = Composite entering all cells.

3.5 Sampling Equipment

The equipment to be used for collecting field and laboratory data is outlined in Table 3-5.

Dissolved oxygen, pH, electrical conductivity, temperature, and oxidation-reduction potential will be determined in the field with hand-held instruments. A YSI 600 sonde will be used to measure DO, pH, EC, and temperature in one probe. Oxidation-reduction potential will be determined by measuring the voltage of a platinum electrode against a standard calomel electrode using an Orion hand-held pH meter with a millivolt scale.

Explosive and explosive byproduct content will be determined in water and sediments collected from the field with high performance liquid chromatography. Total Kjeldahl Nitrogen (TKN), NH_4-N, NO_3-N, and PO_4-P will be determined colorimetrically via an automatic analyzer. Volatile and semi-volatile organics will be determined by gas chromatography/mass spectrometry. Chemical oxygen demand will be determined by a colorimetric analysis. The pH of water samples taken to the laboratory will be analyzed with a glass electrode and pH meter.

All existing procedures are referenced in Appendices B-1 through B-14 of the Milan demonstration test plan. Analyses for total phosphorus, volatile organics, and semi-volatile organics are new to the demonstration program and procedures for these analyses are in Appendices B, C and D respectively.

3.6 Quality Program

3.6.1 Quality Assurance

This plan is to be considered an extension of the existing demonstration test plan. All QA objectives in the existing plan will be followed for these experiments.

Table 3-5

Equipment Used for Data Collection

Field Data	**Equipment**
DO, pH, EC, Temperature	YSI 600 Sonde
Oxidation-reduction potential (Redox Potential)	Orion mV/pH Meter
Laboratory Data	
Explosives and related by-products	Varian HPLC
TKN, NH_4, NO_3, PO_4, and Total Phosphorus	Lachat Quick Chem 8000 or Technicon AutoAnalyzer II
Total organics (volatile and semi-volatile)	Gas Chromatograph/Mass Spectrometry
Organic C	Dohrmann DC 190
BOD	Incubation and YSI DO probe
COD	Hach DR/2000
pH	Orion meter

3.6.2 Quality Control

The analysis to be conducted under this test plan will be performed as an extension of the ongoing demonstration program. All quality control measures to be used in the handling and analysis of these analyses will be identical to those used during the ongoing demonstration. Where new analyses exist, the procedures provided in Appendices B, C, and D will be followed.

Appendix A

Construction and Operational Details

Supplies:

1. Containers: 4 galvanized containers (35"X90"X23") with 300 gallon capacity.

2. Gravel: 5.5 cubic yards is required

3. Plumbing: Headers will be constructed prior to 6/9. Supplies needed for on site construction include:
   - ½ inch pipe-30 feet (all plumbing will be PVC)
   - ½ inch T's-6
   - ½ inch gate valves-4
   - ½ inch L's-12
   - ½ inch (female) threaded adapter-4
   - ½ inch to tubing adapter-4
   - tubing ¼ inch, Tygon-5 feet
   - tubing Y -8
   - 1 inch gate valves-2
   - 1 inch to ½ inch reducer-1
   - PVC glue
   - ½ inch bulkhead-4
   - Flow meter, ½ inch, (total flow)-1
   - 2 and 4 inch slotted PVC pipes for sample ports.

4. Tools:
   - Drill, large
   - Bit
   - Shovels-3
   - 5-gallon buckets-5
   - Pipe cutters
   - Misc. wrenches, pliers, etc.

5. Miscellaneous:
   - Polyethylene plastic-1 roll

Construction

1. Placement: The four containers will be placed (in-line) along the east wall of A1. Some plants will be removed, but will be used later to sod the new cells.

2. Headers: Each cell will contain two inlet headers. The inlet headers will be made of ½ inch PVC pipe with ¼ inch holes (6 holes per header). Placement will be at 6" and 14" from the bottom of the container. The outlet header will be constructed of 1-inch PVC perforated pipe with solid PVC pipe connecting to outlet. The perforated outlet lines will be placed at 6" and 14" from the bottom of the container. The distance between the inlet and outlet headers will be 72."

3. Sample ports: Sample ports will be placed at beginning, middle and end of system. The ports will be of perforated 2" PVC pipe. In addition to sample ports, additional 3" ports will be placed at the extreme ends of the cell to allow for circulating milk powder.

4. Filling: Containers will be filled with 1 ¼ to ¼ inch river gravel.

5. Inoculation: As the cell is filled with clean gravel, inoculated gravel from A1 will be added and mixed. The ratio will be 1 gallon of inoculating gravel for each 2" of clean gravel added to the cell. The inoculating gravel will be obtained from 4 locations in A1 (¼ taken from each location). Upon completion, clean gravel will be added to replace the gravel taken from A1 and marked with a piece of labeled PVC pipe to insure that sampling will not occur at that location in the demonstration program. In addition, 5 gallons of water will be taken from a sample well in A1 and added with each 2" of gravel. The balance of water needed to fill the cells (keeping the gravel covered will prevent the introduction of oxygen

to microbes on the gravel substrate) will be tap water and will be added with each 2" layer of gravel added.

6. Feeding: Two of the new test cells will be fed with milk powder similar to A1. Upon completion of construction, milk will be fed to two cells (286 grams each) and circulated via a drum pump and outlet pipe at the ends of the cells. A solution of sucrose (food grade) will be dissolved in water and fed to the two remaining cells. As with milk powder, 286 grams will be added and the cell circulated. In addition to the sucrose, 5 grams of monoammonium phosphate (27%P) will be added as a source of P (milk powder contains P, but sucrose does not; N is available from ground water).

Operation

1. Following a 7-day acclamation period, the cells will be operated as follows:

   - 286 grams of milk powder will be added to the milk-fed cells as a suspension and circulated as during start-up. The milk powder suspension will be added and recirculated every 2 weeks as in the demonstration test cell A1.

   - For the sugar-fed cells, an aqueous flow of food grade sugar solution (50% w/w) with ammonium phosphate (0.87% w/w) will be used. The sugar solution will be fed intermittently using a piston pump and timer. The sugar solution will be fed into the incoming ground-water feed line and pass through a mixing coil before going to the influent headers. The total amount of sugar fed will be 286 grams in a 14-day period. With a density of 1.23 g/ml for the 50% w/w solution, 286 grams is equivalent to 465 ml. The solution will be delivered at a rate of 33 ml every 24 hours or 16.5 ml every 12 hours.

- When using sewage as the carbon (C) source, the feed rate will depend on the C concentration according to the following equation:

  $$129\ g * 100/(1000 * X * 1 g/ml/) = Y$$

  where 129 g is the amount of C added as milk powder over a 14-day period (assuming 45% C content); X is the percent C concentration in the sewage wastewater (a combination of treated wastewater and sewage sludge); 1 g/ml is the density of the sewage wastewater; and Y is the volume in liters of sewage wastewater to be added over a 14-day period. For example, assuming a 1% C content in the water, 12.9 L would be added over 14 days. The solution will be fed into the cells every two weeks as is done with the milk powder solution. Here 45% carbon is assumed for the milk powder and a density of 1 g/ml is assumed for the sewage wastewater.

- A flow of 38 mL per minute of ground water (nitrobody contaminated) will be initiated to all cells. Flow will be controlled with a gate valve and measured with graduated cylinder/stop watch. Total flow to the four cells will be monitored with a totalizing flow meter.

- Sampling/data acquisition will occur after two weeks and will continue on two-week intervals corresponding to other bi-weekly sampling at the facility.

Appendix B

Total Phosphorus By Flow Injector Analyzer

WP-0022 - Total Phosphorus by Flow Injection Analyzer
June 19, 1997

1.0 Procedure

Perform analysis for total phosphorus in accordance with the procedure for Lachat Quick Chem 8000 flow injection analyzer as attached.

2.0 Recordkeeping

retain all machine printouts, analysis worksheets, preparation worksheets, percent recovery calculation of quality control samples, standards preparation log, and notes as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch, introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or a sample duplicate.

William J. Rogers

Quality Assurance Officer

LACHAT

INSTRUMENTS

QuikChem Method 10-115-01-1-C

Total Phosphorus in Kjeldahl Digests

0.01 to 5.0 mg P/L

-- Principle --

Water samples are digested with sulfuric acid in a block digestor. Using a mercuric oxide catalyst, the samples' phosphorus is converted to the orthophosphate anion. Potassium sulfate is also added to raise the boiling temperature of the digestion and speed the conversion to orthophosphate. The digest is diluted with water.

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

-- Interferences --

1. Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silica concentration of approximately 4000 ppm would be required to produce a 1 ppm positive error in orthophosphate.
2. Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.
3. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

-- Special Apparatus --

1. Heating Unit
2. Block Digestor/75 mL (Lachat Part No. 1800-000)

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QUIKCHEM METHOD 10-115-01-1-C

DETERMINATION OF TOTAL PHOSPHORUS BY FLOW INJECTION ANALYSIS COLORIMETRY

1. SCOPE AND APPLICATION

1.1. This method covers the determination of total phosphorus in Kjeldahl digests.

1.2. The method is based on reactions that are specific for the orthophosphate ion.

1.3. The applicable range is 0.01 to 5.0 mg P/L. The method detection limit (MDL) is 0.003 mg P/L. Approximately 60 samples per hour can be analyzed.

2. SUMMARY OF METHOD

2.1. Water samples are digested with sulfuric acid in a block digestor with a mercuric oxide catalyst, the samples' phosphorus is converted to the orthophosphate anion. Potassium sulfate is also added to raise the boiling temperature of the digestion and speed the conversion to orthophosphate. The digest is diluted with water.

2.2. The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

3. DEFINITIONS

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY FORTIFIED BLANK (LFB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY FORTIFIED SAMPLE MARIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.12. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) -- 26 West Martin Luther King Drive, Cincinnati, Ohio 45268, (513) 569-7453.

4. INTERFERENCES

4.1. Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silica concentration of approximately 4000 ppm would be required to produce a 1 ppm positive error in orthophosphate.

4.2. Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.

4.3 Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1. Mercury

5.3.2. Sulfuric acid

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special apparatus

6.4.1. Heating Unit

6.4.2. Block Digestor/75 mL (Lachat Part No. 1800-000)

6.4.3. 5 mL and 20 mL repipet dispensers

6.4.4. Vortex mixer

7. REAGENTS AND STANDARDS

7.1 PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with helium:

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140 kPa (20 lb/in^2) through a helium degassing tube (Lachat Part 50100). Bubble He vigorously through the solution for one minute.

Reagent 1. Stock Mercuric Sulfate Solution

To a **100 mL** volumetric flask, add **40 mL water**, **10 mL concentrated sulfuric acid** (H_2SO_4), and **8 g red mercuric oxide** (HgO). Stir with a magnetic stirrer at low heat until dissolved, dilute to the mark, and invert to mix. Store up to two months.

Reagent 2. Digestion solution

In a **1 L** volumetric flask, add approximately **700 mL water**, then add **200 mL concentrated sulfuric acid** (H_2SO_4). Add **133 g potassium sulfate** (K_2SO_4). Add **25 mL Stock Mercuric Sulfate Solution (Reagent 1)** and dilute to the mark. Mix with a magnetic stirrer and allow the solution to cool. Dilute to the mark after the solution has cooled. Prepare fresh monthly.

Reagent 3. Diluent 4.8% Sulfuric acid (For simulated standards)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **240 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **240 mL Reagent 2** (Digestion Solution). Invert to mix.

Reagent 4. Stock Ammonium Molybdate Solution

By Volume: In a **1 L** volumetric flask dissolve **40.0 g ammonium molybdate tetrahydrate** [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] in approximately **800 mL water.** Dilute to the mark and mix with a magnetic stirrer for at least four hours. Store up to two months in plastic and refrigerate.

By Weight: To a tared 1 L container add **40.0 g ammonium molybdate tetrahydrate** [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] and 983 g water. Mix with a magnetic stirrer for at least four hours. Store up to two months in plastic and refrigerate.

Reagent 5. Stock Antimony Potassium Tartrate Solution

By Volume: In a **1 L** volumetric flask, dissolve **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6\cdot 1/2H_2O$) in approximately **800 mL water.** Dilute to the mark and mix with a magnetic stirrer until dissolved. Store, up to two months, in a dark bottle and refrigerate.

By Weight: To a **1 L** dark, tared container add **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6\cdot 1/2H_2O$) and **995 g water.** Mix with a magnetic stirrer until dissolved. Store, up to two months, in a dark bottle and refrigerate.

Reagent 6. Molybdate Color Reagent

By Volume: To a **1 L** volumetric flask add about **500 mL water,** and then add **213 mL Ammonium Molybdate Solution (Reagent 4)** and **72 ml Antimony Potassium Tartrate Solution (Reagent 5).** Dilute to the mark and invert to mix. Degas with helium. Prepare weekly.

By Weight: To a tared **1 L** container add **715 g water**, and then **213 g Ammonium Molybdate Solution (Reagent 4)** and **72.0 g Antimony Potassium Tartrate Solution (Reagent 5).** Shake and degas with helium. Prepare weekly.

Reagent 7. Ascorbic Acid Reducing Solution Always Degas

By Volume: In a **1 L** volumetric flask dissolve **60.0 g ascorbic acid** in about **700 mL water.** Dilute to the mark and mix with a magnetic stirrer. Degas this solution with helium. Add **1.0 gm SDS** (sodium dodecyl sulfate Aldrich catalog no. 86,201-0). Mix with a magnetic stirrer. Prepare fresh every two days. Degas before add test

By Weight: To a tared **1 L** container, add **60.0 g ascorbic acid** and **975 g water.** Mix with a magnetic stirrer until dissolved. Degas this solution with helium. Add **1.0 gm SDS** (sodium dodecyl sulfate, Aldrich catalog no. 86,201-0). Mix with a magnetic stirrer. Prepare fresh every two days.

Reagent 8. Sodium Chloride/Sodium Hydroxide Solution

By Volume: In a **1 L** volumetric flask dissolve **160 g sodium chloride** and **20 g sodium hydroxide** in about **600 ml water.** Dilute to the mark and mix with a magnetic stirrer. Degas this solution with helium. Prepare weekly.

By Weight: To a tared **1 L** container, add **160 g sodium chloride** and **20 g sodium hydroxide,** and **916 g water.** Mix with a magnetic stirrer until dissolved. Degas this solution with helium. Prepare weekly.

Reagent 9. Sulfuric Acid/Potassium Sulfate solution (Carrier)

The sulfuric acid concentration in the carrier needs to match the digestion matrix. The table below shows the quantity of sulfuric acid required to prepare 1 L of the carrier so that it will match some of the common digestion matrices. This table assumes 5 mL of digestion solution is added to each sample. Prepare weekly.

Required Reagents

final volume of digestate (mL)	*sulfuric acid (%v/v)*	potassium sulfate (K_2SO_4)(g)	DI water (mL)	**By Weight** sulfuric acid (H_2SO_4) g	**By Volume** sulfuric acid (H_2SO_4) (mL)
20	*5.0*	31.7	938	92.0	50
21*	***4.8***	**31.7**	**940**	**88.3**	**48**
25	*4.0*	31.7	948	73.6	40

* used in Lachat Method Support Data

Reagent 10. Sodium Hydroxide - EDTA Rinse

Dissolve **65 g sodium hydroxide** (NaOH) and **6 g tetrasodium ethylenediamine tetraacetic acid** (Na_4EDTA) in 1.0 L or 1.0 kg water. Prepare fresh monthly.

7.2 PREPARATION OF STANDARDS

Non-Digested Standards

Standard 1. Stock Standard 250.0 mg P/L

In a **1 L** volumetric flask dissolve **1.099 g primary standard grade anhydrous potassium dihydrogen phosphate** (KH_2PO_4) that has been dried for two hours at 110°C in about **800 mL water**. Dilute to the mark with **DI water** and invert to mix.

Standard 2. Working Stock Standard Solution 5.00 mg P/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard** (Standard 1) to the mark with **Diluent** (Reagent 3). Invert to mix.

Note:

Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg P/L for a 5.00 mg P/L non-digested standard. *If the digestion volumes used by your laboratory require the digested equivalent adjustment, the digested equivalents will be the values entered into the data system (e.g. 5.25 rather than 5.00 mg P/L).*

$$\text{Labeled non-digested standard concentration} = \frac{\text{final digestate volume}}{\text{initial sample volume}} \times \text{standard concentration}$$

These standards will not be digested.

Working Standards (Prepare Daily)	A	B	C	D	E	F
Concentration (mg P/L)	5.00	2.00	1.00	0.50	0.10	0.00

By Volume

Volume (mL) of **Standard 2** diluted to 250 mL with Reagent 3	250	100	50	25	5	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with Reagent 3.	250.0	100	50	25	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.02	0

Digested Standards

Standards prepared in DI water must be carried through the digestion procedure (see section 11).

Standard 3. Working Stock Standard Solution 5.00 mg P/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard** (Standard 1) to the mark with **DI water**. Invert to mix.

By Weight: To a tared **250 mL** container add about **5.0 g Stock Standard** (Standard 1). Divide the actual weight of the solution added by **0.02** and make up to this resulting total weight with **DI water**. Invert to mix.

These standards will be digested

Working Standards (Prepare Daily)	**A**	**B**	**C**	**D**	**E**	**F**
Concentration mg P/L	5.00	2.00	1.00	0.50	0.10	0.00

By Volume

Volume (mL) of **Standard 3** diluted to 250 mL with DI water.	250	100	50	25	5	0

By Weight

Weight (g) of **Standard 3** diluted to final weight (~250 g) divide by **factor** below with DI water.	250.0	100	50	25	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.02	0

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2. Samples may be preserved by addition of a maximum of 2 mL of concentrated H_2SO_4 per liter to produce a pH less than 2 and stored at 4°C. Acid preserved samples have a holding time of 28 days. Sample digests should be run within one week of digestion.

9. QUALITY CONTROL (USEPA GUIDELINE)

9.1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2. INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every six months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = tS$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates]. S = standard deviation of the replicate analyses.

MDLs should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must used LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \overline{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \overline{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going

precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \text{ X100}$$

Where, R = percent recovery

C_s = fortified sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of at least three standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in Section 7.2).

10.2. Calibrate the instrument as described in Section 11.

10.3. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.4. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/-10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11. PROCEDURE

11.1. DIGESTION PROCEDURE

11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.

11.1.2. To a **20.0 mL** sample add **5 mL digestion solution** (Reagent 2) and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)

11.1.3. Add two to four Hengar granules to each tube. Hengar (Alundum) granules are effective for smooth boiling. They are available from Fisher Scientific, cat. no. S145-500. Teflon boiling chips may be used as an alternative.

11.1.4. Place tubes in the preheated block digestor for one hour at 160°C. Water from the sample should have boiled off before increasing the temperature in step 11.1.6.

11.1.5. Place the cold fingers on the top of the sample tube.

11.1.6. Continue to digest for 1.5 additional hours with the controller set to 380 °C. This time includes the ramp time for the block temperature to come up to 380

^{o}C. The typical ramp time is 50-60 minutes and 380 ^{o}C must be maintained for 30 minutes.

11.1.7. Remove the samples from the block and allow about 10 minutes to cool.

11.1.8. Add **20.0 mL** water to each tube and vortex to mix. The final volume should be **21 mL**.

11.1.9. Transfer the digestate into a clean labeled container. If samples are not run immediately they should be covered tightly.

11.2. SYSTEM START-UP PROCEDURE

11.2.1. Prepare reagent and standards as described in Section 7.

11.2.2. Set up manifold as shown in Section 17.1.

11.2.3. Input peak timing and integration window parameters as suggested in Section 17.

11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.2.5. Place standards in the sampler, and fill the sample tray. Input the information required by data system, such as concentration, replicates and QC scheme.

11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.2.7. After a stable baseline has been obtained, start the sampler and perform analysis.

11.3. SYSTEM NOTES

11.3.1. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

11.3.2. Allow 15 min for heating unit to warm up to 37°C.

11.3.3. If necessary, at end of run place the color reagent and ascorbic acid transmission lines into the NaOH - EDTA solution (Reagent 10). Pump this solution for approximately five minutes to remove any precipitated reaction products. Then place these lines in water and pump for an additional five minutes. Then pump dry all lines.

11.3.4. If sample concentrations are greater than the high standard, the digested sample should be diluted with **diluent** (Reagent 3). When the digital diluter is used, **Diluent** (Reagent 3) should be used. Do not dilute digested samples or standards with DI water.

11.3.5. To analyzing digestion sample, prepare a standard curve by plotting heights of digested standards against concentration values. Compute the concentrations by comparing the sample peak heights with the standard curve.

11.3.6. If simulation standards are used to prepare a standard curve, the response curve has to be obtained by comparing to a digested standard curve.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve.

12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed. Any sample whose computed value is less than 5% of its immediate predecessor must be rerun.

12.3. Report results in mg P/L.

13. METHOD PERFORMANCE

13.1. The method performance data are presented as method support data in Section 17.2. This data was generated according to Lachat Standard Operating Procedure J001WI, Lachat FIA Support Data Generation.

14. POLLUTION PREVENTION

14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's

Department of Government Regulations and Science Policy", 115 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. The USEPA requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

16.1. U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Revised March 1983, Method 365.4

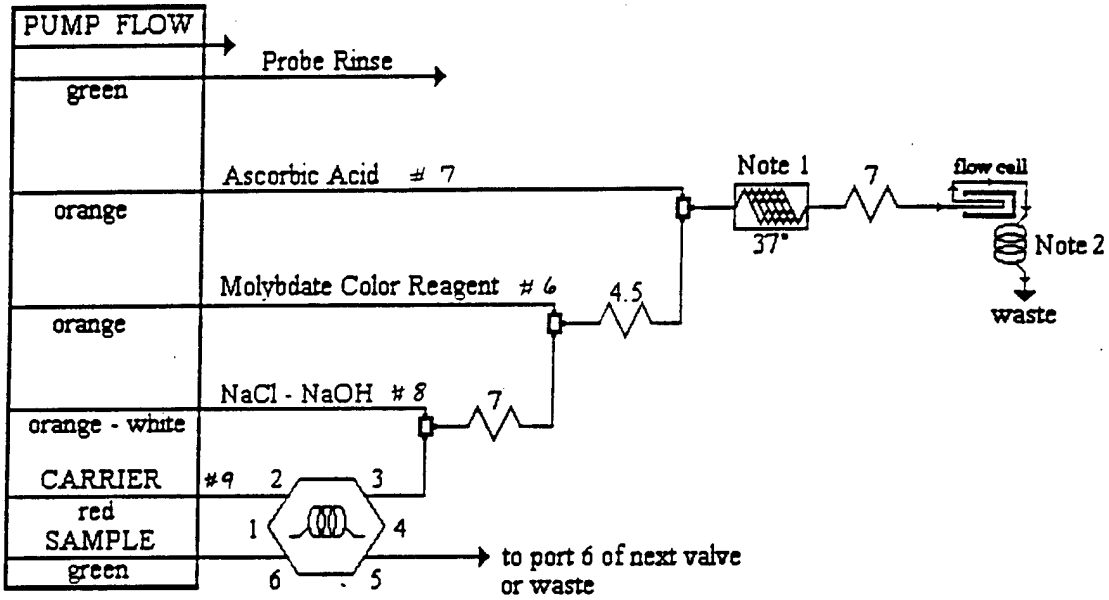
16.2. Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-78.

16.3. Lachat Instruments, QuikChem Method 10-115-01-1-C revised by Ann Zuehlke and Kevin Switala on 13 July 1992.

16.4. Guideline and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. TOTAL KJELDAHL PHOSPHORUS MANIFOLD DIAGRAM:



CARRIER is sulfuric acid/potassium sulfate solution (Reagent 9).

4.5 is **70.0** cm of tubing on a 4.5 cm coil support

7 is **135** cm of tubing on a 7 cm coil support

APPARATUS: Standard valve, flow cell, and detector head modules are used.

All manifold tubing is **0.8 mm (0.032 in.) i.d.** This is **5.2 uL/cm.**

Note 1: 175 cm of heated tubing.

Note 2: 2 meter restrictor coil, 0.52 mm (0.022 in.) i.d.

17.2 Data System Parameters for the QuikChem AE

Sample throughput:		60 samples/hour; 60 s/sample
Pump speed:		35
Cycle Period:		60 s
Inject to start of peak period:		28 s
Inject to end of peak period:		86 s
Presentation, Data Window		
Top Scale Response:		0.50 abs
Bottom Scale Response:		0.00 abs
Segment/Boundaries:	A:	5.00 mg P/L
	E:	0.10 mg P/L
	F:	0.00 mg P/L

Series 4000/System IV Settings: Gain = 330 x 1

17.3. QUIKCHEM AE SUPPORT DATA

17.3.1. Support Data for Non-Digested Standards

QuikChem AE Calibration Report for Calibration 93081002
Method: TKP low level
This calibration was first done or last modified on 08/10/93 at 02:38 pm
This report prepared on 08/10/93 at 05:14 pm

	Analyte	Units	Average Concentrations Known	Determined	% Residual	Baseline Corrected Average Absorbance
Standard A,	TKP	mg P/L	5.000	4.993	0.14	0.3844
Standard B,	TKP	mg P/L	2.000	2.014	-0.71	0.1551
Standard C,	TKP	mg P/L	1.000	1.009	-0.93	0.0777
Standard D,	TKP	mg P/L	0.500	0.498	0.40	0.0383
Standard E,	TKP	mg P/L	0.100	0.100	0.00	0.0067
Standard F,	TKP	mg P/L	0.000	0.000	***.**	-0.0002

This calibration may have been modified since it was first run!
If there is any question about this, see the calibration graph displayed in "Calib Graphs and Stats" menu selection, Results/Approval.

End of Calibration Report

Calibration Statistics Report 08/10/93 05:19 pm

Cal Ref: 93081002
Method: TKP

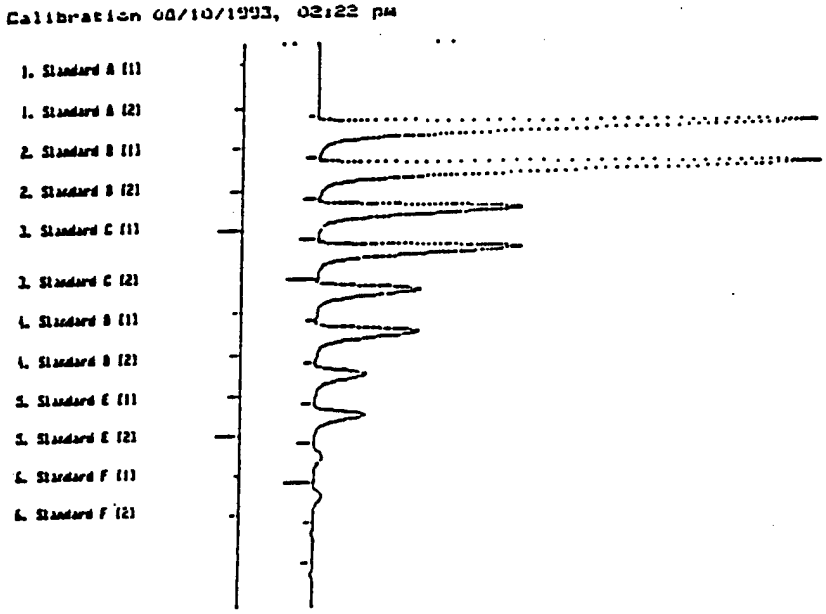
Channel: TKP

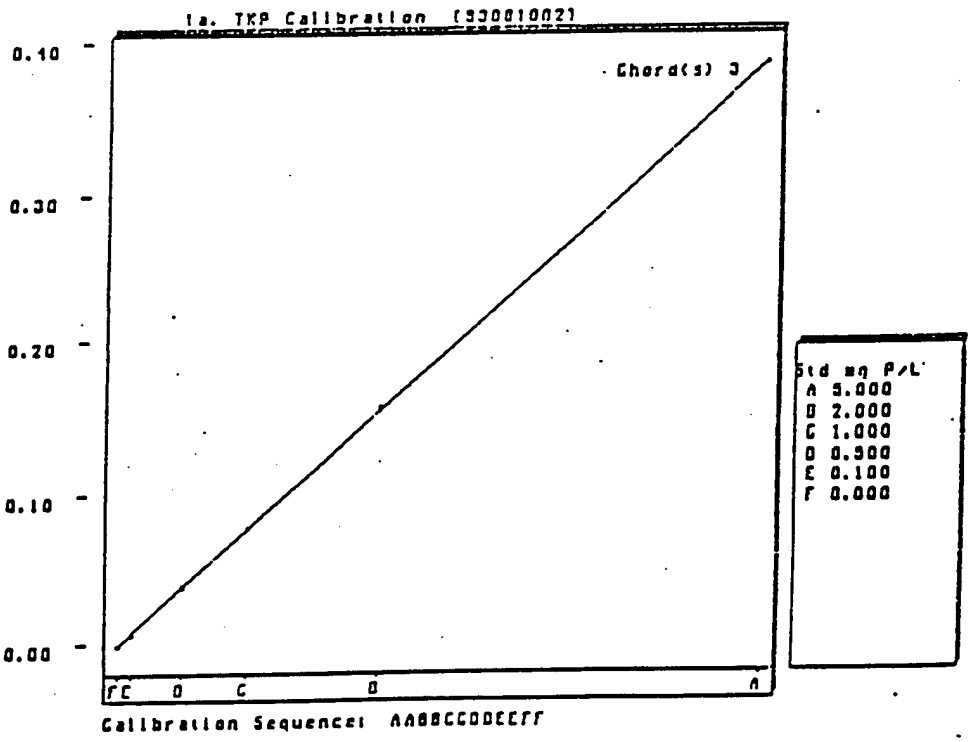
Correlation Coefficients

Seq	Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-E	0.9999	0.9225 ?	0.9999	1.0000	0.9994	0.9200 ?
2	E-F	0.9986	0.1017 ?	0.9979	0.9999	0.9927	0.6885 ?

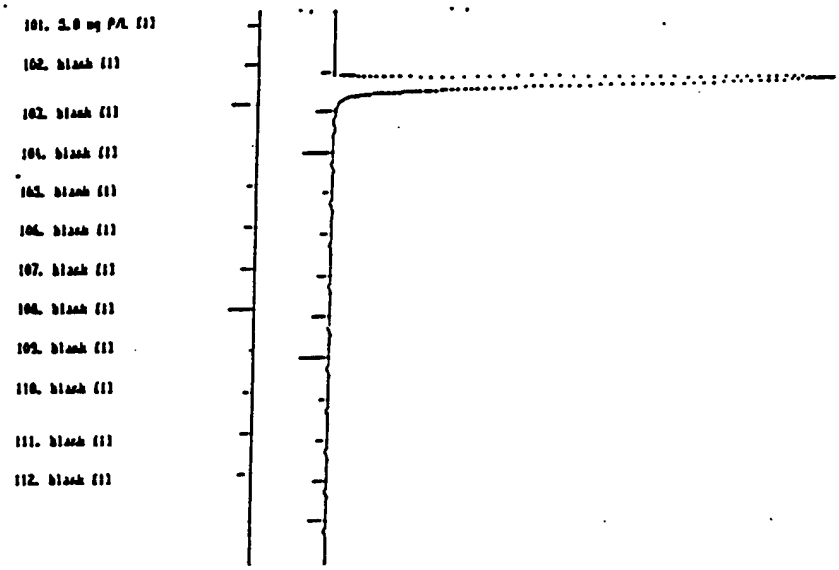
Percent Standard Deviation in Slope

1	A-E	0.3	7.6	0.3	0.2	0.9	10.4
2	E-F	2.6	210.2 ?	3.3	0.7	6.1	47.6


Calibration 08/10/1993, 02:22 pm
1. Standard A (1)
1. Standard A (2)
2. Standard B (1)
2. Standard B (2)
3. Standard C (1)
3. Standard C (2)
4. Standard D (1)
4. Standard D (2)
5. Standard E (1)
5. Standard E (2)
6. Standard F (1)
6. Standard F (2)

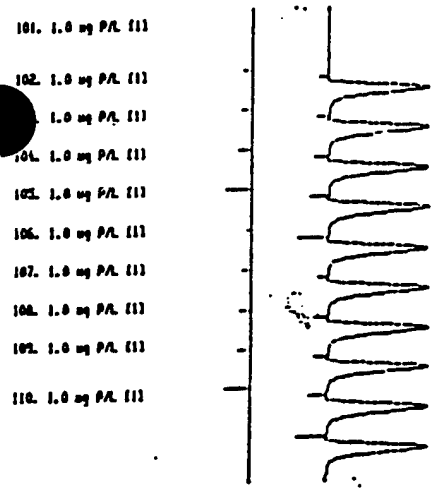

1a. TKP Calibration (930010021)
Chord(s) 3
0.10
0.30
0.20
0.10
0.00
F E D C B A
Std mg P/L
A 5.000
B 2.000
C 1.000
D 0.500
E 0.100
F 0.000
Calibration Sequence: AABBCCDDEEFF

Rack 1 (Ref: 93081002) 08/10/1993, 02:33 pm



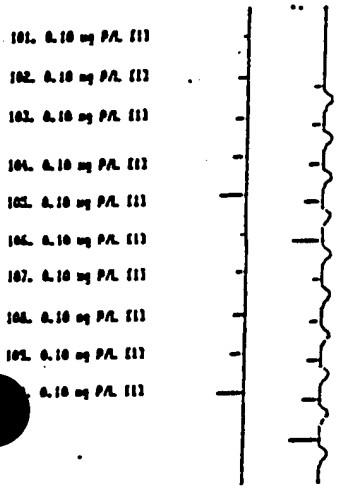
TKP(simulated) mg P/L	Carryover:
4.994	x = 0.000 mg P/L
-0.014	s = 0.001 mg P/L
0.000	t = 2.262
-0.001	95%CI = 0.000 +/- 0.001 mg P/L
-0.001	
0.001	EMDL:
0.001	s = 0.001 mg P/L
0.001	t = 4.65
-0.001	EMDL = 0.005 mg P/L
0.001	
0.001	
0.000	

Rack 1 (Ref: 93081003) 08/10/1993, 03:35 pm



mg P/L	Precision:
1.006	x = 1.010 mg P/L
1.003	s = 0.003 mg P/L
1.010	%RSD = 0.3%
1.011	
1.009	
1.009	
1.013	
1.012	
1.011	
1.011	

Rack 1 (Ref: 93081010) 08/10/1993, 04:29 pm



mg P/L	MDL:
0.103	x = 0.102 mg P/L
0.100	s = 0.001 mg P/L
0.103	t = 2.821
0.102	MDL = 0.003 mg P/L
0.102	
0.100	
0.101	
0.101	
0.102	
0.102	

Rack 1 (Ref: 93081004) 08/10/1993, 03:08 pm

	mg P/L
101. 0.05 mg P/L [1]	0.049
102. 0.05 mg P/L [1]	0.048
103. 0.05 mg P/L [1]	0.049
104. 0.05 mg P/L [1]	0.048
105. 0.05 mg P/L [1]	0.049
106. 0.05 mg P/L [1]	0.048
107. 0.05 mg P/L [1]	0.051
	0.049
108. 0.05 mg P/L [1]	0.049
109. 0.05 mg P/L [1]	0.048
110. 0.05 mg P/L [1]	0.048
111. 0.05 mg P/L [1]	0.048
112. 0.05 mg P/L [1]	0.049
113. 0.05 mg P/L [1]	0.048
114. 0.05 mg P/L [1]	0.047
115. 0.05 mg P/L [1]	0.046
116. 0.05 mg P/L [1]	0.049
117. 0.05 mg P/L [1]	0.048
118. 0.05 mg P/L [1]	0.049
119. 0.05 mg P/L [1]	0.048
120. 0.05 mg P/L [1]	0.048
121. 0.05 mg P/L [1]	

MDL:

x = 0.048 mg P/L

s = 0.001 mg P/L

t = 2.528

MDL = 0.003 mg P/L

17.3.2. Support Data for Digested Standards

DuoChem AE Calibration Report for Calibration 93081003
Method: TKP low level
This calibration was first done or last modified on 08/10/93 at 04:04 pm
This report prepared on 08/10/93 at 05:25 pm

	Analyte	Units	Average Concentrations Known	Determined	% Residual	Baseline Corrected Average Absorbance
Standard A,	TKP	mg P/L	5.000	4.992	0.15	0.3425
Standard B,	TKP	mg P/L	2.000	2.019	-0.93	0.1386
Standard C,	TKP	mg P/L	1.000	1.006	-0.57	0.0691
Standard D,	TKP	mg P/L	0.500	0.491	1.85	0.0338
Standard E,	TKP	mg P/L	0.100	0.100	0.00	0.0065
Standard F,	TKP	mg P/L	0.000	-0.003	***.**	0.0014

This calibration may have been modified since it was first run!
If there is any question about this, see the calibration graph displayed in "Calib Graphs and Stats" menu selection, Results/Approval.

End of Calibration Report

Calibration Statistics Report

Cal Ref: 93081003 08/10/93 05:30 pm
Method: TKP

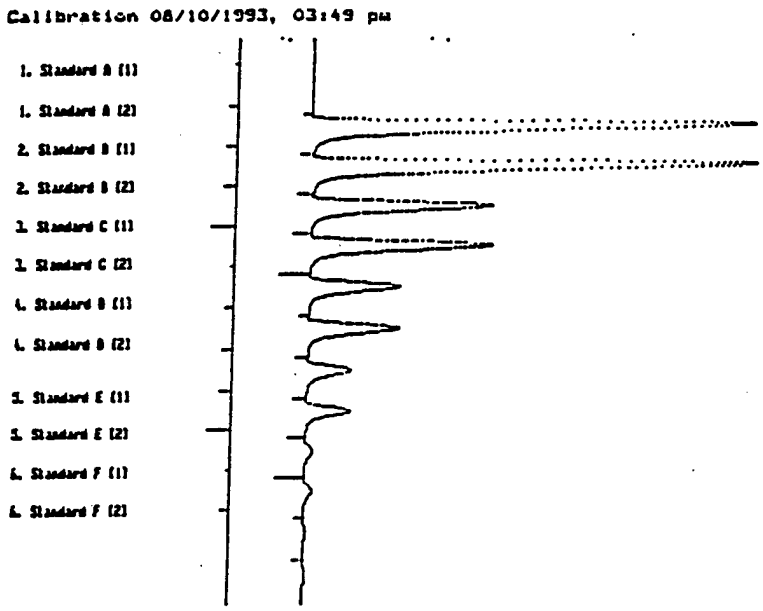
Channel: TKP

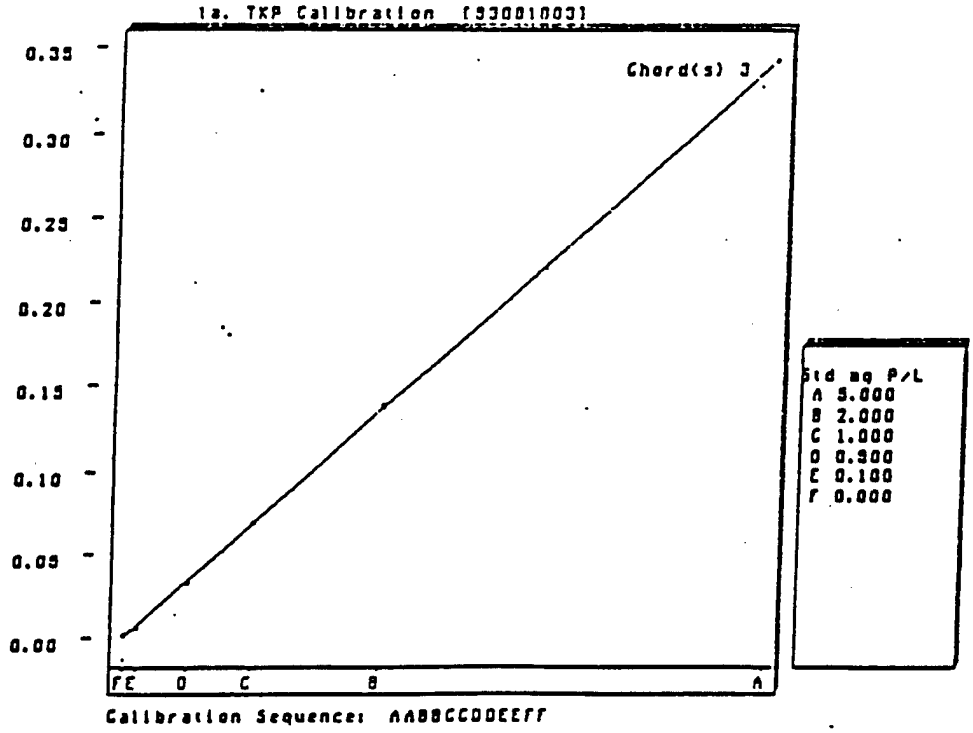
Correlation Coefficients

Seq	Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-E	0.9999	0.8393 ?	0.9990	1.0000	0.9996	0.9014 ?
2	E-F	0.9977	0.3948 ?	0.9902	0.9999	0.9891 ?	0.0312 ?

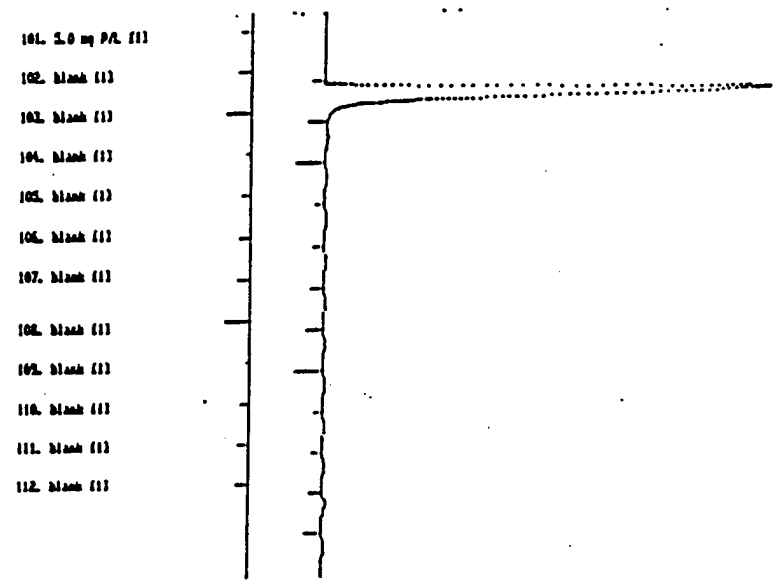
Percent Standard Deviation in Slope

1	A-E	0.3	11.0	0.5	0.2	0.7	11.7
2	E-F	3.4	87.5 ?	3.0	0.7	7.4	394.0 ?


Calibration 08/10/1993, 03:49 pm
1. Standard A (1)
1. Standard A (2)
2. Standard B (1)
2. Standard B (2)
3. Standard C (1)
3. Standard C (2)
4. Standard D (1)
4. Standard D (2)
5. Standard E (1)
5. Standard E (2)
6. Standard F (1)
6. Standard F (2)

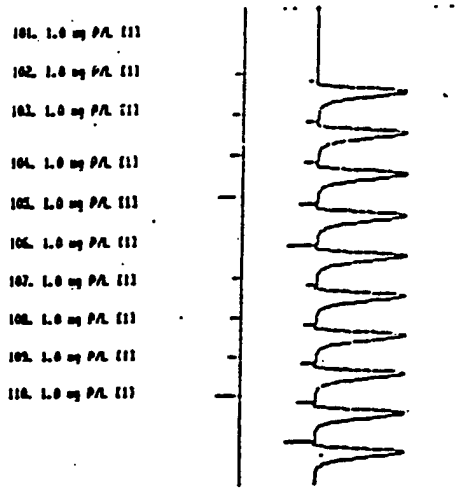

1a. TKP Calibration [93081003]
Chord(s) 3
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
FE D C B A
Std mg P/L
A 5.000
B 2.000
C 1.000
D 0.500
E 0.100
F 0.000
Calibration Sequence: AABBCCDDEEFF

Rack 1 (Ref: 92081006) 08/10/1993, 04:04 pm

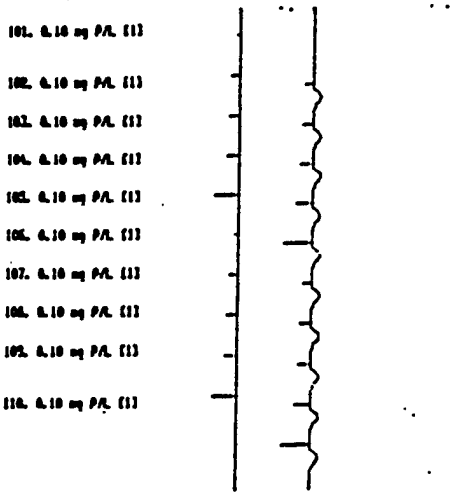


TKP(digested) mg P/L	Carryover:
4.986	x = -0.001 mg P/L
-0.020	s = 0.002 mg P/L
-0.001	t = 2.262
-0.001	95%CI = -0.001 +/- 0.001 mg P/L
-0.001	
0.000	EMDL:
-0.002	s = 0.002 mg P/L
-0.002	t = 4.65
-0.002	EMDL = 0.009 mg P/L
-0.004	
0.005	
0.001	

Rack 1 (Ref: 92081007) 08/10/1993, 04:19 pm

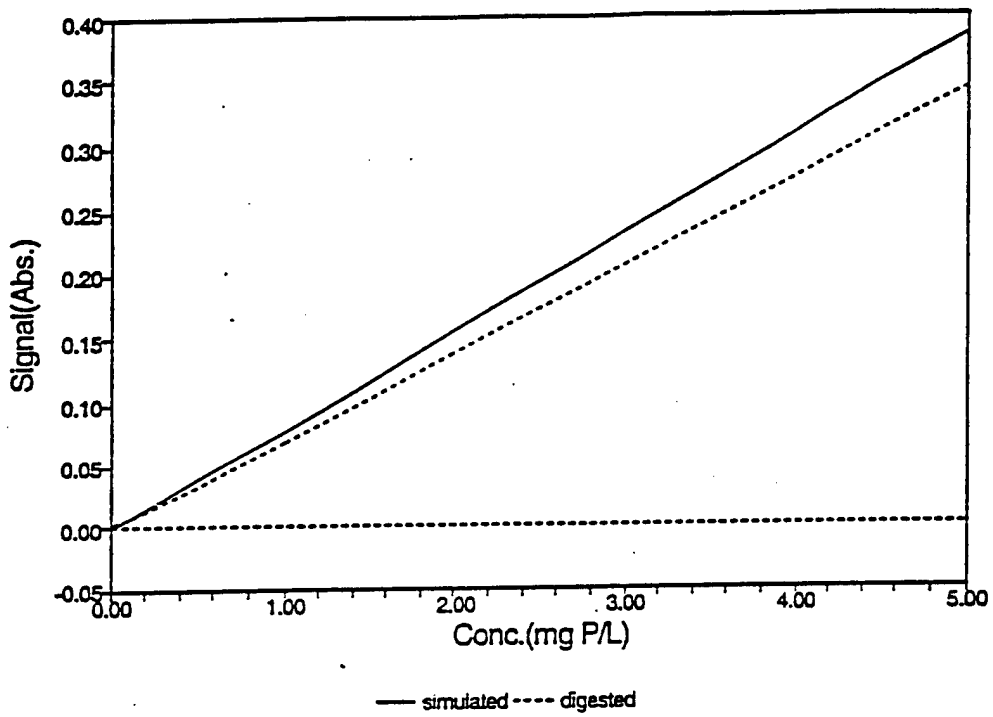


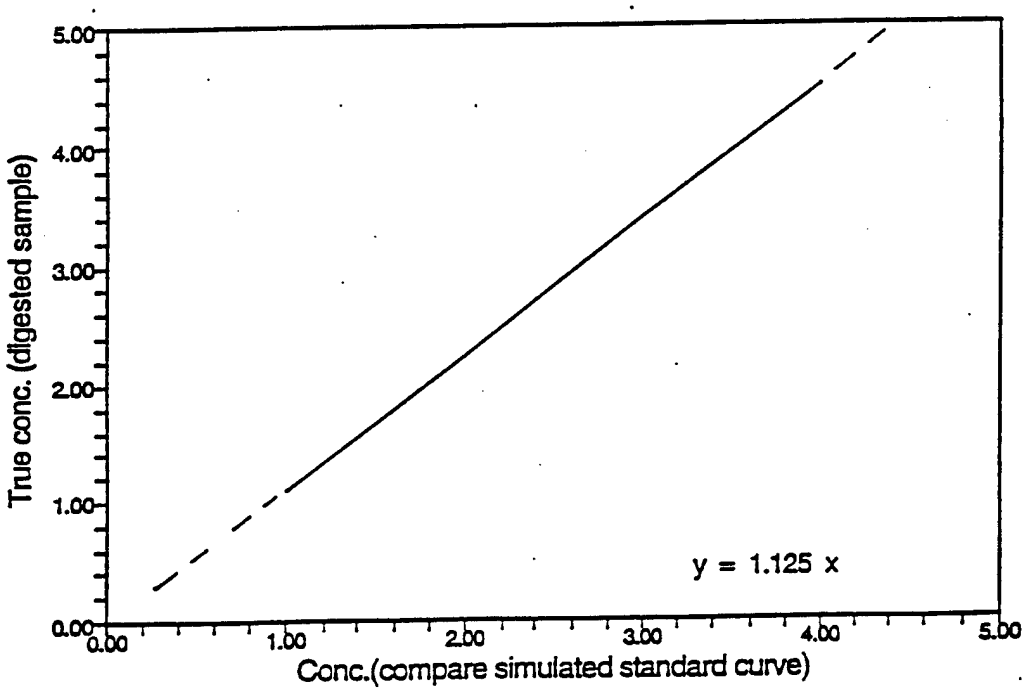
mg P/L	Precision:
1.002	x = 1.010 mg P/L
1.016	s = 0.004 mg P/L
1.017	%RSD = 0.4%
1.011	
1.008	
1.006	
1.008	
1.009	
1.010	
1.010	



mg P/L	MDL:
0.100	x = 0.100 mg P/L
0.098	s = 0.001 mg P/L
0.099	t = 2.821
0.099	MDL = 0.003 mg P/L
0.099	
0.099	Precision:
0.100	x = 0.100 mg P/L
0.101	s = 0.001 mg P/L
0.100	%RSD = 1.0%
0.100	

17.3.3. Response Curve





17.4. DATA PARAMETERS FOR THE QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Analyte data:

Peak Base Width: 48 s

% Width Tolerance: 100

Threshold: 14000

Inject to Peak Start: 23.4 s

Chemistry: Direct

Calibration Data:

Levels	1	2	3	4	5	6
Concentrations mg P/L	5.00	2.00	1.00	0.50	0.10	0.00

If digested equivalents are used (see page 9 for details) enter these values in the above concentrations table.

Calibration Fit Type: 1st Order Polynomial

Weighting Method: none

Sampler Timing:

Min. Probe in Wash Period: ~~20 s~~

Probe in Sample Period: ~~20 s~~

Valve Timing:

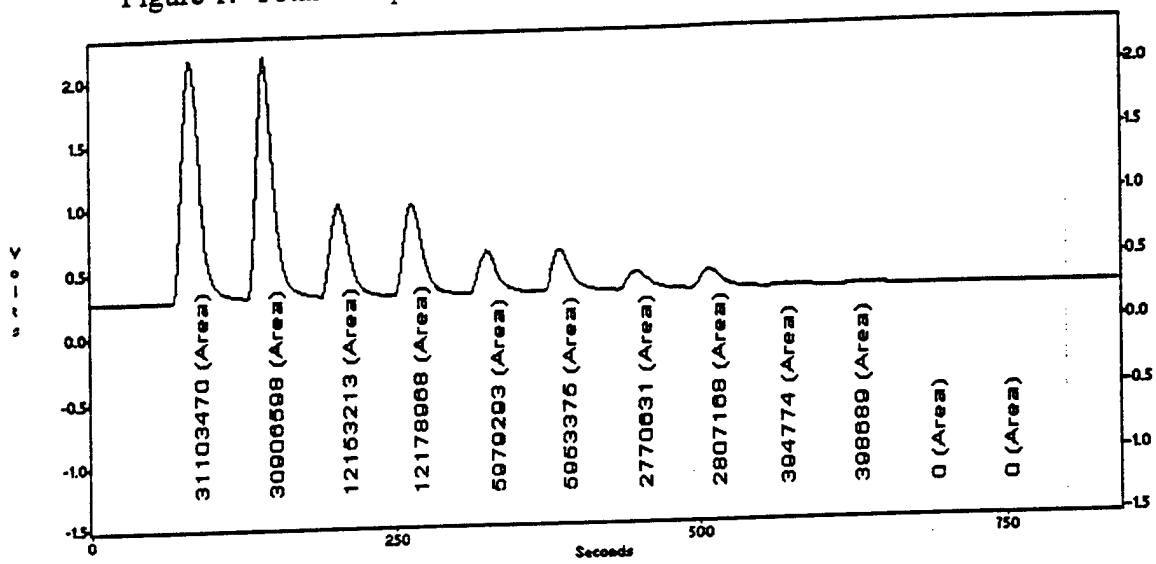
Load Period: ~~12~~ s

Inject Period: 25 s

Load Time: 0.0 s

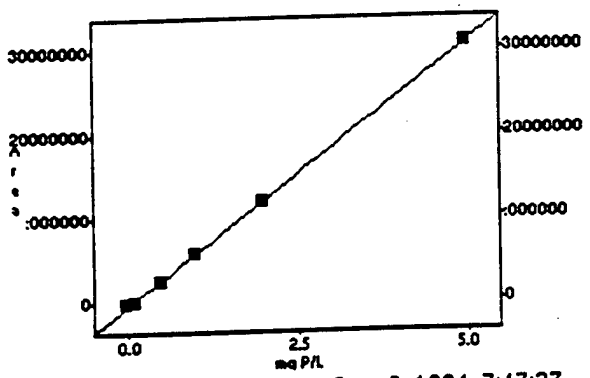
17.5. QUIKCHEM 8000 METHOD SUPPORT DATA

Figure 1. Total Phosphorus Calibration Peaks



Calibration Graph and Statistics

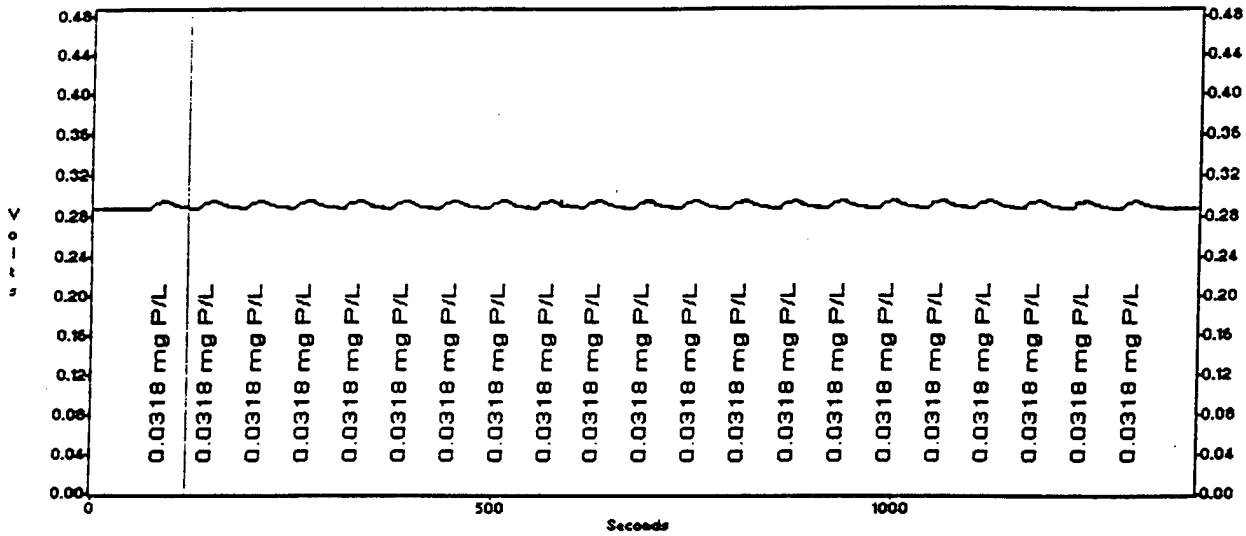
Level	Area	mg P/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	30906598	5	5.001	30906598	31103470	107831.2	0.3	-0.1
2	12178968	2	1.996	12178968	12153213	14106.6	0.1	0.4
3	5953375	1	0.989	5953375	5979293	14195.9	0.2	1.1
4	2827391	0.5	0.4855	2827391	2810339	9339.6	0.3	2.9
5	412386	0.1	0.0968	412386	413693	716.1	0.2	3.2
6	0	0	0	0	0.0	0.0	0.0	—



Scaling: None
Weighting: None
1st order poly
Conc = 1.610e-007area + 3.037e-002

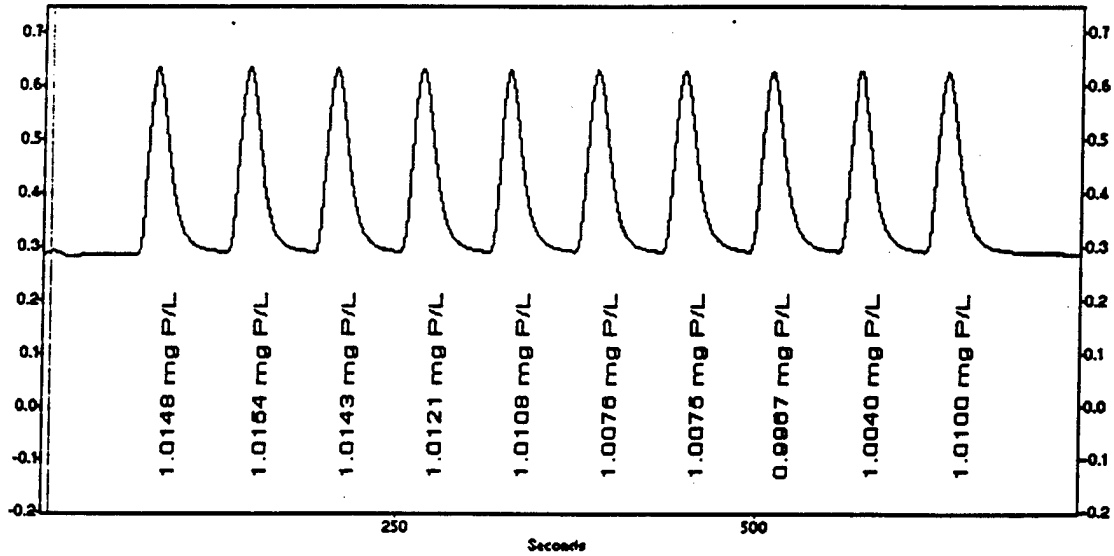
ACQ. TIME: Sep 8, 1994 7:47:37
DATA FILENAME: C:\OMNION\DATA\1011511C\090894C1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1011511C.MET

Figure 2. Method Detection Limit for Total Phosphorus



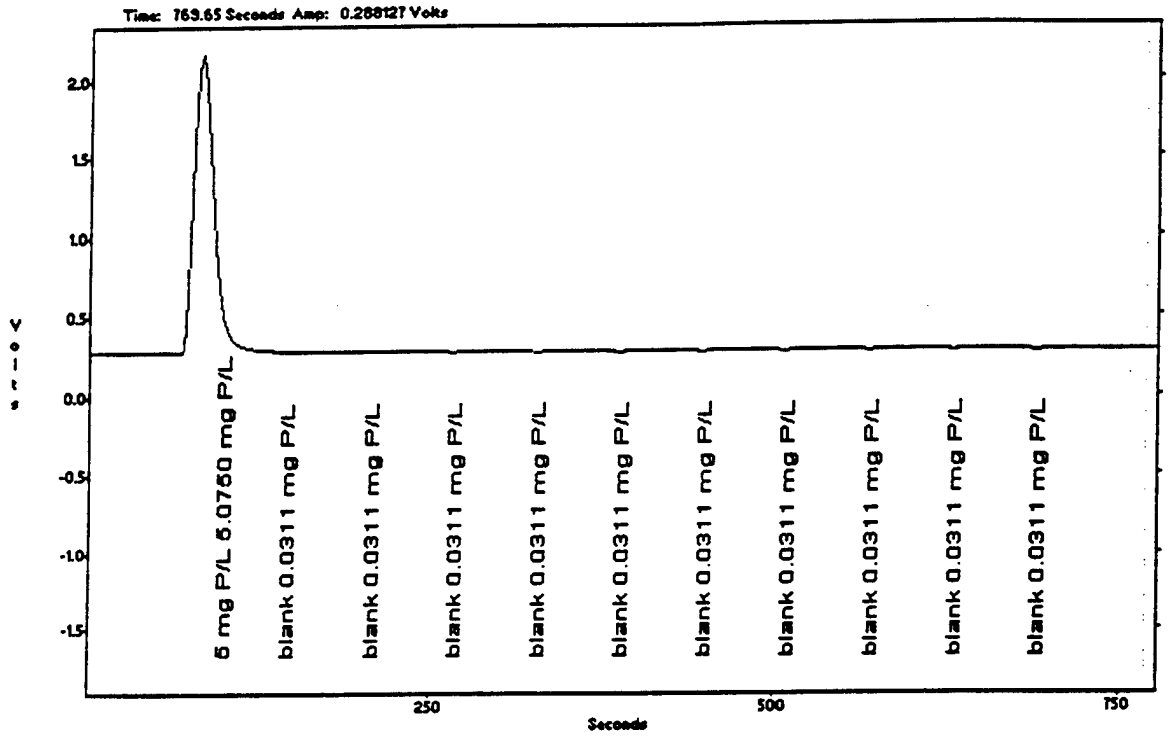
MDL = 0.0026 ug P/L
n = 20
ACQ. TIME: Sep 8, 1994 8:05:19
DATA FILENAME: C:\OMNION\DATA\1011511C\090894M1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1011511C.MET

Figure 3. Precision for Total Phosphorus



Precison = 0.59 % RSD
ACQ. TIME: Sep 8, 1994 10:02:34
DATA FILENAME: C:\OMNION\DATA\1011511C\090894P1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1011511C.MET

Figure 4. Carryover for Total Phosphorus



Carryover passed

ACQ. TIME:	Sep 8, 1994 10:15:41
DATA FILENAME:	C:\OMNION\DATA\1011511C\090894R1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1011511C.MET

Appendix C

Volatile Organic Compounds - Method 8260A

VOLATILE ORGANIC COMPOUNDS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS): CAPILLARY COLUMN TECHNIQUE

1.0 SCOPE AND APPLICATION

1.1 Method 8260 is used to determine volatile organic compounds in a variety of solid waste matrices. This method is applicable to nearly all types of samples, regardless of water content, including ground water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments. The following compounds can be determined by this method:

Analyte	CAS No.[b]	Appropriate Technique	
		Purge-and-Trap	Direct Injection
Acetone	67-64-1	pp	a
Acetonitrile	75-05-8	pp	a
Acrolein (Propenal)	107-02-8	pp	a
Acrylonitrile	107-13-1	pp	a
Allyl alcohol	107-18-6	ht	a
Allyl chloride	107-05-1	a	a
Benzene	71-43-2	a	a
Benzyl chloride	100-44-7	a	a
Bromoacetone	598-31-2	pp	a
Bromochloromethane (I.S.)	74-97-5	a	a
Bromodichloromethane	75-27-4	a	a
4-Bromofluorobenzene (surr.)	460-00-4	a	a
Bromoform	75-25-2	a	a
Bromomethane	74-83-9	a	a
n-Butanol	71-36-3	ht	a
2-Butanone (MEK)	78-93-3	pp	a
Carbon disulfide	75-15-0	pp	a
Carbon tetrachloride	56-23-5	a	a
Chloral hydrate	302-17-0	pp	a
Chlorobenzene	108-90-7	a	a
2-Chloro-1,3-butadiene	126-99-8	a	a
Chlorodibromomethane	124-48-1	a	a
Chloroethane	75-00-3	a	a
2-Chloroethanol	107-07-3	pp	a
bis-(2-Chloroethyl) sulfide	505-60-2	pp	a
2-Chloroethyl vinyl ether	110-75-8	a	a
Chloroform	67-66-3	a	a
Chloromethane	74-87-3	a	a
Chloroprene	126-99-8	a	pc

Analyte	CAS No.[b]	Appropriate Technique	
		Purge-and-Trap	Direct Injection
3-Chloropropene	107-05-1	a	a
3-Chloropropionitrile	542-76-7	i	pc
1,2-Dibromo-3-chloropropane	96-12-8	pp	a
1,2-Dibromoethane	106-93-4	a	a
Dibromomethane	74-95-3	a	a
1,2-Dichlorobenzene	95-50-1	a	a
1,3-Dichlorobenzene	541-73-1	a	a
1,4-Dichlorobenzene	106-46-7	a	a
cis-1,4-Dichloro-2-butene	1476-11-5	a	a
trans-1,4-Dichloro-2-butene	110-57-6	pp	a
Dichlorodifluoromethane	75-71-8	a	a
1,1-Dichloroethane	75-34-3	a	a
1,2-Dichloroethane	107-06-2	a	a
1,1-Dichloroethene	75-35-4	a	a
trans-1,2-Dichloroethene	156-60-5	a	a
1,2-Dichloropropane	78-87-5	a	a
1,3-Dichloro-2-propanol	96-23-1	pp	a
cis-1,3-Dichloropropene	10061-01-5	a	a
trans-1,3-Dichloropropene	10061-02-6	a	a
1,2,3,4-Diepoxybutane	1464-53-5	a	a
Diethyl ether	60-29-7	a	a
1,4-Difluorobenzene (I.S.)	540-36-3	a	a
1,4-Dioxane	123-91-1	pp	a
Epichlorohydrin	106-89-8	i	a
Ethanol	64-17-5	i	a
Ethyl acetate	141-78-6	i	a
Ethylbenzene	100-41-4	a	a
Ethylene oxide	75-21-8	pp	a
Ethyl methacrylate	97-63-2	a	a
Hexachlorobutadiene	87-68-3	a	a
Hexachloroethane	67-72-1	i	a
2-Hexanone	591-78-6	pp	a
2-Hydroxypropionitrile	78-97-7	i	pc
Iodomethane	74-88-4	a	a
Isobutyl alcohol	78-83-1	pp	a
Isopropylbenzene	98-82-8	a	a
Malononitrile	109-77-3	pp	a
Methacrylonitrile	126-98-7	pp	a
Methanol	67-56-1	i	a
Methylene chloride (DCM)	75-09-2	a	a
Methyl methacrylate	80-62-6	a	a
4-Methyl-2-pentanone (MIBK)	108-10-1	pp	a
Naphthalene	91-20-3	a	a
Nitrobenzene	98-95-3	a	a
2-Nitropropane	79-46-9	a	a

Analyte	Appropriate Technique		
	CAS No.[b]	Purge-and-Trap	Direct Injection
Pentachloroethane	76-01-7	i	a
2-Picoline	109-06-8	pp	a
Propargyl alcohol	107-19-7	pp	a
ß-Propiolactone	57-57-8	pp	a
Propionitrile (ethyl cyanide)	107-12-0	ht	pc
n-Propylamine	107-10-8	a	a
Pyridine	110-86-1	i	a
Styrene	100-42-5	a	a
1,1,1,2-Tetrachloroethane	630-20-6	a	a
1,1,2,2-Tetrachloroethane	79-34-5	a	a
Tetrachloroethene	127-18-4	a	a
Toluene	108-88-3	a	a
1,2,4-Trichlorobenzene	120-82-1	a	a
1,1,1-Trichloroethane	71-55-6	a	a
1,1,2-Trichloroethane	79-00-5	a	a
Trichloroethene	79-01-6	a	a
Trichlorofluoromethane	75-69-4	a	a
1,2,3-Trichloropropane	96-18-4	a	a
Vinyl acetate	108-05-4	a	a
Vinyl chloride	75-01-4	a	a
o-Xylene	95-47-6	a	a
m-Xylene	108-38-3	a	a
p-Xylene	106-42-3	a	a

a Adequate response by this technique.
b Chemical Abstract Services Registry Number.
ht Method analyte only when purged at 80°C
i Inappropriate technique for this analyte.
pc Poor chromatographic behavior.
pp Poor purging efficiency resulting in high EQLs.
surr Surrogate
I.S. Internal Standard

1.2 Method 8260 can be used to quantitate most volatile organic compounds that have boiling points below 200°C and that are insoluble or slightly soluble in water. Volatile water-soluble compounds can be included in this analytical technique. However, for the more soluble compounds, quantitation limits are approximately ten times higher because of poor purging efficiency. Such compounds include low-molecular-weight halogenated hydrocarbons, aromatics, ketones, nitriles, acetates, acrylates, ethers, and sulfides. See Tables 1 and 2 for lists of analytes and retention times that have been evaluated on a purge-

and-trap GC/MS system. Also, the method detection limits for 25 mL sample volumes are presented. The following analytes are also amenable to analysis by Method 8260:

Bromobenzene	1-Chlorohexane
n-Butylbenzene	2-Chlorotoluene
sec-Butylbenzene	4-Chlorotoluene
tert-Butylbenzene	Crotonaldehyde
Chloroacetonitrile	Dibromofluoromethane
1-Chlorobutane	cis-1,2-Dichloroethene
1,3-Dichloropropane	Methyl-t-butyl ether
2,2-Dichloropropane	Pentafluorobenzene
1,1-Dichloropropene	n-Propylbenzene
Fluorobenzene	1,2,3-Trichlorobenzene
p-Isopropyltoluene	1,2,4-Trimethylbenzene
Methyl acrylate	1,3,5-Trimethylbenzene

1.3 The estimated quantitation limit (EQL) of Method 8260 for an individual compound is somewhat instrument dependent. Using standard quadrupole instrumentation, limits should be approximately 5 µg/kg (wet weight) for soil/sediment samples, 0.5 mg/kg (wet weight) for wastes, and 5 µg/L for ground water (see Table 3). Somewhat lower limits may be achieved using an ion trap mass spectrometer or other instrumentation of improved design. No matter which instrument is used, EQLs will be proportionately higher for sample extracts and samples that require dilution or reduced sample size to avoid saturation of the detector.

1.4 Method 8260 is based upon a purge-and-trap, gas chromatographic/mass spectrometric (GC/MS) procedure. This method is restricted to use by, or under the supervision of, analysts experienced in the use of purge-and-trap systems and gas chromatograph/mass spectrometers, and skilled in the interpretation of mass spectra and their use as a quantitative tool.

1.5 An additional method for sample introduction is direct injection. This technique has been tested for the analysis of waste oil diluted with hexadecane 1:1 (vol/vol) and may have application for the analysis of some alcohols and aldehydes in aqueous samples.

2.0 SUMMARY OF METHOD

2.1 The volatile compounds are introduced into the gas chromatograph by the purge-and-trap method or by direct injection (in limited applications). Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated and backflushed with helium to desorb trapped sample components. The analytes are desorbed directly to a large bore capillary or cryofocussed on a capillary precolumn before being flash evaporated to a narrow bore capillary for analysis. The column is temperature programmed to separate the analytes which are then detected with a mass spectrometer (MS) interfaced to the gas chromatograph. Wide bore capillary columns require a jet separator, whereas narrow bore capillary columns can be directly interfaced to the ion source.

2.2 If the above sample introduction techniques are not applicable, a portion of the sample is dispersed in solvent to dissolve the volatile organic constituents. A portion of the solution is combined with organic-free reagent water in the purge chamber. It is then analyzed by purge-and-trap GC/MS following the normal water method.

2.3 Analytes eluted from the capillary column are introduced into the mass spectrometer via a jet separator or a direct connection. Identification of target analytes is accomplished by comparing their mass spectra with the electron impact (or electron impact-like) spectra of authentic standards. Quantitation is accomplished by comparing the response of a major (quantitation) ion relative to an internal standard with a five-point calibration curve.

2.4 The method includes specific calibration and quality control steps that replace the general requirements in Method 8000.

3.0 INTERFERENCES

3.1 Major contaminant sources are volatile materials in the laboratory and impurities in the inert purging gas and in the sorbent trap. The use of non-polytetrafluoroethylene (PTFE) thread sealants, plastic tubing, or flow controllers with rubber components should be avoided since such materials out-gas organic compounds which will be concentrated in the trap during the purge operation. Analyses of calibration and reagent blanks provide information about the presence of contaminants. When potential interfering peaks are noted in blanks, the analyst should change the purge gas source and regenerate the molecular sieve purge gas filter (Figure 1). Subtracting blank values from sample results is not permitted. If reporting values not corrected for blanks result in what the laboratory feels is a false positive for a sample, this should be fully explained in text accompanying the uncorrected data.

3.2 Interfering contamination may occur when a sample containing low concentrations of volatile organic compounds is analyzed immediately after a sample containing high concentrations of volatile organic compounds. The preventive technique is rinsing of the purging apparatus and sample syringes with two portions of organic-free reagent water between samples. After analysis of a sample containing high concentrations of volatile organic compounds, one or more calibration blanks should be analyzed to check for cross contamination. For samples containing large amounts of water soluble materials, suspended solids, high boiling compounds or high concentrations of compounds being determined, it may be necessary to wash the purging device with a soap solution, rinse it with organic-free reagent water, and then dry the purging device in an oven at 105°C. In extreme situations, the whole purge and trap device may require dismantling and cleaning. Screening of the samples prior to purge and trap GC/MS analysis is highly recommended to prevent contamination of the system. This is especially true for soil and waste samples. Screening may be accomplished with an automated headspace technique or by Method 3820 (Hexadecane Extraction and Screening of Purgeable Organics).

3.2.1 The low purging efficiency of many analytes from a 25 mL sample often results in significant concentrations remaining in the sample purge vessel after analysis. After removal of the analyzed sample aliquot

and three rinses of the purge vessel with analyte free water, it is required that the empty vessel be subjected to a heated purge cycle prior to the analysis of another sample in the same purge vessel to reduce sample to sample carryover.

3.3 Special precautions must be taken to analyze for methylene chloride. The analytical and sample storage area should be isolated from all atmospheric sources of methylene chloride. Otherwise random background levels will result. Since methylene chloride will permeate through PTFE tubing, all gas chromatography carrier gas lines and purge gas plumbing should be constructed from stainless steel or copper tubing. Laboratory clothing worn by the analyst should be clean since clothing previously exposed to methylene chloride fumes during liquid/liquid extraction procedures can contribute to sample contamination.

3.4 Samples can be contaminated by diffusion of volatile organics (particularly methylene chloride and fluorocarbons) through the septum seal into the sample during shipment and storage. A trip blank prepared from organic-free reagent water and carried through the sampling and handling protocol can serve as a check on such contamination.

3.5 Use of sensitive mass spectrometers to achieve lower detection level will increase the potential to detect laboratory contaminants as interferences.

3.6 Direct injection - Some contamination may be eliminated by baking out the column between analyses. Changing the injector liner will reduce the potential for cross-contamination. A portion of the analytical column may need to be removed in the case of extreme contamination. Use of direct injection will result in the need for more frequent instrument maintenance.

3.7 If hexadecane is added to samples or petroleum samples are analyzed, some chromatographic peaks will elute after the target analytes. The oven temperature program must include a post-analysis bake out period to ensure that semi-volatile hydrocarbons are volatilized.

4.0 APPARATUS AND MATERIALS

4.1 Purge-and-trap device - aqueous samples, described in Method 5030.

4.2 Purge-and-trap device - solid samples, described in Method 5030.

4.3 Injection port liners (HP catalogue #18740-80200, or equivalent) are modified for direct injection analysis by placing a 1-cm plug of pyrex wool approximately 50-60 mm down the length of the injection port towards the oven. An 0.53 mm ID column is mounted 1 cm into the liner from the oven side of the injection port, according to manufacturer's specifications.

Modified Injector

4.4 Gas chromatography/mass spectrometer/data system

4.4.1 Gas chromatograph - An analytical system complete with a temperature-programmable gas chromatograph suitable for splitless injection or interface to purge-and-trap apparatus. The system includes all required accessories, including syringes, analytical columns, and gases. The GC should be equipped with variable constant differential flow controllers so that the column flow rate will remain constant throughout desorption and temperature program operation. For some column configurations, the column oven must be cooled to < 30°C, therefore, a subambient oven controller may be required. The capillary column should be directly coupled to the source.

4.4.1.1 Capillary precolumn interface when using cryogenic cooling - This device interfaces the purge and trap concentrator to the capillary gas chromatograph. The interface condenses the desorbed sample components and focuses them into a narrow band on an uncoated fused silica capillary precolumn. When the interface is flash heated, the sample is transferred to the analytical capillary column.

4.4.1.1.1 During the cryofocussing step, the temperature of the fused silica in the interface is maintained at -150°C under a stream of liquid nitrogen. After the desorption period, the interface must be capable of rapid heating to 250°C in 15 seconds or less to complete the transfer of analytes.

4.4.2 Gas chromatographic columns

4.4.2.1 Column 1 - 60 m x 0.75 mm ID capillary column coated with VOCOL (Supelco), 1.5 µm film thickness, or equivalent.

4.4.2.2 Column 2 - 30 - 75 m x 0.53 mm ID capillary column coated with DB-624 (J&W Scientific), Rt_x-502.2 (RESTEK), or VOCOL (Supelco), 3 µm film thickness, or equivalent.

4.4.2.3 Column 3 - 30 m x 0.25 - 0.32 mm ID capillary column coated with 95% dimethyl - 5% diphenyl polysiloxane (DB-5, Rt_x-5, SPB-5, or equivalent), 1 µm film thickness.

4.4.2.4 Column 4 - 60 m x 0.32 mm ID capillary column coated with DB-624 (J&W Scientific), 1.8 µm film thickness, or equivalent.

4.4.3 Mass spectrometer - Capable of scanning from 35 to 300 amu every 2 sec or less, using 70 volts (nominal) electron energy in the electron impact ionization mode. The mass spectrometer must be capable of producing a mass spectrum for p-Bromofluorobenzene (BFB) which meets all of the criteria in Table 4 when 5-50 ng of the GC/MS tuning standard (BFB) is injected through the GC. To ensure sufficient precision of mass spectral data, the desirable MS scan rate allows acquisition of at least five spectra while a sample component elutes from the GC.

4.4.3.1 The ion trap mass spectrometer may be used if it is capable of axial modulation to reduce ion-molecule reactions and can produce electron impact-like spectra that match those in the EPA/NIST Library. In an ion trap mass spectrometer, because ion-molecule reactions with water and methanol may produce interferences that coelute with chloromethane and chloroethane, the base peak for both of these analytes will be at m/z 49. This ion should be used as the quantitation ion in this case. The mass spectrometer must be capable of producing a mass spectrum for BFB which meets all of the criteria in Table 3 when 5 or 50 ng are introduced.

4.4.4 GC/MS interface - Two alternatives are used to interface the GC to the mass spectrometer.

4.4.4.1 Direct coupling by inserting the column into the mass spectrometer is generally used for 0.25-0.32 mm id columns.

4.4.4.2 A separator including an all-glass transfer line and glass enrichment device or split interface is used with an 0.53 mm column.

4.4.4.3 Any enrichment device or transfer line can be used if all of the performance specifications described in Sec. 8 (including acceptable calibration at 50 ng or less) can be achieved. GC-to-MS interfaces constructed entirely of glass or of glass-lined materials are recommended. Glass can be deactivated by silanizing with dichlorodimethylsilane.

4.4.5 Data system - A computer system that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program must be interfaced to the mass spectrometer. The computer must have software that allows searching any GC/MS data file for ions of a specified mass and plotting such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. The most recent version of the EPA/NIST Mass Spectral Library should also be available.

4.5 Microsyringes - 10, 25, 100, 250, 500, and 1,000 µL.

4.6 Syringe valve - Two-way, with Luer ends (three each), if applicable to the purging device.

4.7 Syringes - 5, 10, or 25 mL, gas-tight with shutoff valve.

4.8 Balance - Analytical, 0.0001 g, and top-loading, 0.1 g.

4.9 Glass scintillation vials - 20 mL, with Teflon lined screw-caps or glass culture tubes with Teflon lined screw-caps.

4.10 Vials - 2 mL, for GC autosampler.

4.11 Disposable pipets - Pasteur.

4.12 Volumetric flasks, Class A - 10 mL and 100 mL, with ground-glass stoppers.

4.13 Spatula - Stainless steel.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all inorganic reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Methanol, CH_3OH - Pesticide quality or equivalent, demonstrated to be free of analytes. Store apart from other solvents.

5.4 Reagent Hexadecane - Reagent hexadecane is defined as hexadecane in which interference is not observed at the method detection limit of compounds of interest.

5.4.1 In order to demonstrate that all interfering volatiles have been removed from the hexadecane, a direct injection blank must be analyzed.

5.5 Polyethylene glycol, $H(OCH_2CH_2)_nOH$ - Free of interferences at the detection limit of the target analytes.

5.6 Hydrochloric acid (1:1 v/v), HCl - Carefully add a measured volume of concentrated HCl to an equal volume of organic-free reagent water.

5.7 Stock solutions - Stock solutions may be prepared from pure standard materials or purchased as certified solutions. Prepare stock standard solutions in methanol, using assayed liquids or gases, as appropriate.

5.7.1 Place about 9.8 mL of methanol in a 10 mL tared ground-glass-stoppered volumetric flask. Allow the flask to stand, unstoppered, for about 10 minutes or until all alcohol-wetted surfaces have dried. Weigh the flask to the nearest 0.0001 g.

5.7.2 Add the assayed reference material, as described below.

5.7.2.1 Liquids - Using a 100 μL syringe, immediately add two or more drops of assayed reference material to the flask; then reweigh. The liquid must fall directly into the alcohol without contacting the neck of the flask.

5.7.2.2 Gases - To prepare standards for any compounds that boil below 30°C (e.g. bromomethane, chloroethane, chloromethane, or vinyl chloride), fill a 5 mL valved gas-tight syringe with the reference standard to the 5.0 mL mark. Lower the needle to 5 mm above the methanol meniscus. Slowly introduce the reference standard above the surface of the liquid. The heavy gas will rapidly dissolve in the methanol. Standards may also be prepared by using a lecture bottle equipped with a Hamilton Lecture Bottle Septum (#86600). Attach Teflon tubing to the side arm relief valve and direct a gentle stream of gas into the methanol meniscus.

5.7.3 Reweigh, dilute to volume, stopper, and then mix by inverting the flask several times. Calculate the concentration in milligrams per liter (mg/L) from the net gain in weight. When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

5.7.4 Transfer the stock standard solution into a bottle with a Teflon lined screw-cap. Store, with minimal headspace, at -10°C to -20°C and protect from light.

5.7.5 Prepare fresh standards for gases weekly or sooner if comparison with check standards indicates a problem. Reactive compounds such as 2-chloroethyl vinyl ether and styrene may need to be prepared more frequently. All other standards must be replaced after six months, or sooner if comparison with check standards indicates a problem. Both gas and liquid standards must be monitored closely by comparison to the initial calibration curve and by comparison to QC check standards. It may be necessary to replace the standards more frequently if either check exceeds a 20% drift.

5.7.6 Optionally calibration using a certified gaseous mixture can be accomplished daily utilizing commercially available gaseous analyte mixture of bromomethane, chloromethane, chloroethane, vinyl chloride, dichlorodifluoromethane and trichlorofluoromethane in nitrogen. These mixtures of documented quality are stable for as long as six months without refrigeration. (VOA-CYL III, RESTEK Corporation, Cat. #20194 or equivalent).

5.7.6.1 Preparation of Calibration Standards From a Gas Mixture

5.7.6.1.1 Before removing the cylinder shipping cap, be sure the valve is completely closed (turn clockwise). The contents are under pressure and should be used in a well-ventilated area.

5.7.6.1.2 Wrap the pipe thread end of the Luer fitting with Teflon tape. Remove the shipping cap from the cylinder and replace it with the Luer fitting.

5.7.6.1.3 Transfer half the working standard containing other analytes, internal standards, and surrogates to the purge apparatus.

5.7.6.1.4 Purge the Luer fitting and stem on the gas cylinder prior to sample removal using the following sequence:

a) Connect either the 100 µL or 500 µL Luer syringe to the inlet fitting of the cylinder.

b) Make sure the on/off valve on the syringe is in the open position.

c) Slowly open the valve on the cylinder and withdraw a full syringe volume.

d) **Be sure to close the valve on the cylinder before you withdraw the syringe from the Luer fitting.**

e) Expel the gas from the syringe into a well-ventilated area.

f) Repeat steps a through e one more time to fully purge the fitting.

5.7.6.1.5 Once the fitting and stem have been purged, quickly withdraw the volume of gas you require using steps 5.6.6.1.4(a) through (d). Be sure to close the valve on the cylinder and syringe before you withdraw the syringe from the Luer fitting.

5.7.6.1.6 Open the syringe on/off valve for 5 seconds to reduce the syringe pressure to atmospheric pressure. The pressure in the cylinder is -30 psi.

5.7.6.1.7 The gas mixture should be quickly transferred into the reagent water through the female Luer fitting located above the purging vessel.

NOTE: Make sure the arrow on the 4-way valve is pointing toward the female Luer fitting when transferring the sample from the syringe. **Be sure to switch the 4-way valve back to the closed position before removing the syringe from the Luer fitting.**

5.7.6.1.8 Transfer the remaining half of the working standard into the purging vessel. This procedure insures that the total volume of gas mix is flushed into the purging vessel, with none remaining in the valve or lines.

5.7.6.1.9 Concentration of each compound in the cylinder is typically 0.0025 µg/µL.

5.7.6.1.10 The following are the recommended gas volumes spiked into 5 mL of water to produce a typical 5-point calibration:

Gas Volume	Calibration Concentration
40 µL	20 µg/L
100 µL	50 µg/L
200 µL	100 µg/L
300 µL	150 µg/L
400 µL	200 µg/L

5.7.6.1.11 The following are the recommended gas volumes spiked into 25 mL of water to produce a typical 5-point calibration:

Gas Volume	Calibration Concentration
10 µL	1 µg/L
20 µL	2 µg/L
50 µL	5 µg/L
100 µL	10 µg/L
250 µL	25 µg/L

5.8 Secondary dilution standards - Using stock standard solutions, prepare in methanol, secondary dilution standards containing the compounds of interest, either singly or mixed together. Secondary dilution standards must be stored with minimal headspace and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Store in a vial with no headspace for one week only.

5.9 Surrogate standards - The surrogates recommended are toluene-d_8, 4-bromofluorobenzene, 1,2-dichloroethane-d_4, and dibromofluoromethane. Other compounds may be used as surrogates, depending upon the analysis requirements. A stock surrogate solution in methanol should be prepared as described above, and a surrogate standard spiking solution should be prepared from the stock at a concentration of 50-250 µg/10 mL in methanol. Each water sample undergoing GC/MS analysis must be spiked with 10 µL of the surrogate spiking solution prior to analysis.

5.9.1 If a more sensitive mass spectrometer is employed to achieve lower detection levels, more dilute surrogate solutions may be required.

5.10 Internal standards - The recommended internal standards are fluorobenzene, chlorobenzene-d_5, and 1,4-dichlorobenzene-d_4. Other compounds may be used as internal standards as long as they have retention times similar to the compounds being detected by GC/MS. Prepare internal standard stock and secondary dilution standards in methanol using the procedures described in Secs. 5.7 and 5.8. It is recommended that the secondary dilution standard should be prepared at a concentration of 25 mg/L of each internal standard compound. Addition of 10 µL of this standard to 5.0 mL of sample or calibration standard would be the equivalent of 50 µg/L.

5.10.1 If a more sensitive mass spectrometer is employed to achieve lower detection levels, more dilute internal standard solutions may be required. Area counts of the internal standard peaks should be between 50-200% of the area of the target analytes in the mid-point calibration analysis.

5.11 4-Bromofluorobenzene (BFB) standard - A standard solution containing 25 ng/µL of BFB in methanol should be prepared.

5.11.1 If a more sensitive mass spectrometer is employed to achieve lower detection levels, a more dilute BFB standard solution may be required.

5.12 Calibration standards - Calibration standards at a minimum of five concentrations should be prepared from the secondary dilution of stock standards (see Secs. 5.7 and 5.8). Prepare these solutions in organic-free reagent water. One of the concentrations should be at a concentration near, but above, the method detection limit. The remaining concentrations should correspond to the expected range of concentrations found in real samples but should not exceed the working range of the GC/MS system. Each standard should contain each analyte for detection by this method. It is EPA's intent that all target analytes for a particular analysis be included in the calibration standard(s). However, these target analytes may not include the entire List of Analytes (Sec. 1.1) for which the method has been demonstrated. However, the laboratory shall not report a quantitative result for a target analyte that was not included in the calibration standard(s). Calibration standards must be prepared daily.

5.13 Matrix spiking standards - Matrix spiking standards should be prepared from volatile organic compounds which will be representative of the compounds being investigated. At a minimum, the matrix spike should include 1,1-dichloroethene, trichloroethene, chlorobenzene, toluene, and benzene. It is desirable to perform a matrix spike using compounds found in samples. Some permits may require spiking specific compounds of interest, especially if they are polar and would not be represented by the above listed compounds. The standard should be prepared in methanol, with each compound present at a concentration of 250 µg/10.0 mL.

5.13.1 If a more sensitive mass spectrometer is employed to achieve lower detection levels, more dilute matrix spiking solutions may be required.

5.14 Great care must be taken to maintain the integrity of all standard solutions. It is recommended all standards in methanol be stored at -10°C to -20°C in amber bottles with Teflon lined screw-caps.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

7.0 PROCEDURE

7.1 Three alternate methods are provided for sample introduction. All internal standards, surrogates, and matrix spikes (when applicable) must be added to samples before introduction.

7.1.1 Direct injection - in very limited application, (e.g., volatiles in waste oil or aqueous process wastes) direct injection of aqueous samples or samples diluted according to Method 3585 may be appropriate. Direct injection has been used for the analysis of volatiles in waste oil (diluted 1:1 with hexadecane) and for determining if the sample is ignitable (aqueous injection, Methods 1010 or 1020). Direct injection is only permitted for the determination of volatiles at the toxicity characteristic (TC) regulatory limits, at concentrations in excess of 10,000 µg/L, or for water-soluble compounds that do not purge.

7.1.2 Purge-and-trap for aqueous samples, see Method 5030 for details.

7.1.3 Purge-and-trap for solid samples, see Method 5030 for details.

7.2 Recommended Chromatographic conditions

7.2.1 General:

Injector temperature:	200-225°C
Transfer line temperature:	250-300°C

7.2.2 Column 1 (A sample chromatogram is presented in Figure 5)

Carrier gas (He) flow rate:	15 mL/min
Initial temperature:	10°C, hold for 5 minutes
Temperature program:	6°C/min to 160°C
Final temperature:	160°C, hold until all expected compounds have eluted.

7.2.3 Column 2, Cryogenic cooling (A sample chromatogram is presented in Figure 6)

Carrier gas (He) flow rate:	15 mL/min
Initial temperature:	10°C, hold for 5 minutes
Temperature program:	6°C/min to 160°C
Final temperature:	160°C, hold until all expected compounds have eluted.

7.2.4 Column 2, Non-cryogenic cooling (A sample chromatogram is presented in Figure 7). It is recommended that carrier gas flow and split and make-up gases be set using performance of standards as guidance. Set the carrier gas head pressure to ≈ 10 psi and the split to ≈ 30 mL/min. Optimize the make-up gas flow for the separator (approximately 30 mL/min) by injecting BFB, and determining the optimum response when varying the make-up gas. This will require several injections of BFB. Next, make several injections of the volatile working standard with all analytes of

interest. Adjust the carrier and split to provide optimum chromatography and response. This is an especially critical adjustment for the volatile gas analytes. The head pressure should optimize between 8-12 psi and the split between 20-60 mL/min. The use of the splitter is important to minimize the effect of water on analyte response, to allow the use of a larger volume of helium during trap desorption, and to slow column flow.

Initial temperature:	45°C, hold for 2 minutes
Temperature program:	8°C/min to 200°C
Final temperature:	200°C, hold for 6 minutes.

A trap preheated to 150°C prior to trap desorption is required to provide adequate chromatography of the gas analytes.

7.2.5 Column 3 (A sample chromatogram is presented in Figure 8)

Carrier gas (He) flow rate:	4 mL/min
Initial temperature:	10°C, hold for 5 minutes
Temperature program:	6°C/min to 70°C, then 15°C/min to 145°C
Final temperature:	145°C, hold until all expected compounds have eluted.

7.2.6 Direct injection - Column 2

Carrier gas (He) flow rate:	4 mL/min
Column:	J&W DB-624, 70m x 0.53 mm
Initial temperature:	40°C, hold for 3 minutes
Temperature program:	8°C/min
Final temperature:	260°C, hold until all expected compounds have eluted.
Column Bake out (direct inj):	75 minutes
Injector temperature:	200-225°C
Transfer line temperature:	250-300°C

7.2.7 Direct Split Interface - Column 4

Carrier gas (He) flow rate:	1.5 mL/min
Initial temperature:	35°C, hold for 2 minutes
Temperature program:	4°C/min to 50°C 10°C/min to 220°C
Final temperature:	220°C, hold until all expected compounds have eluted
Split ratio:	100:1
Injector temperature:	125°C

7.3 Initial calibration - the recommended MS operating conditions

Mass range:	35-260 amu
Scan time:	0.6-2 sec/scan
Source temperature:	According to manufacturer's specifications

Ion trap only: Set axial modulation, manifold temperature, and emission current to manufacturer's recommendations

7.3.1 Each GC/MS system must be hardware-tuned to meet the criteria in Table 4 for a 5-50 ng injection or purging of 4-bromofluorobenzene (2 µL injection of the BFB standard). Analyses must not begin until these criteria are met.

7.3.2 Set up the purge-and-trap system as outlined in Method 5030 if purge-and-trap analysis is to be utilized. A set of at least five calibration standards containing the method analytes is needed. One calibration standard should contain each analyte at a concentration approaching but greater than the method detection limit (Table 1) for that compound; the other calibration standards should contain analytes at concentrations that define the range of the method. Calibration should be done using the sample introduction technique that will be used for samples. For Method 5030, the purging efficiency for 5 mL of water is greater than for 25 mL. Therefore, develop the standard curve with whichever volume of sample that will be analyzed.

7.3.2.1 To prepare a calibration standard for purge-and-trap or aqueous direct injection, add an appropriate volume of a secondary dilution standard solution to an aliquot of organic-free reagent water in a volumetric flask. Use a microsyringe and rapidly inject the alcoholic standard into the expanded area of the filled volumetric flask. Remove the needle as quickly as possible after injection. Mix by inverting the flask three times only. Discard the contents contained in the neck of the flask. Aqueous standards are not stable and should be prepared daily. Transfer 5.0 mL (or 25 mL if lower detection limits are required) of each standard to a gas tight syringe along with 10 µL of internal standard. Then transfer the contents to a purging device or syringe. Perform purge-and-trap or direct injection as outlined in Method 5030.

7.3.2.2 To prepare a calibration standard for direct injection analysis of oil, dilute standards in hexadecane.

7.3.3 Tabulate the area response of the characteristic ions (see Table 5) against concentration for each compound and each internal standard. Calculate response factors (RF) for each compound relative to one of the internal standards. The internal standard selected for the calculation of the RF for a compound should be the internal standard that has a retention time closest to the compound being measured (Sec. 7.6.2). The RF is calculated as follows:

$$RF = (A_x C_{is})/(A_{is} C_x)$$

where:

A_x	=	Area of the characteristic ion for the compound being measured.
A_{is}	=	Area of the characteristic ion for the specific internal standard.
C_{is}	=	Concentration of the specific internal standard.
C_x	=	Concentration of the compound being measured.

7.3.4 The average RF must be calculated and recorded for each compound using the five RF values calculated for each compound from the initial (5-point) calibration curve. A system performance check should be made before this calibration curve is used. Five compounds (the System Performance Check Compounds, or SPCCs) are checked for a minimum average relative response factor. These compounds are chloromethane; 1,1-dichloroethane; bromoform; 1,1,2,2-tetrachloroethane; and chlorobenzene. These compounds are used to check compound instability and to check for degradation caused by contaminated lines or active sites in the system. Examples of these occurrences are:

7.3.4.1 Chloromethane - This compound is the most likely compound to be lost if the purge flow is too fast.

7.3.4.2 Bromoform - This compound is one of the compounds most likely to be purged very poorly if the purge flow is too slow. Cold spots and/or active sites in the transfer lines may adversely affect response. Response of the quantitation ion (m/z 173) is directly affected by the tuning of BFB at ions m/z 174/176. Increasing the m/z 174/176 ratio relative to m/z 95 may improve bromoform response.

7.3.4.3 Tetrachloroethane and 1,1-dichloroethane - These compounds are degraded by contaminated transfer lines in purge-and-trap systems and/or active sites in trapping materials.

7.3.5 Using the RFs from the initial calibration, calculate and record the percent relative standard deviation (%RSD) for all compounds. The percent RSD is calculated as follows:

$$\% \text{ RSD} = \frac{SD}{\overline{RF}_x} \times 100\%$$

where:

RSD	=	Relative standard deviation.
RF_x	=	mean of 5 initial RFs for a compound.
SD	=	standard deviation of the 5 initial RFs for a compound.

$$SD = \sqrt{\sum_{i=1}^{n} \frac{(RF_i - \overline{RF})^2}{n-1}}$$

where:

RF_i = RF for each of the 5 calibration levels
N = number of RF values (i.e., 5)

The percent relative standard deviation should be less than 15% for each compound. However, the %RSD for each individual Calibration Check Compound (CCC) must be less than 30%. The CCCs are:

1,1-Dichloroethene,
Chloroform,
1,2-Dichloropropane,
Toluene,
Ethylbenzene, and
Vinyl chloride.

7.3.5.1 If a %RSD greater than 30 percent is measured for any CCC, then corrective action to eliminate a system leak and/or column reactive sites is required before reattempting calibration.

7.3.6 Linearity - If the %RSD of any compound is 15% or less, then the relative response factor is assumed to be constant over the calibration range, and the average relative response factor may be used for quantitation.

7.3.6.1 If the %RSD of any compound is greater than 15%, construct calibration curves of area ratio (A/A_{is}) versus concentration using first or higher order regression fit of the five calibration points. The analyst should select the regression order which introduces the least calibration error into the quantitation. The use of calibration curves is a recommended alternative to average response factor calibration (Sec. 7.6.2.4), and a useful diagnostic of standard preparation accuracy and absorption activity in the chromatographic system.

7.3.7 These curves are verified each shift by purging a performance standard. Recalibration is required only if calibration and on-going performance criteria cannot be met.

7.4 GC/MS calibration verification

7.4.1 Prior to the analysis of samples, inject or purge 5-50 ng of the 4-bromofluorobenzene standard following Method 5030. The resultant mass spectra for the BFB must meet all of the criteria given in Table 4 before sample analysis begins. These criteria must be demonstrated each 12-hour shift.

7.4.2 The initial calibration curve (Sec. 7.3) for each compound of interest must be checked and verified once every 12 hours during analysis with the introduction technique used for samples. This is accomplished by analyzing a calibration standard that is at a concentration near the midpoint concentration for the working range of the GC/MS by checking the SPCC and CCC.

7.4.3 System Performance Check Compounds (SPCCs) - A system performance check must be made each 12 hours. If the SPCC criteria are met, a comparison of relative response factors is made for all compounds. This is the same check that is applied during the initial calibration. If the minimum relative response factors are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Some possible problems are standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column, and active sites in the column or chromatographic system.

7.4.3.1 The minimum relative response factor for volatile SPCCs are as follows:

Chloromethane	0.10
1,1-Dichloroethane	0.10
Bromoform	>0.10
Chlorobenzene	0.30
1,1,2,2-Tetrachloroethane	0.30

7.4.4 Calibration Check Compounds (CCCs) - After the system performance check is met, CCCs listed in Sec. 7.3.5 are used to check the validity of the initial calibration.

Calculate the percent drift using the following equation:

$$\% \text{ Drift} = (C_I - C_C)/C_I \times 100$$

where:

C_I = Calibration Check Compound standard concentration.
C_c = Measured concentration using selected quantitation method.

If the percent drift for each CCC is less than 20%, the initial calibration is assumed to be valid. If the criterion is not met (> 20% drift), for any one CCC, corrective action must be taken. Problems similar to those listed under SPCCs could affect this criterion. If no source of the problem can be determined after corrective action has been taken, a new five point calibration MUST be generated. This criterion MUST be met before quantitative sample analysis begins. If the CCCs are not required analytes by the permit, then all required analytes must meet the 20% drift criterion.

7.4.5 The internal standard responses and retention times in the check calibration standard must be evaluated immediately after or during data acquisition. If the retention time for any internal standard changes

by more than 30 seconds from the last calibration check (12 hours), the chromatographic system must be inspected for malfunctions and corrections must be made, as required. If the EICP area for any of the internal standards changes by a factor of two (-50% to +100%) from the last daily calibration check standard, the mass spectrometer must be inspected for malfunctions and corrections must be made, as appropriate. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is necessary.

7.5 GC/MS analysis

7.5.1 It is highly recommended that the extract be screened on a headspace-GC/FID (Methods 3810/8015), headspace-GC/PID/ELCD (Methods 3810/8021), or waste dilution-GC/PID/ELCD (Methods 3585/8021) using the same type of capillary column. This will minimize contamination of the GC/MS system from unexpectedly high concentrations of organic compounds. Use of screening is particularly important when this method is used to achieve low detection levels.

7.5.2 All samples and standard solutions must be allowed to warm to ambient temperature before analysis. Set up the purge-and-trap system as outlined in Method 5030 if purge-and-trap introduction will be used.

7.5.3 BFB tuning criteria and GC/MS calibration verification criteria must be met before analyzing samples.

7.5.3.1 Remove the plunger from a 5 mL syringe and attach a closed syringe valve. If lower detection limits are required, use a 25 mL syringe. Open the sample or standard bottle, which has been allowed to come to ambient temperature, and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL.

7.5.4 The process of taking an aliquot destroys the validity of aqueous and soil samples for future analysis; therefore, if there is only one VOA vial, the analyst should prepare a second aliquot for analysis at this time to protect against possible loss of sample integrity. This second sample is maintained only until such time when the analyst has determined that the first sample has been analyzed properly. For aqueous samples, filling one 20 mL syringe would require the use of only one syringe. If a second analysis is needed from a syringe, it must be analyzed within 24 hours. Care must be taken to prevent air from leaking into the syringe.

7.5.4.1 The following procedure is appropriate for diluting aqueous purgeable samples. All steps must be performed without delays until the diluted sample is in a gas-tight syringe.

7.5.4.1.1 Dilutions may be made in volumetric flasks (10 to 100 mL). Select the volumetric flask that will allow for the necessary dilution. Intermediate dilutions may be necessary for extremely large dilutions.

7.5.4.1.2 Calculate the approximate volume of organic-free reagent water to be added to the volumetric flask selected and add slightly less than this quantity of organic-free reagent water to the flask.

7.5.4.1.3 Inject the proper aliquot of sample from the syringe into the flask. Aliquots of less than 1 mL are not recommended. Dilute the sample to the mark with organic-free reagent water. Cap the flask, invert, and shake three times. Repeat above procedure for additional dilutions.

7.5.4.1.4 Fill a 5 mL syringe with the diluted sample.

7.5.4.2 Compositing aqueous samples prior to GC/MS analysis

7.5.4.2.1 Add 5 mL or equal larger amounts of each sample (up to 5 samples are allowed) to a 25 mL glass syringe. Special precautions must be made to maintain zero headspace in the syringe.

7.5.4.2.2 The samples must be cooled at 4°C during this step to minimize volatilization losses.

7.5.4.2.3 Mix well and draw out a 5 mL aliquot for analysis.

7.5.4.2.4 Follow sample introduction, purging, and desorption steps described in Method 5030.

7.5.4.2.5 If less than five samples are used for compositing, a proportionately smaller syringe may be used unless a 25 mL sample is to be purged.

7.5.5 Add 10.0 µL of surrogate spiking solution and 10 µL of internal standard spiking solution to each sample. The surrogate and internal standards may be mixed and added as a single spiking solution. The addition of 10 µL of the surrogate spiking solution to 5 mL of sample is equivalent to a concentration of 50 µg/L of each surrogate standard. The addition of 10 µL of the surrogate spiking solution to 5 g of sample is equivalent to a concentration of 50 µg/kg of each surrogate standard.

7.5.5.1 If a more sensitive mass spectrometer is employed to achieve lower detection levels, more dilute surrogate and internal standard solutions may be required.

7.5.6 Perform purge-and-trap or direct injection by Method 5030. If the initial analysis of sample or a dilution of the sample has a concentration of analytes that exceeds the initial calibration range, the sample must be reanalyzed at a higher dilution. Secondary ion quantitation is allowed only when there are sample interferences with the primary ion. When a sample is analyzed that has saturated ions from a compound, this analysis must be followed by a blank organic-free reagent

water analysis. If the blank analysis is not free of interferences, the system must be decontaminated. Sample analysis may not resume until the blank analysis is demonstrated to be free of interferences.

7.5.6.1. All dilutions should keep the response of the major constituents (previously saturated peaks) in the upper half of the linear range of the curve. Proceed to Secs. 7.6.1 and 7.6.2 for qualitative and quantitative analysis.

7.5.7 For matrix spike analysis, add 10 µL of the matrix spike solution (Sec. 5.13) to the 5 mL of sample to be purged. Disregarding any dilutions, this is equivalent to a concentration of 50 µg/L of each matrix spike standard.

7.6 Data interpretation

7.6.1 Qualitative analysis

7.6.1.1 The qualitative identification of compounds determined by this method is based on retention time, and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined to be the three ions of greatest relative intensity, or any ions over 30% relative intensity if less than three such ions occur in the reference spectrum. Compounds should be identified as present when the criteria below are met.

7.6.1.1.1 The intensities of the characteristic ions of a compound maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention time will be accepted as meeting this criterion.

7.6.1.1.2 The RRT of the sample component is within ± 0.06 RRT units of the RRT of the standard component.

7.6.1.1.3 The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum. (Example: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 20% and 80%.)

7.6.1.1.4 Structural isomers that produce very similar mass spectra should be identified as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the sum of

the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

7.6.1.1.5 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When gas chromatographic peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important. Examination of extracted ion current profiles of appropriate ions can aid in the selection of spectra, and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria can be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

7.6.1.2 For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The necessity to perform this type of identification will be determined by the type of analyses being conducted. Guidelines for making tentative identification are:

(1) Relative intensities of major ions in the reference spectrum (ions > 10% of the most abundant ion) should be present in the sample spectrum.

(2) The relative intensities of the major ions should agree within ± 20%. (Example: For an ion with an abundance of 50% in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70%).

(3) Molecular ions present in the reference spectrum should be present in the sample spectrum.

(4) Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.

(5) Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or coeluting peaks. Data system library reduction programs can sometimes create these discrepancies.

Computer generated library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other. Only after visual comparison of sample with the nearest library searches will the mass spectral interpretation specialist assign a tentative identification.

7.6.2 Quantitative analysis

7.6.2.1 When a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion. Quantitation will take place using the internal standard technique. The internal standard used shall be the one nearest the retention time of that of a given analyte.

7.6.2.2 When MS response is linear and passes through the origin, calculate the concentration of each identified analyte in the sample as follows:

Water

$$\text{concentration } (\mu g/L) = \frac{(A_x)(I_s)}{(A_{is})(\overline{RF})(V_o)}$$

where:

A_x = Area of characteristic ion for compound being measured.
I_s = Amount of internal standard injected (ng).
A_{is} = Area of characteristic ion for the internal standard.
$\overline{RF}$ = Mean relative response factor for compound being measured.
V_o = Volume of water purged (mL), taking into consideration any dilutions made.

Sediment/Soil Sludge (on a dry-weight basis) and Waste (normally on a wet-weight basis)

$$\text{concentration } (\mu g/kg) = \frac{(A_x)(I_s)(V_t)}{(A_{is})(\overline{RF})(V_i)(W_s)(D)}$$

where:

A_x, I_s, A_{is}, $\overline{RF}$, = Same as for water.
V_t = Volume of total extract (μL) (use 10,000 μL or a factor of this when dilutions are made).
V_i = Volume of extract added (μL) for purging.
W_s = Weight of sample extracted or purged (g).
D = % dry weight of sample/100, or 1 for a wet-weight basis.

7.6.2.3 Where applicable, an estimate of concentration for noncalibrated components in the sample should be made. The formulae given above should be used with the following modifications: The areas A_x and A_{is} should be from the total ion chromatograms, and the RF for the compound should be assumed to be 1. The concentration

obtained should be reported indicating (1) that the value is an estimate and (2) which internal standard was used to determine concentration. Use the nearest internal standard free of interferences.

7.6.2.4 Alternatively, the regression line fitted to the initial calibration (Sec. 7.3.6.1) may be used for determination of analyte concentration.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for general quality control procedures.

8.2 Additional required instrument QC is found in the Secs. 7.3 and 7.4:

8.2.1 The GC/MS system must be tuned to meet the BFB specifications.

8.2.2 There must be an initial calibration of the GC/MS system

8.2.3 The GC/MS system must meet the SPCC criteria and the CCC criteria, each 12 hours.

8.3 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.3.1 A quality control (QC) reference sample concentrate is required containing each analyte at a concentration of 10 mg/L or less in methanol. The QC reference sample concentrate may be prepared from pure standard materials or purchased as certified solutions. If prepared by the laboratory, the QC reference sample concentrate must be made using stock standards prepared independently from those used for calibration.

8.3.2 Prepare a QC reference sample to contain 20 µg/L or less of each analyte by adding 200 µL of QC reference sample concentrate to 100 mL of organic-free reagent water.

8.3.3 Four 5-mL aliquots of the well mixed QC reference sample are analyzed according to the method beginning in Sec. 7.5.1.

8.3.4 Calculate the average recovery ($\bar{x}$) in µg/L, and the standard deviation of the recovery (s) in µg/L, for each analyte using the four results.

8.3.5 Tables 7 and 8 provide single laboratory recovery and precision data obtained for the method analytes from water. Similar results from dosed water should be expected by any experienced laboratory. Compare s and $\bar{x}$ (Sec. 8.3.4) for each analyte to the single laboratory recovery and precision data. Results are comparable if the calculated standard deviation of the recovery does not exceed 2.6 times the single laboratory RSD or 20%, whichever is greater, and the mean recovery lies within the interval $\bar{x} \pm 3s$ or $\bar{x} \pm 30\%$, whichever is greater.

NOTE: The large number of analytes in Tables 7 and 8 present a substantial probability that one or more will fail at least one of the acceptance criteria when all analytes of a given method are determined.

8.3.6 When one or more of the analytes tested are not comparable to the data in Table 6 or 7, the analyst must proceed according to Sec. 8.3.6.1 or 8.3.6.2.

8.3.6.1 Locate and correct the source of the problem and repeat the test for all analytes beginning with Sec. 8.3.2.

8.3.6.2 Beginning with Sec. 8.3.2, repeat the test only for those analytes that are not comparable. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning with Sec. 8.3.2.

8.4 For aqueous and soil matrices, laboratory established surrogate control limits should be compared with the control limits listed in Table 8.

8.4.1 If recovery is not within limits, the following procedures are required.

8.4.1.1 Check to be sure that there are no errors in the calculations, surrogate solutions or internal standards. If errors are found, recalculate the data accordingly.

8.4.1.2 Check instrument performance. If an instrument performance problem is identified, correct the problem and re-analyze the extract.

8.4.1.3 If no problem is found, re-extract and re-analyze the sample.

8.4.1.4 If, upon re-analysis, the recovery is again not within limits, flag the data as "estimated concentration".

8.4.2 At a minimum, each laboratory should update surrogate recovery limits on a matrix-by-matrix basis, annually.

9.0 METHOD PERFORMANCE

9.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

9.2 This method has been tested in a single laboratory using spiked water. Using a wide-bore capillary column, water was spiked at concentrations between 0.5 and 10 μg/L. Single laboratory accuracy and precision data are

presented for the method analytes in Table 6. Calculated MDLs are presented in Table 1.

9.3 The method was tested using water spiked at 0.1 to 0.5 µg/L and analyzed on a cryofocussed narrow-bore column. The accuracy and precision data for these compounds are presented in Table 7. MDL values were also calculated from these data and are presented in Table 2.

9.4 Direct injection has been used for the analysis of waste motor oil samples using a wide-bore column. The accuracy and precision data for these compounds are presented in Table 10.

10.0 REFERENCES

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TABLE 1.
CHROMATOGRAPHIC RETENTION TIMES AND METHOD DETECTION LIMITS (MDL)
FOR VOLATILE ORGANIC COMPOUNDS ON WIDE-BORE CAPILLARY COLUMNS

ANALYTE	RETENTION TIME (minutes)			MDL[d] (µg/L)
	Column 1[a]	Column 2[b]	Column 2'[c]	
Dichlorodifluoromethane	1.35	0.70	3.13	0.10
Chloromethane	1.49	0.73	3.40	0.13
Vinyl Chloride	1.56	0.79	3.93	0.17
Bromomethane	2.19	0.96	4.80	0.11
Chloroethane	2.21	1.02	--	0.10
Trichlorofluoromethane	2.42	1.19	6.20	0.08
Acrolein	3.19			
Iodomethane	3.56			
Acetonitrile	4.11			
Carbon disulfide	4.11			
Allyl chloride	4.11			
Methylene chloride	4.40	2.06	9.27	0.03
1,1-Dichloroethene	4.57	1.57	7.83	0.12
Acetone	4.57			
trans-1,2-Dichloroethene	4.57	2.36	9.90	0.06
Acrylonitrile	5.00			
1,1-Dichloroethane	6.14	2.93	10.80	0.04
Vinyl acetate	6.43			
2,2-Dichloropropane	8.10	3.80	11.87	0.35
2-Butanone	--			
cis-1,2-Dichloroethene	8.25	3.90	11.93	0.12
Propionitrile	8.51			
Chloroform	9.01	4.80	12.60	0.03
Bromochloromethane	--	4.38	12.37	0.04
Methacrylonitrile	9.19			
1,1,1-Trichloroethane	10.18	4.84	12.83	0.08
Carbon tetrachloride	11.02	5.26	13.17	0.21
1,1-Dichloropropene	--	5.29	13.10	0.10
Benzene	11.50	5.67	13.50	0.04
1,2-Dichloroethane	12.09	5.83	13.63	0.06
Trichloroethene	14.03	7.27	14.80	0.19
1,2-Dichloropropane	14.51	7.66	15.20	0.04
Bromodichloromethane	15.39	8.49	15.80	0.08
Dibromomethane	15.43	7.93	15.43	0.24
Methyl methacrylate	15.50			
1,4-Dioxane	16.17			
2-Chloroethyl vinyl ether	--			
4-Methyl-2-pentanone	17.32			
trans-1,3-Dichloropropene	17.47	--	16.70	--
Toluene	18.29	10.00	17.40	0.11
cis-1,3-Dichloropropene	19.38	--	17.90	--
1,1,2-Trichloroethane	19.59	11.05	18.30	0.10

TABLE 1.
(Continued)

ANALYTE	RETENTION TIME (minutes)			MDL[d] (µg/L)
	Column 1[a]	Column 2[b]	Column 2'[c]	
Ethyl methacrylate	20.01			
2-Hexanone	20.30			
Tetrachloroethene	20.26	11.15	18.60	0.14
1,3-Dichloropropane	20.51	11.31	18.70	0.04
Dibromochloromethane	21.19	11.85	19.20	0.05
1,2-Dibromoethane	21.52	11.83	19.40	0.06
1-Chlorohexane	--	13.29	--	0.05
Chlorobenzene	23.17	13.01	20.67	0.04
1,1,1,2-Tetrachloroethane	23.36	13.33	20.87	0.05
Ethylbenzene	23.38	13.39	21.00	0.06
p-Xylene	23.54	13.69	21.30	0.13
m-Xylene	23.54	13.68	21.37	0.05
o-Xylene	25.16	14.52	22.27	0.11
Styrene	25.30	14.60	22.40	0.04
Bromoform	26.23	14.88	22.77	0.12
Isopropylbenzene (Cumene)	26.37	15.46	23.30	0.15
cis-1,4-Dichloro-2-butene	27.12			
1,1,2,2-Tetrachloroethane	27.29	16.35	24.07	0.04
Bromobenzene	27.46	15.86	24.00	0.03
1,2,3-Trichloropropane	27.55	16.23	24.13	0.32
n-Propylbenzene	27.58	16.41	24.33	0.04
2-Chlorotoluene	28.19	16.42	24.53	0.04
trans-1,4-Dichloro-2-butene	28.26			
1,3,5-Trimethylbenzene	28.31	16.90	24.83	0.05
4-Chlorotoluene	28.33	16.72	24.77	0.06
Pentachloroethane	29.41			
1,2,4-Trimethylbenzene	29.47	17.70	31.50	0.13
sec-Butylbenzene	30.25	18.09	26.13	0.13
tert-Butylbenzene	30.59	17.57	26.60	0.14
p-Isopropyltoluene	30.59	18.52	26.50	0.12
1,3-Dichlorobenzene	30.56	18.14	26.37	0.12
1,4-Dichlorobenzene	31.22	18.39	26.60	0.03
Benzyl chloride	32.00			
n-Butylbenzene	32.23	19.49	27.32	0.11
1,2-Dichlorobenzene	32.31	19.17	27.43	0.03
1,2-Dibromo-3-chloropropane	35.30	21.08	--	0.26
1,2,4-Trichlorobenzene	38.19	23.08	31.50	0.04
Hexachlorobutadiene	38.57	23.68	32.07	0.11
Naphthalene	39.05	23.52	32.20	0.04
1,2,3-Trichlorobenzene	40.01	24.18	32.97	0.03

TABLE 1.
(Continued)

ANALYTE	RETENTION TIME (minutes)			MDL[d] (µg/L)
	Column 1[a]	Column 2[b]	Column 2'[c]	
INTERNAL STANDARDS/SURROGATES				
1,4-Difluorobenzene	13.26			
Chlorobenzene-d_5	23.10			
1,4-Dichlorobenzene-d_4	31.16			
4-Bromofluorobenzene	27.83	15.71	23.63	
1,2-Dichlorobenzene-d_4	32.30	19.08	27.25	
Dichloroethane-d_4	12.08			
Dibromofluoromethane	--			
Toluene-d_8	18.27			
Pentafluorobenzene	--			
Fluorobenzene	13.00	6.27	14.06	

[a] Column 1 - 60 meter x 0.75 mm ID VOCOL capillary. Hold at 10°C for 8 minutes, then program to 180°C at 4°/min.

[b] Column 2 - 30 meter x 0.53 mm ID DB-624 wide-bore capillary using cryogenic oven. Hold at 10°C for 5 minutes, then program to 160°C at 6°/min.

[c] Column 2' - 30 meter x 0.53 mm ID DB-624 wide-bore capillary, cooling GC oven to ambient temperatures. Hold at 10°C for 6 minutes, program to 70°C at 10°/min, program to 120°C at 5°/min, then program to 180°C at 8°/min.

[d] MDL based on a 25 mL sample volume.

TABLE 2.
CHROMATOGRAPHIC RETENTION TIMES AND METHOD DETECTION LIMITS (MDL)
FOR VOLATILE ORGANIC COMPOUNDS ON NARROW-BORE CAPILLARY COLUMNS

ANALYTE	RETENTION TIME (minutes) Column 3[a]	MDL[b] (µg/L)
Dichlorodifluoromethane	0.88	0.11
Chloromethane	0.97	0.05
Vinyl chloride	1.04	0.04
Bromomethane	1.29	0.06
Chloroethane	1.45	0.02
Trichlorofluoromethane	1.77	0.07
1,1-Dichloroethene	2.33	0.05
Methylene chloride	2.66	0.09
trans-1,2-Dichloroethene	3.54	0.03
1,1-Dichloroethane	4.03	0.03
cis-1,2-Dichloroethene	5.07	0.06
2,2-Dichloropropane	5.31	0.08
Chloroform	5.55	0.04
Bromochloromethane	5.63	0.09
1,1,1-Trichloroethane	6.76	0.04
1,2-Dichloroethane	7.00	0.02
1,1-Dichloropropene	7.16	0.12
Carbon tetrachloride	7.41	0.02
Benzene	7.41	0.03
1,2-Dichloropropane	8.94	0.02
Trichloroethene	9.02	0.02
Dibromomethane	9.09	0.01
Bromodichloromethane	9.34	0.03
Toluene	11.51	0.08
1,1,2-Trichloroethane	11.99	0.08
1,3-Dichloropropane	12.48	0.08
Dibromochloromethane	12.80	0.07
Tetrachloroethene	13.20	0.05
1,2-Dibromoethane	13.60	0.10
Chlorobenzene	14.33	0.03
1,1,1,2-Tetrachloroethane	14.73	0.07
Ethylbenzene	14.73	0.03
p-Xylene	15.30	0.06
m-Xylene	15.30	0.03
Bromoform	15.70	0.20
o-Xylene	15.78	0.06
Styrene	15.78	0.27
1,1,2,2-Tetrachloroethane	15.78	0.20
1,2,3-Trichloropropane	16.26	0.09
Isopropylbenzene	16.42	0.10

TABLE 2.
(Continued)

ANALYTE	RETENTION TIME (minutes) Column 3[a]	MDL[b] (µg/L)
Bromobenzene	16.42	0.11
2-Chlorotoluene	16.74	0.08
n-Propylbenzene	16.82	0.10
4-Chlorotoluene	16.82	0.06
1,3,5-Trimethylbenzene	16.99	0.06
tert-Butylbenzene	17.31	0.33
1,2,4-Trimethylbenzene	17.31	0.09
sec-Butylbenzene	17.47	0.12
1,3-Dichlorobenzene	17.47	0.05
p-Isopropyltoluene	17.63	0.26
1,4-Dichlorobenzene	17.63	0.04
1,2-Dichlorobenzene	17.79	0.05
n-Butylbenzene	17.95	0.10
1,2-Dibromo-3-chloropropane	18.03	0.50
1,2,4-Trichlorobenzene	18.84	0.20
Naphthalene	19.07	0.10
Hexachlorobutadiene	19.24	0.10
1,2,3-Trichlorobenzene	19.24	0.14

[a] Column 3 - 30 meter x 0.32 mm ID DB-5 capillary with 1 µm film thickness.

[b] MDL based on a 25 mL sample volume.

TABLE 3.
ESTIMATED QUANTITATION LIMITS FOR VOLATILE ANALYTES[a]

	Estimated Quantitation Limits (All Analytes in Table 1)	
	Ground water µg/L	Low Soil/Sediment[b] µg/kg
Purging 5 mL of water	5	--
Purging 25 mL of water	1	--
Soil/Sediment	-	5

[a] Estimated Quantitation Limit (EQL) - The lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. The EQL is generally 5 to 10 times the MDL. However, it may be nominally chosen within these guidelines to simplify data reporting. For many analytes the EQL is selected from the lowest non-zero standard in the calibration curve. Sample EQLs are highly matrix-dependent. The EQLs listed herein are provided for guidance and may not always be achievable.

[b] EQLs listed for soil/sediment are based on wet weight. Normally data are reported on a dry weight basis; therefore, EQLs will be higher, based on the percent dry weight in each sample.

Other Matrices	Factor[c]
Water miscible liquid waste	50
High-concentration soil and sludge	125
Non-water miscible waste	500

[c]EQL = [EQL for low soil/sediment (see Table 3)] X [Factor]. For non-aqueous samples, the factor is on a wet-weight basis.

TABLE 4.
BFB MASS - INTENSITY SPECIFICATIONS (4-BROMOFLUOROBENZENE)[a]

Mass	Intensity Required (relative abundance)
50	15 to 40% of mass 95
75	30 to 60% of mass 95
95	base peak, 100% relative abundance
96	5 to 9% of mass 95
173	less than 2% of mass 174
174	greater than 50% of mass 95
175	5 to 9% of mass 174
176	greater than 95% but less than 101% of mass 174
177	5 to 9% of mass 176

[a] Alternate tuning criteria may be used (e.g. CLP, Method 524.2, or manufacturers' instructions), provided that method performance is not adversely affected.

TABLE 5.
CHARACTERISTIC MASSES (M/Z) FOR PURGEABLE ORGANIC COMPOUNDS

Analyte	Primary Characteristic Ion	Secondary Characteristic Ion(s)
Acetone	58	43
Acetonitrile	41	41, 40, 39
Acrolein	56	55, 58
Acrylonitrile	53	52, 51
Allyl alcohol	57	57, 58, 39
Allyl chloride	76	76, 41, 39, 78
Benzene	78	-
Benzyl chloride	91	91, 126, 65, 128
Bromoacetone	136	43, 136, 138, 93, 95
Bromobenzene	156	77, 158
Bromochloromethane	128	49, 130
Bromodichloromethane	83	85, 127
Bromoform	173	175, 254
Bromomethane	94	96
iso-Butanol	74	43
n-Butanol	56	41
2-Butanone	72	43, 72
n-Butylbenzene	91	92, 134
sec-Butylbenzene	105	134
tert-Butylbenzene	119	91, 134
Carbon disulfide	76	78
Carbon tetrachloride	117	119
Chloral hydrate	82	44, 84, 86, 111
Chloroacetonitrile	48	75
Chlorobenzene	112	77, 114
1-Chlorobutane	56	49
Chlorodibromomethane	129	208, 206
Chloroethane	64(49*)	66(51*)
2-Chloroethanol	49	49, 44, 43, 51, 80
bis-(2-chloroethyl) sulfide	109	111, 158, 160
2-Chloroethyl vinyl ether	63	65, 106
Chloroform	83	85
Chloromethane	50(49*)	52(51*)
Chloroprene	53	53, 88, 90, 51
3-Chloropropionitrile	54	54, 49, 89, 91
2-Chlorotoluene	91	126
4-Chlorotoluene	91	126
1,2-Dibromo-3-chloropropane	75	155, 157
Dibromochloromethane	129	127
1,2-Dibromoethane	107	109, 188
Dibromomethane	93	95, 174
1,2-Dichlorobenzene	146	111, 148
1,2-Dichlorobenzene-d_4	152	115, 150

TABLE 5.(continued)

Analyte	Primary Characteristic Ion	Secondary Characteristic Ion(s)
1,3-Dichlorobenzene	146	111, 148
1,4-Dichlorobenzene	146	111, 148
cis-1,4-Dichloro-2-butene	75	75, 53, 77, 124, 89
trans-1,4-Dichloro-2-butene	53	88, 75
Dichlorodifluoromethane	85	87
1,1-Dichloroethane	63	65, 83
1,2-Dichloroethane	62	98
1,1-Dichloroethene	96	61, 63
cis-1,2-Dichloroethene	96	61, 98
trans-1,2-Dichloroethene	96	61, 98
1,2-Dichloropropane	63	112
1,3-Dichloropropane	76	78
2,2-Dichloropropane	77	97
1,3-Dichloro-2-propanol	79	79, 43, 81, 49
1,1-Dichloropropene	75	110, 77
cis-1,3-Dichloropropene	75	77, 39
trans-1,3-Dichloropropene	75	77, 39
1,2,3,4-Diepoxybutane	55	55, 57, 56
Diethyl ether	74	45, 59
1,4-Dioxane	88	88, 58, 43, 57
Epichlorohydrin	57	57, 49, 62, 51
Ethanol	31	45, 27, 46
Ethyl acetate	88	43, 45, 61
Ethylbenzene	91	106
Ethylene oxide	44	44, 43, 42
Ethyl methacrylate	69	69, 41, 99, 86, 114
Hexachlorobutadiene	225	223, 227
Hexachloroethane	201	166, 199, 203
2-Hexanone	43	58, 57, 100
2-Hydroxypropionitrile	44	44, 43, 42, 53
Iodomethane	142	127, 141
Isobutyl alcohol	43	43, 41, 42, 74
Isopropylbenzene	105	120
p-Isopropyltoluene	119	134, 91
Malononitrile	66	66, 39, 65, 38
Methacrylonitrile	41	41, 67, 39, 52, 66
Methyl acrylate	55	85
Methyl-t-butyl ether	73	57
Methylene chloride	84	86, 49
Methyl ethyl ketone	72	43
Methyl iodide	142	142, 127, 141
Methyl methacrylate	69	69, 41, 100, 39
4-Methyl-2-pentanone	100	43, 58, 85
Naphthalene	128	-
Nitrobenzene	123	51, 77

TABLE 5.(continued)

Analyte	Primary Characteristic Ion	Secondary Characteristic Ion(s)
2-Nitropropane	46	-
2-Picoline	93	93, 66, 92, 78
Pentachloroethane	167	167, 130, 132, 165, 169
Propargyl alcohol	55	55, 39, 38, 53
β-Propiolactone	42	42, 43, 44
Propionitrile (ethyl cyanide)	54	54, 52, 55, 40
n-Propylamine	59	59, 41, 39
n-Propylbenzene	91	120
Pyridine	79	52
Styrene	104	78
1,2,3-Trichlorobenzene	180	182, 145
1,2,4-Trichlorobenzene	180	182, 145
1,1,1,2-Tetrachloroethane	131	133, 119
1,1,2,2-Tetrachloroethane	83	131, 85
Tetrachloroethene	164	129, 131, 166
Toluene	92	91
1,1,1-Trichloroethane	97	99, 61
1,1,2-Trichloroethane	83	97, 85
Trichloroethene	95	97, 130, 132
Trichlorofluoromethane	151	101, 153
1,2,3-Trichloropropane	75	77
1,2,4-Trimethylbenzene	105	120
1,3,5-Trimethylbenzene	105	120
Vinyl acetate	43	86
Vinyl chloride	62	64
o-Xylene	106	91
m-Xylene	106	91
p-Xylene	106	91
INTERNAL STANDARDS/SURROGATES		
1,4-Difluorobenzene	114	
Chlorobenzene-d_5	117	
1,4-Dichlorobenzene-d_4	152	115, 150
4-Bromofluorobenzene	95	174, 176
Dibromofluoromethane	113	
Dichloroethane-d_4	102	
Toluene-d_8	98	
Pentafluorobenzene	168	
Fluorobenzene	96	77

* - characteristic ion for an ion trap mass spectrometer (to be used when ion-molecule reactions are observed)

TABLE 6.
SINGLE LABORATORY ACCURACY AND PRECISION DATA FOR VOLATILE ORGANIC COMPOUNDS IN WATER DETERMINED WITH A WIDE-BORE CAPILLARY COLUMN

Analyte	Conc. Range, µg/L	Number of Samples	Recovery[a] %	Standard Deviation of Recovery[b]	Percent RSD
Benzene	0.1 - 10	31	97	6.5	5.7
Bromobenzene	0.1 - 10	30	100	5.5	5.5
Bromochloromethane	0.5 - 10	24	90	5.7	6.4
Bromodichloromethane	0.1 - 10	30	95	5.7	6.1
Bromoform	0.5 - 10	18	101	6.4	6.3
Bromomethane	0.5 - 10	18	95	7.8	8.2
n-Butylbenzene	0.5 - 10	18	100	7.6	7.6
sec-Butylbenzene	0.5 - 10	16	100	7.6	7.6
tert-Butylbenzene	0.5 - 10	18	102	7.4	7.3
Carbon tetrachloride	0.5 - 10	24	84	7.4	8.8
Chlorobenzene	0.1 - 10	31	98	5.8	5.9
Chloroethane	0.5 - 10	24	89	8.0	9.0
Chloroform	0.5 - 10	24	90	5.5	6.1
Chloromethane	0.5 - 10	23	93	8.3	8.9
2-Chlorotoluene	0.1 - 10	31	90	5.6	6.2
4-Chlorotoluene	0.1 - 10	31	99	8.2	8.3
1,2-Dibromo-3-Chloropropane	0.5 - 10	24	83	16.6	19.9
Dibromochloromethane	0.1 - 10	31	92	6.5	7.0
1,2-Dibromoethane	0.5 - 10	24	102	4.0	3.9
Dibromomethane	0.5 - 10	24	100	5.6	5.6
1,2-Dichlorobenzene	0.1 - 10	31	93	5.8	6.2
1,3-Dichlorobenzene	0.5 - 10	24	99	6.8	6.9
1,4-Dichlorobenzene	0.2 - 20	31	103	6.6	6.4
Dichlorodifluoromethane	0.5 - 10	18	90	6.9	7.7
1,1-Dichlorobenzene	0.5 - 10	24	96	5.1	5.3
1,2-Dichlorobenzene	0.1 - 10	31	95	5.1	5.4
1,1-Dichloroethene	0.1 - 10	34	94	6.3	6.7
cis-1,2-Dichloroethene	0.5 - 10	18	101	6.7	6.7
trans-1,2-Dichloroethene	0.1 - 10	30	93	5.2	5.6
1,2-Dichloropropane	0.1 - 10	30	97	5.9	6.1
1,3-Dichloropropane	0.1 - 10	31	96	5.7	6.0
2,2-Dichloropropane	0.5 - 10	12	86	14.6	16.9
1,1-Dichloropropene	0.5 - 10	18	98	8.7	8.9
Ethylbenzene	0.1 - 10	31	99	8.4	8.6
Hexachlorobutadiene	0.5 - 10	18	100	6.8	6.8
Isopropylbenzene	0.5 - 10	16	101	7.7	7.6
p-Isopropyltoluene	0.1 - 10	23	99	6.7	6.7
Methylene chloride	0.1 - 10	30	95	5.0	5.3
Naphthalene	0.1 -100	31	104	8.6	8.2
n-Propylbenzene	0.1 - 10	31	100	5.8	5.8
Styrene	0.1 -100	39	102	7.3	7.2

TABLE 6.
(Continued)

Analyte	Conc. Range, µg/L	Number of Samples	Recovery[a] %	Standard Deviation of Recovery[b]	Percent RSD
1,1,1,2-Tetrachloroethane	0.5 - 10	24	90	6.1	6.8
1,1,2,2-Tetrachloroethane	0.1 - 10	30	91	5.7	6.3
Tetrachloroethene	0.5 - 10	24	89	6.0	6.8
Toluene	0.5 - 10	18	102	8.1	8.0
1,2,3-Trichlorobenzene	0.5 - 10	18	109	9.4	8.6
1,2,4-Trichlorobenzene	0.5 - 10	18	108	9.0	8.3
1,1,1-Trichloroethane	0.5 - 10	18	98	7.9	8.1
1,1,2-Trichloroethane	0.5 - 10	18	104	7.6	7.3
Trichloroethene	0.5 - 10	24	90	6.5	7.3
Trichlorofluoromethane	0.5 - 10	24	89	7.2	8.1
1,2,3-Trichloropropane	0.5 - 10	16	108	15.6	14.4
1,2,4-Trimethylbenzene	0.5 - 10	18	99	8.0	8.1
1,3,5-Trimethylbenzene	0.5 - 10	23	92	6.8	7.4
Vinyl chloride	0.5 - 10	18	98	6.5	6.7
o-Xylene	0.1 - 31	18	103	7.4	7.2
m-Xylene	0.1 - 10	31	97	6.3	6.5
p-Xylene	0.5 - 10	18	104	8.0	7.7

[a] Recoveries were calculated using internal standard method. Internal standard was fluorobenzene.

[b] Standard deviation was calculated by pooling data from three concentrations.

TABLE 7.
SINGLE LABORATORY ACCURACY AND PRECISION DATA FOR
VOLATILE ORGANIC COMPOUNDS IN WATER DETERMINED
WITH A NARROW-BORE CAPILLARY COLUMN

Analyte	Conc. µg/L	Number of Samples	Recovery[a] %	Standard Deviation of Recovery	Percent RSD
Benzene	0.1	7	99	6.2	6.3
Bromobenzene	0.5	7	97	7.4	7.6
Bromochloromethane	0.5	7	97	5.8	6.0
Bromodichloromethane	0.1	7	100	4.6	4.6
Bromoform	0.5	7	101	5.4	5.3
Bromomethane	0.5	7	99	7.1	7.2
n-Butylbenzene	0.5	7	94	6.0	6.4
sec-Butylbenzene	0.5	7	110	7.1	6.5
tert-Butylbenzene	0.5	7	110	2.5	2.3
Carbon tetrachloride	0.1	7	108	6.8	6.3
Chlorobenzene	0.1	7	91	5.8	6.4
Chloroethane	0.1	7	100	5.8	5.8
Chloroform	0.1	7	105	3.2	3.0
Chloromethane	0.5	7	101	4.7	4.7
2-Chlorotoluene	0.5	7	99	4.6	4.6
4-Chlorotoluene	0.5	7	96	7.0	7.3
1,2-Dibromo-3-chloropropane	0.5	7	92	10.0	10.9
Dibromochloromethane	0.1	7	99	5.6	5.7
1,2-Dibromoethane	0.5	7	97	5.6	5.8
Dibromomethane	0.5	7	93	5.6	6.0
1,2-Dichlorobenzene	0.1	7	97	3.5	3.6
1,3-Dichlorobenzene	0.1	7	101	6.0	5.9
1,4-Dichlorobenzene	0.1	7	106	6.5	6.1
Dichlorodifluoromethane	0.1	7	99	8.8	8.9
1,1-Dichloroethane	0.5	7	98	6.2	6.3
1,2-Dichloroethane	0.1	7	100	6.3	6.3
1,1-Dichloroethene	0.1	7	95	9.0	9.5
cis-1,2-Dichloroethene	0.1	7	100	3.7	3.7
trans-1,2-Dichloroethene	0.1	7	98	7.2	7.3
1,2-Dichloropropane	0.5	7	96	6.0	6.3
1,3-Dichloropropane	0.5	7	99	5.8	5.9
2,2-Dichloropropane	0.5	7	99	4.9	4.9
1,1-Dichloropropene	0.5	7	102	7.4	7.3
Ethylbenzene	0.5	7	99	5.2	5.3
Hexachlorobutadiene	0.5	7	100	6.7	6.7
Isopropylbenzene	0.5	7	102	6.4	6.3
p-Isopropyltoluene	0.5	7	113	13.0	11.5
Methylene chloride	0.5	7	97	13.0	13.4
Naphthalene	0.5	7	98	7.2	7.3
n-Propylbenzene	0.5	7	99	6.6	6.7

TABLE 7.
(Continued)

Analyte	Conc. µg/L	Number of Samples	Recovery[a] %	Standard Deviation of Recovery	Percent RSD
Styrene	0.5	7	96	19.0	19.8
1,1,1,2-Tetrachloroethane	0.5	7	100	4.7	4.7
1,1,2,2-Tetrachloroethane	0.5	7	100	12.0	12.0
Tetrachloroethene	0.1	7	96	5.0	5.2
Toluene	0.5	7	100	5.9	5.9
1,2,3-Trichlorobenzene	0.5	7	102	8.9	8.7
1,2,4-Trichlorobenzene	0.5	7	91	16.0	17.6
1,1,1-Trichloroethane	0.5	7	100	4.0	4.0
1,1,2-Trichloroethane	0.5	7	102	4.9	4.8
Trichloroethene	0.1	7	104	2.0	1.9
Trichlorofluoromethane	0.1	7	97	4.6	4.7
1,2,3-Trichloropropane	0.5	7	96	6.5	6.8
1,2,4-Trimethylbenzene	0.5	7	96	6.5	6.8
1,3,5-Trimethylbenzene	0.5	7	101	4.2	4.2
Vinyl chloride	0.1	7	104	0.2	0.2
o-Xylene	0.5	7	106	7.5	7.1
m-Xylene	0.5	7	106	4.6	4.3
p-Xylene	0.5	7	97	6.1	6.3

[a] Recoveries were calculated using internal standard method. Internal standard was fluorobenzene.

TABLE 8.
SURROGATE SPIKE RECOVERY LIMITS FOR WATER AND SOIL/SEDIMENT SAMPLES

Surrogate Compound	Percent Recovery	
	Low/High Water	Low/High Soil/Sediment
4-Bromofluorobenzene[a]	86-115	74-121
Dibromofluoromethane[a]	86-118	80-120
Toluene-d_8[a]	88-110	81-117
Dichloroethane-d_4[a]	80-120	80-120

[a] Single laboratory data, for guidance only.

TABLE 9.
QUANTITY OF EXTRACT REQUIRED FOR ANALYSIS OF HIGH-CONCENTRATION SAMPLES

Approximate Concentration Range	Volume of Extract[a]
500 - 10,000 µg/kg	100 µL
1,000 - 20,000 µg/kg	50 µL
5,000 - 100,000 µg/kg	10 µL
25,000 - 500,000 µg/kg	100 µL of 1/50 dilution[b]

Calculate appropriate dilution factor for concentrations exceeding this table.

[a] The volume of solvent added to 5 mL of water being purged should be kept constant. Therefore, add to the 5 mL syringe whatever volume of solvent is necessary to maintain a volume of 100 µL added to the syringe.

[b] Dilute an aliquot of the solvent extract and then take 100 µL for analysis.

TABLE 10
DIRECT INJECTION ANALYSIS OF NEW OIL AT 5 PPM

Compound	Recovery (%)	%RSD	Blank (ppm)	Spike (ppm)
Acetone	91	14.8	1.9	5.0
Benzene	86	21.3	0.1	0.5
n-Butanol*,**	107	27.8	0.5	5.0
iso-Butanol*,**	95	19.5	0.9	5.0
Carbon tetrachloride	86	44.7	0.0	0.5
Carbon disulfide**	53	22.3	0.0	5.0
Chlorobenzene	81	29.3	0.0	5.0
Chloroform	84	29.3	0.0	6.0
1,4-Dichlorobenzene	98	24.9	0.0	7.5
1,2-Dichloroethane	101	23.1	0.0	0.5
1,1-Dichloroethene	97	45.3	0.0	0.7
Diethyl ether	76	24.3	0.0	5.0
Ethyl acetate	113	27.4	0.0	5.0
Ethylbenzene	83	30.1	0.2	5.0
Hexachloroethane	71	30.3	0.0	3.0
Methylene chloride	98	45.3	0.0	5.0
Methyl ethyl ketone	79	24.6	0.4	5.0
MIBK	93	31.4	0.0	5.0
Nitrobenzene	89	30.3	0.0	2.0
Pyridine	31	35.9	0.0	5.0
Tetrachloroethene	82	27.1	0.0	0.7
Trichlorofluoromethane	76	27.6	0.0	5.0
1,1,2-Cl_3F_3ethane	69	29.2	0.0	5.0
Toluene	73	21.9	0.6	5.0
Trichloroethene	66	28.0	0.0	0.5
Vinyl chloride	63	35.2	0.0	0.2
o-Xylene	83	29.5	0.4	5.0
m/p-Xylene	84	29.5	0.6	10.0

* Alternate mass employed
** IS quantitation
Data are taken from Reference 9.

FIGURE 1.
PURGING DEVICE

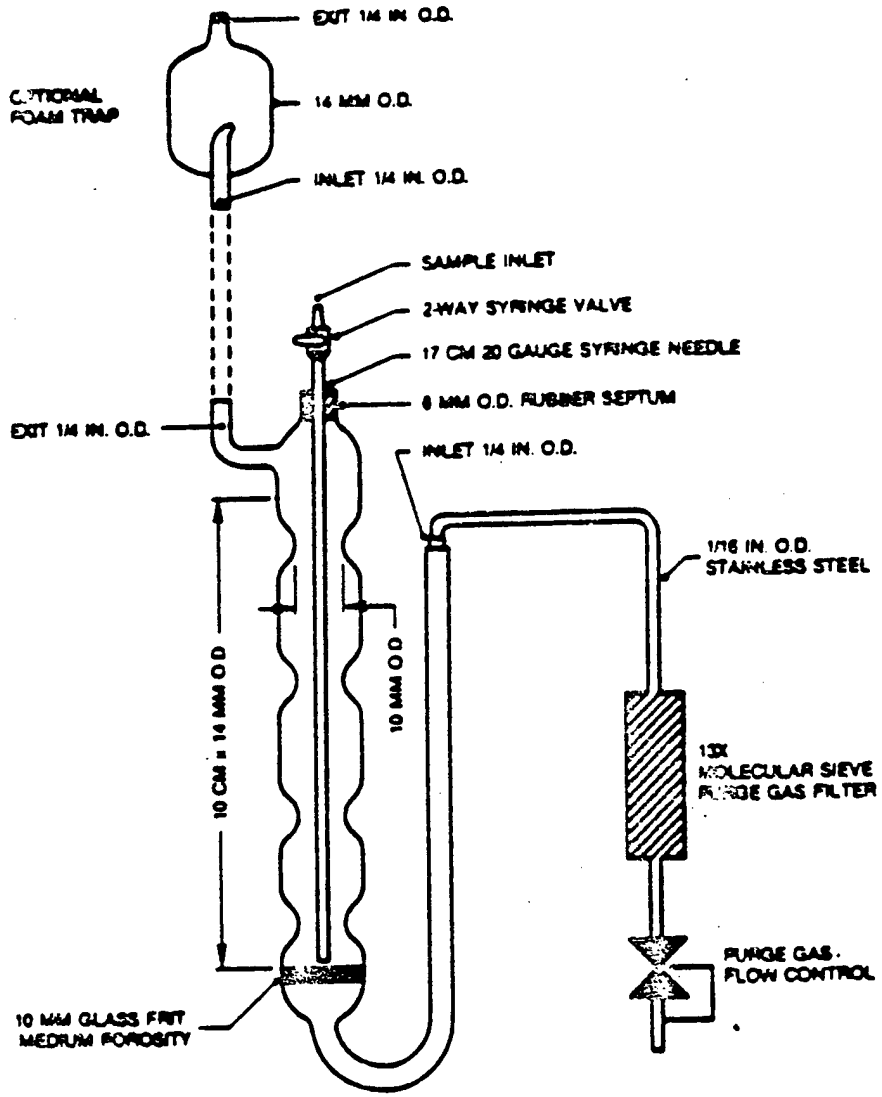


FIGURE 2.
TRAP PACKING AND CONSTRUCTION TO INCLUDE DESORB CAPABILITY

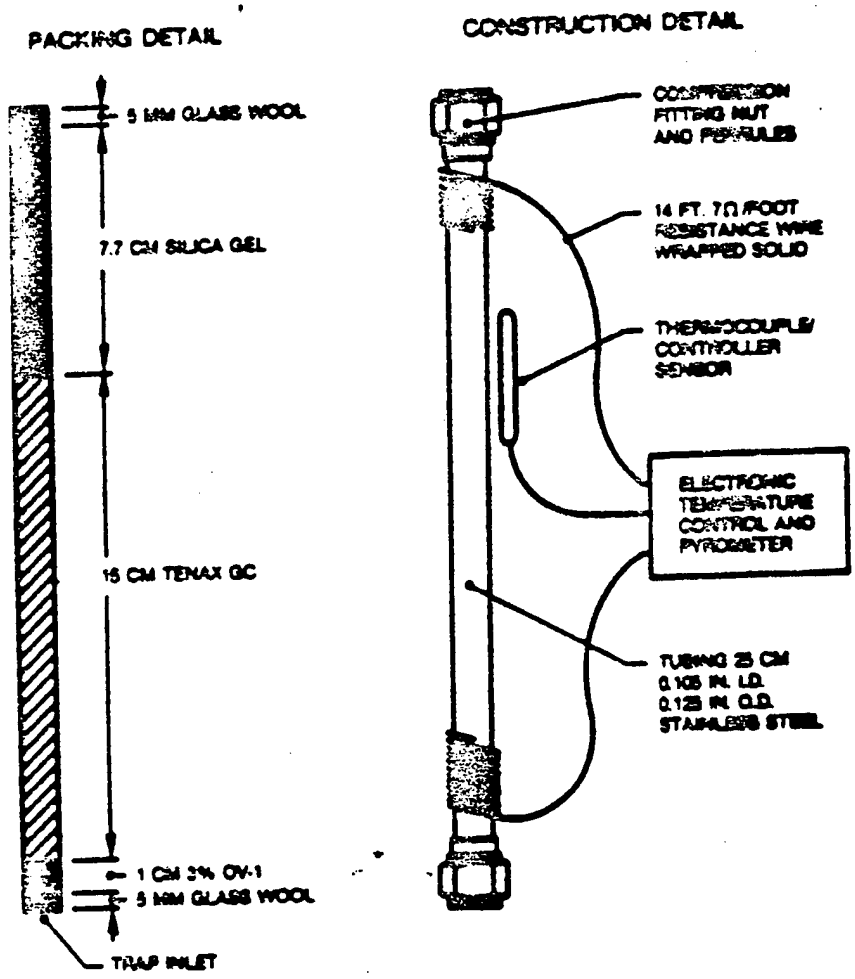


FIGURE 3.
SCHEMATIC OF PURGE-AND-TRAP DEVICE - PURGE MODE

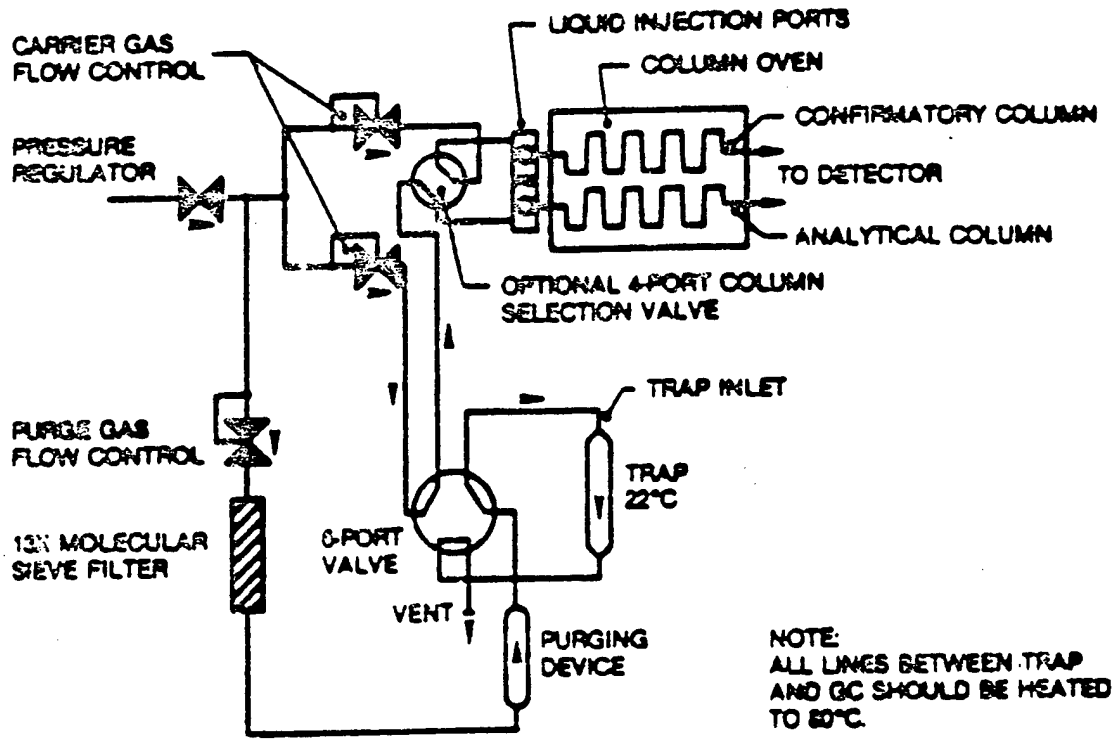


FIGURE 4.
SCHEMATIC OF PURGE-AND-TRAP DEVICE - DESORB MODE

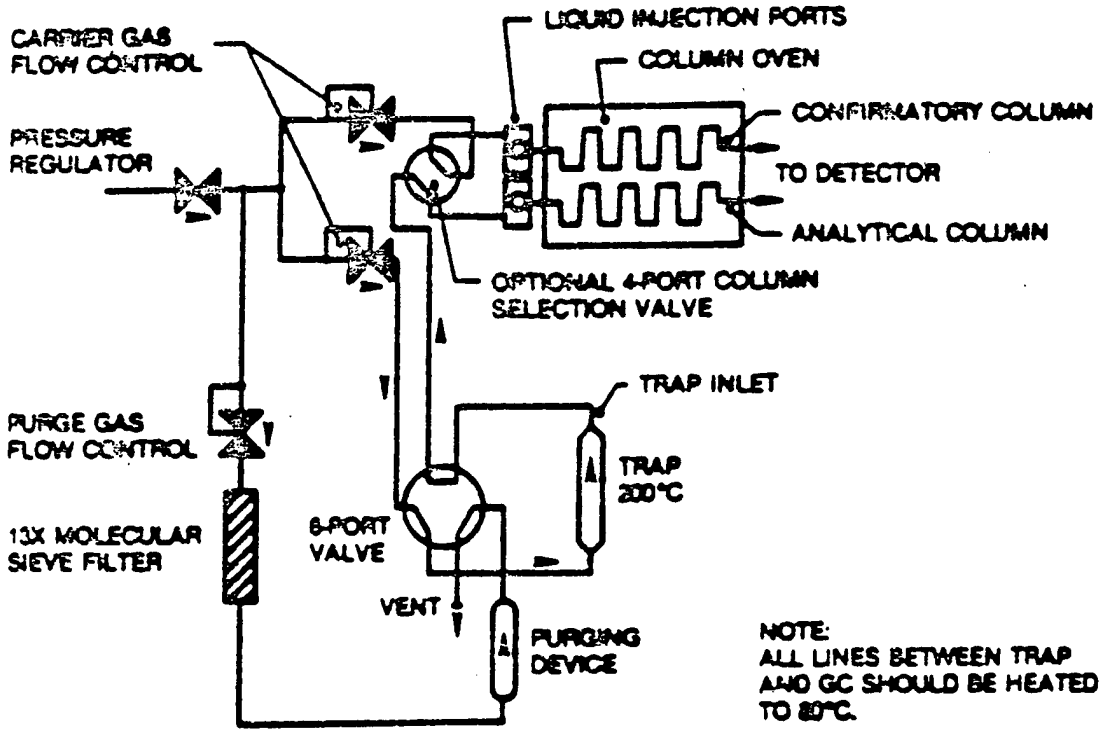


FIGURE 5.
GAS CHROMATOGRAM OF VOLATILE ORGANICS

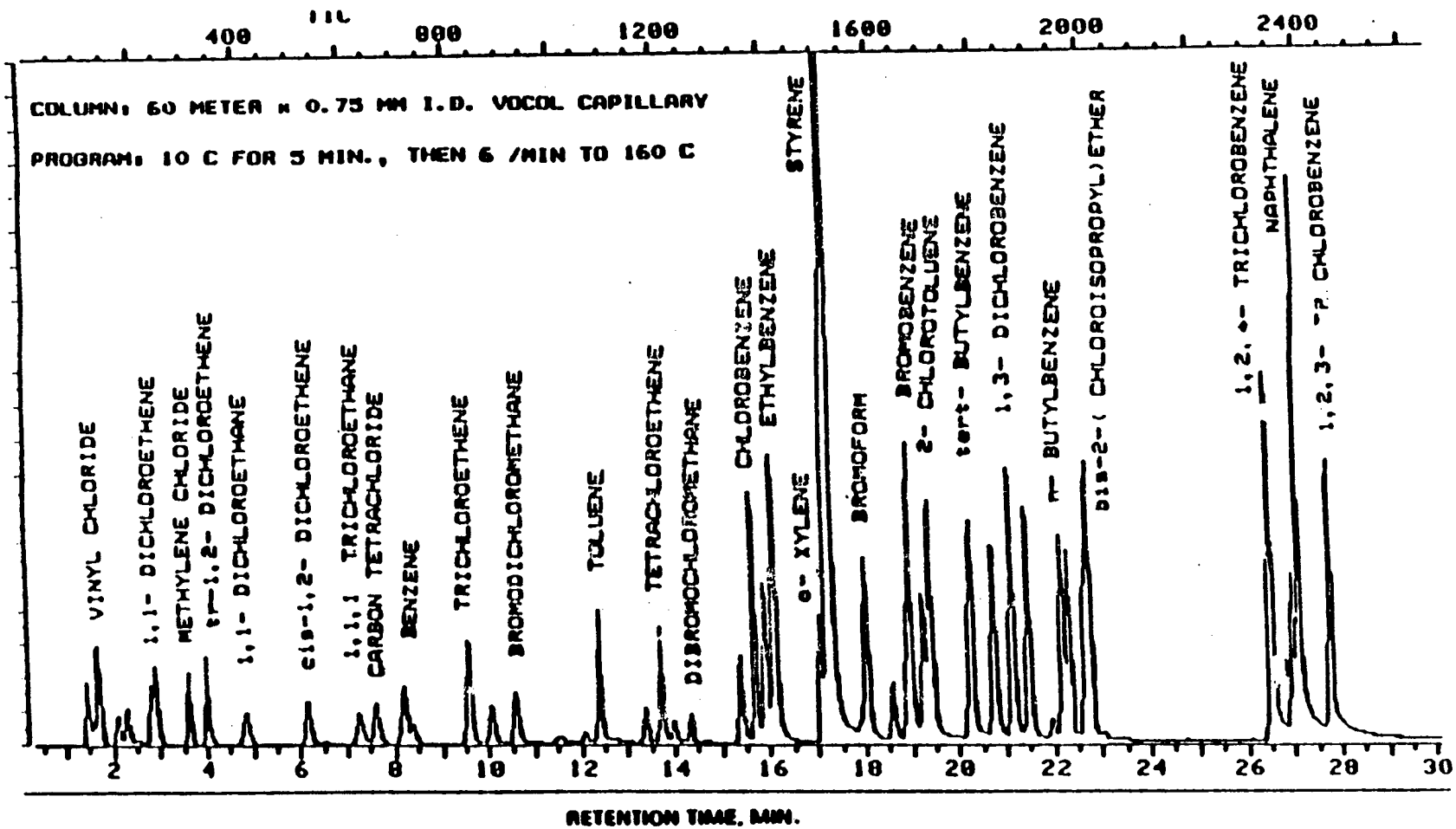


FIGURE
GAS CHROMATOGRAM OF VOLATILE ORGANICS

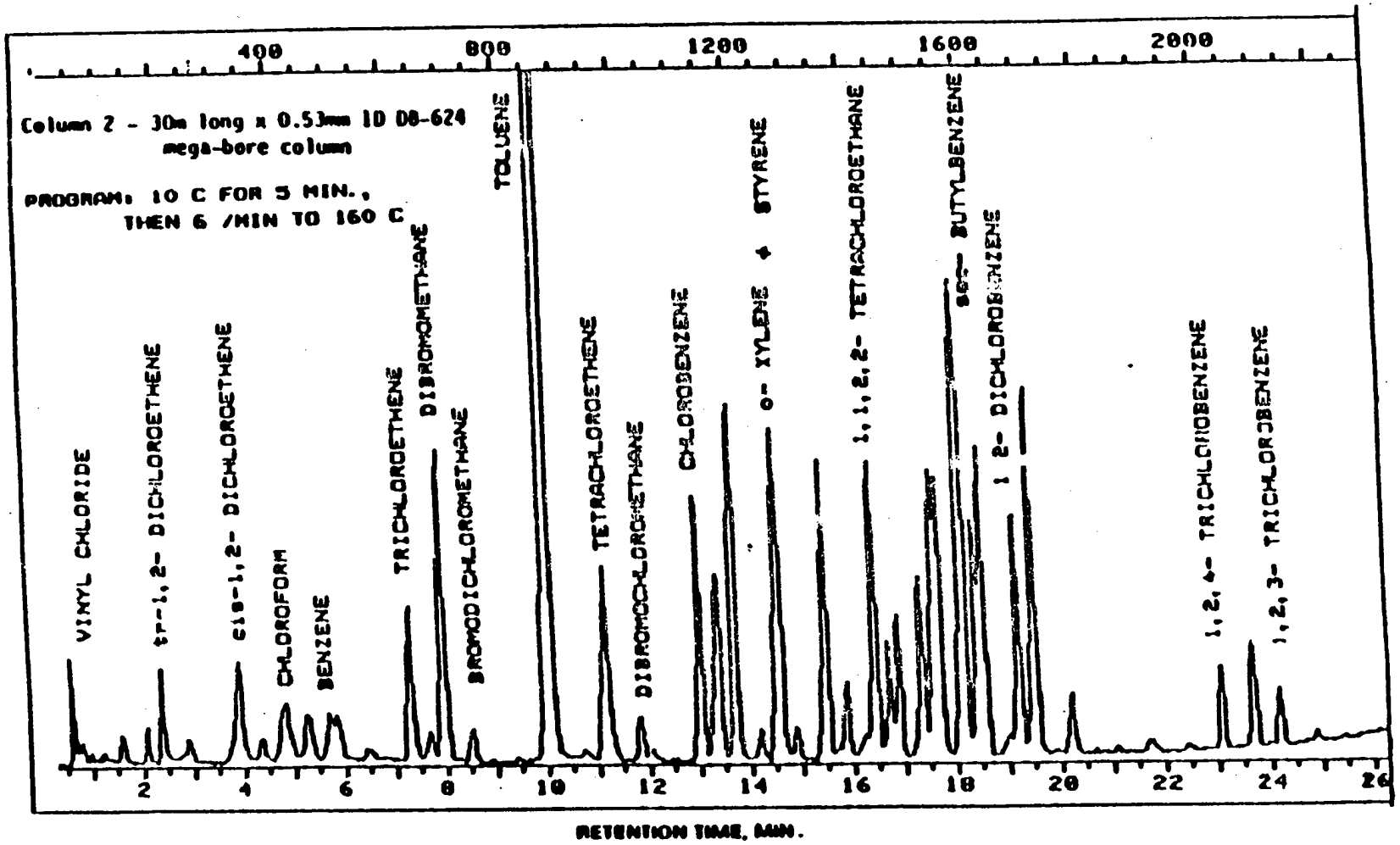


FIGURE
GAS CHROMATOGRAM OF VOLATILE ORGANICS

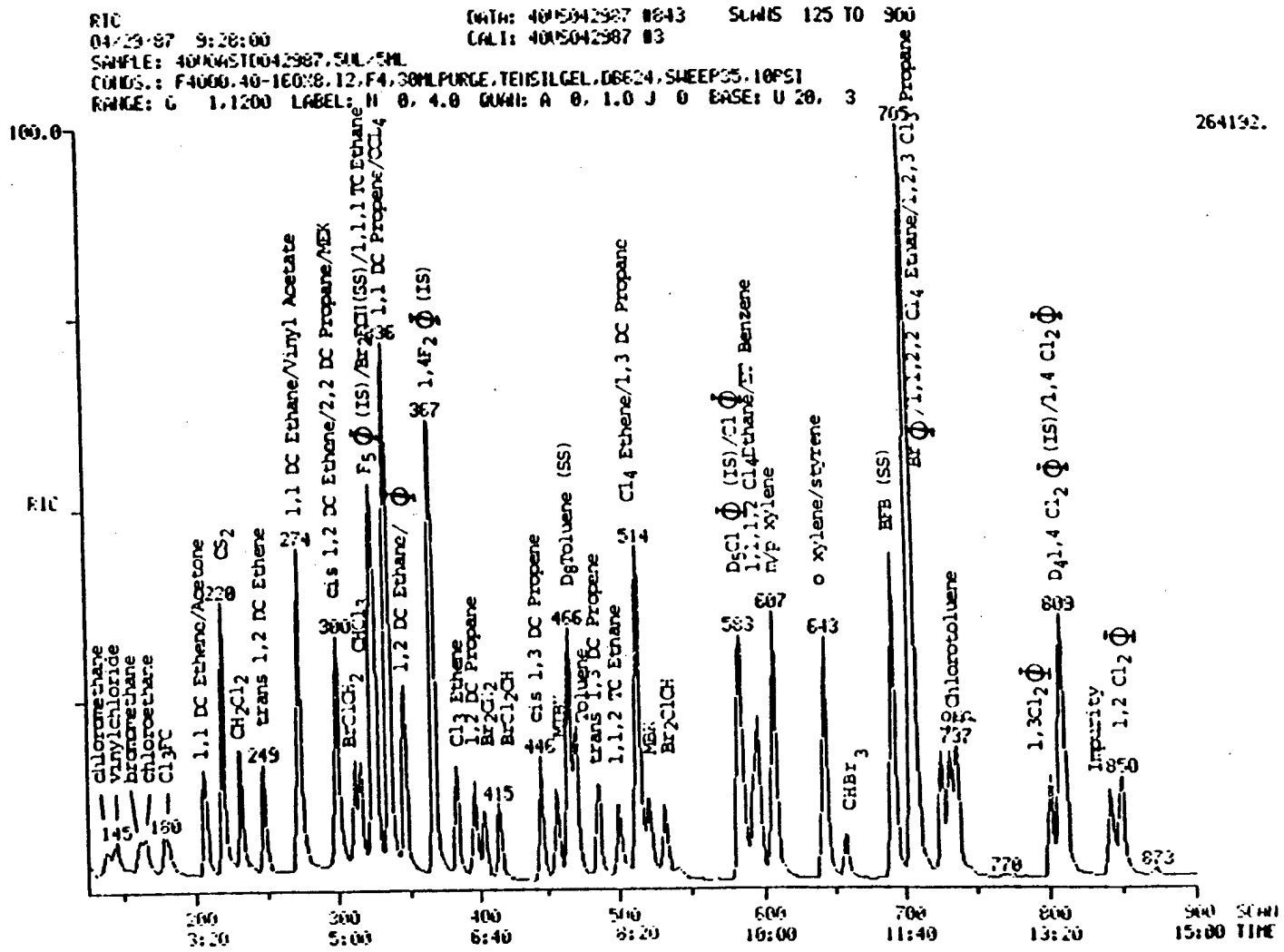
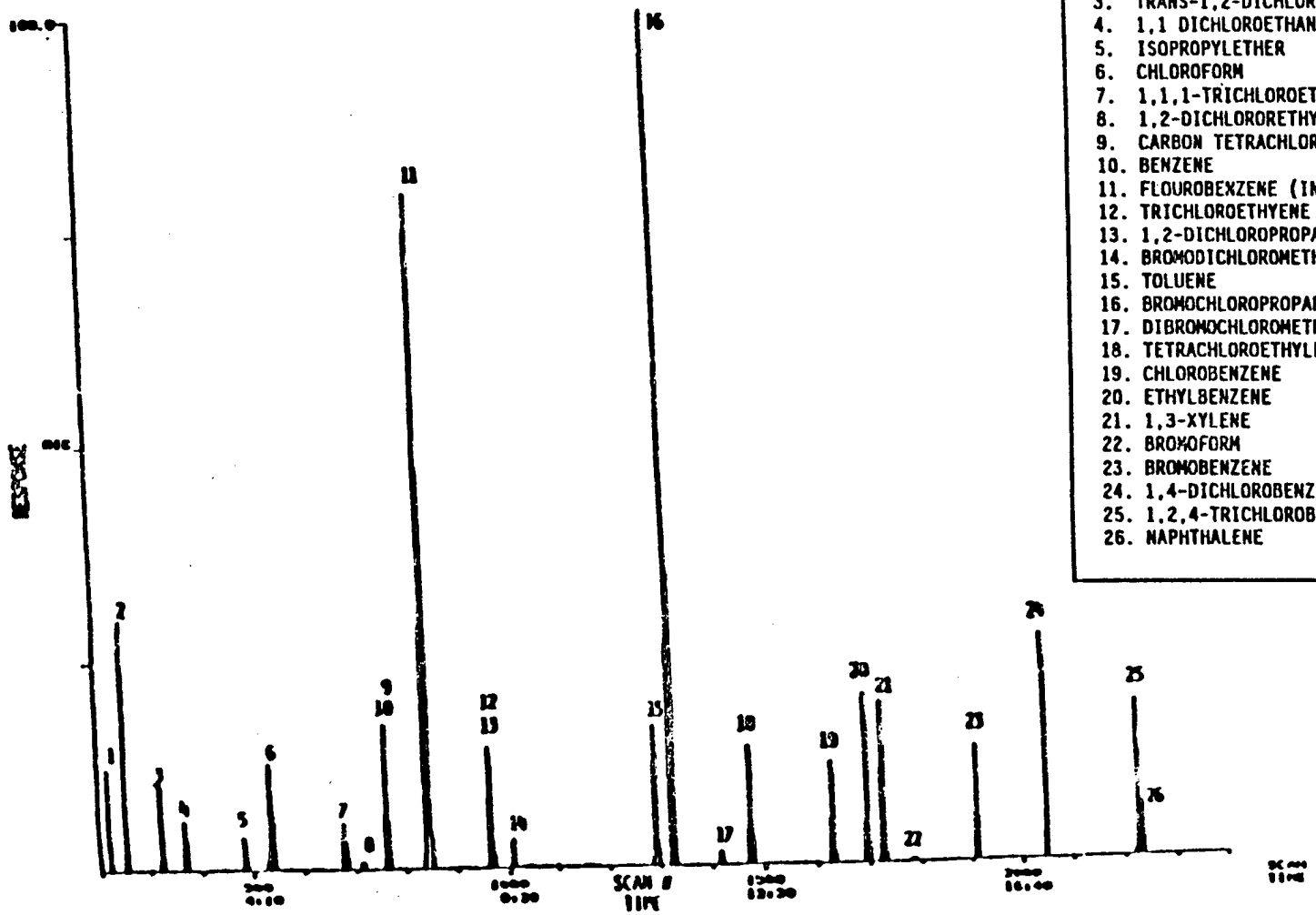


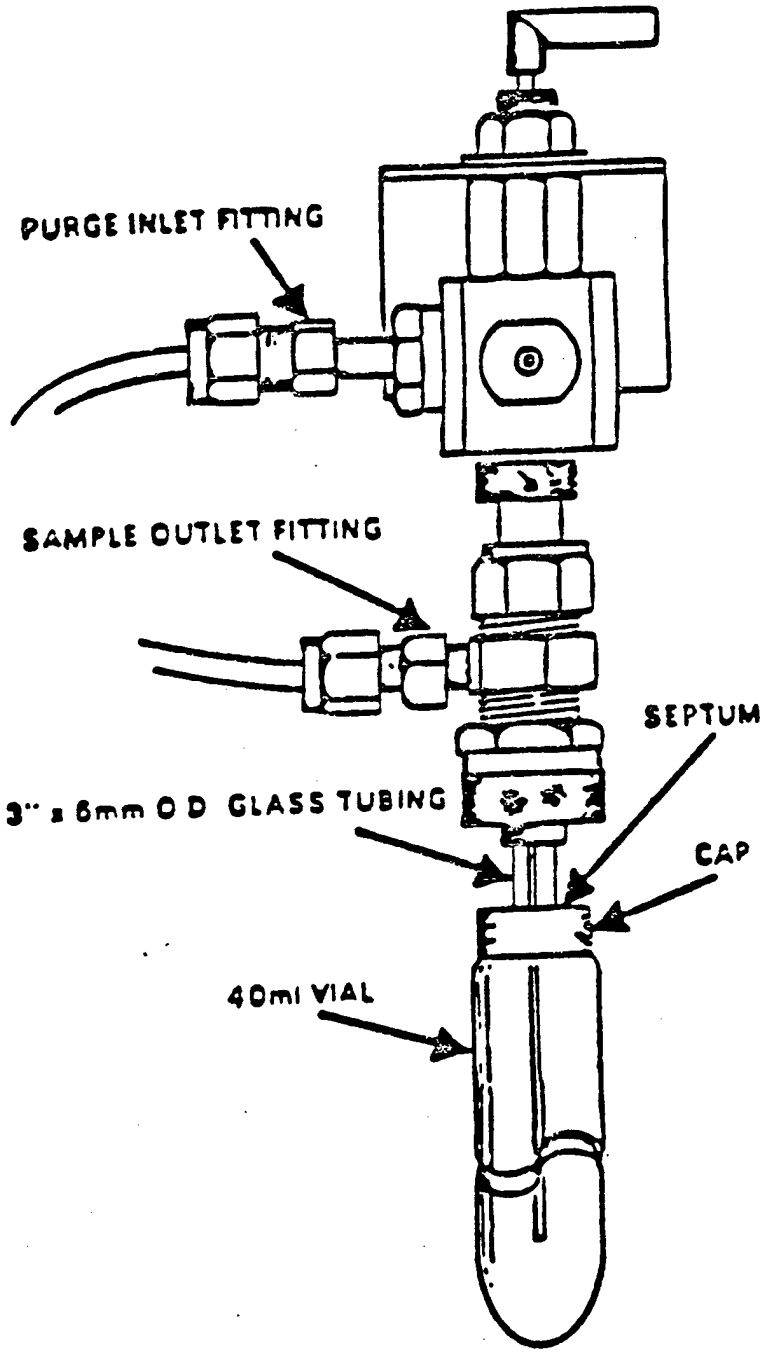
FIGURE
GAS CHROMATOGRAM O[illegible]ST MIXTURE

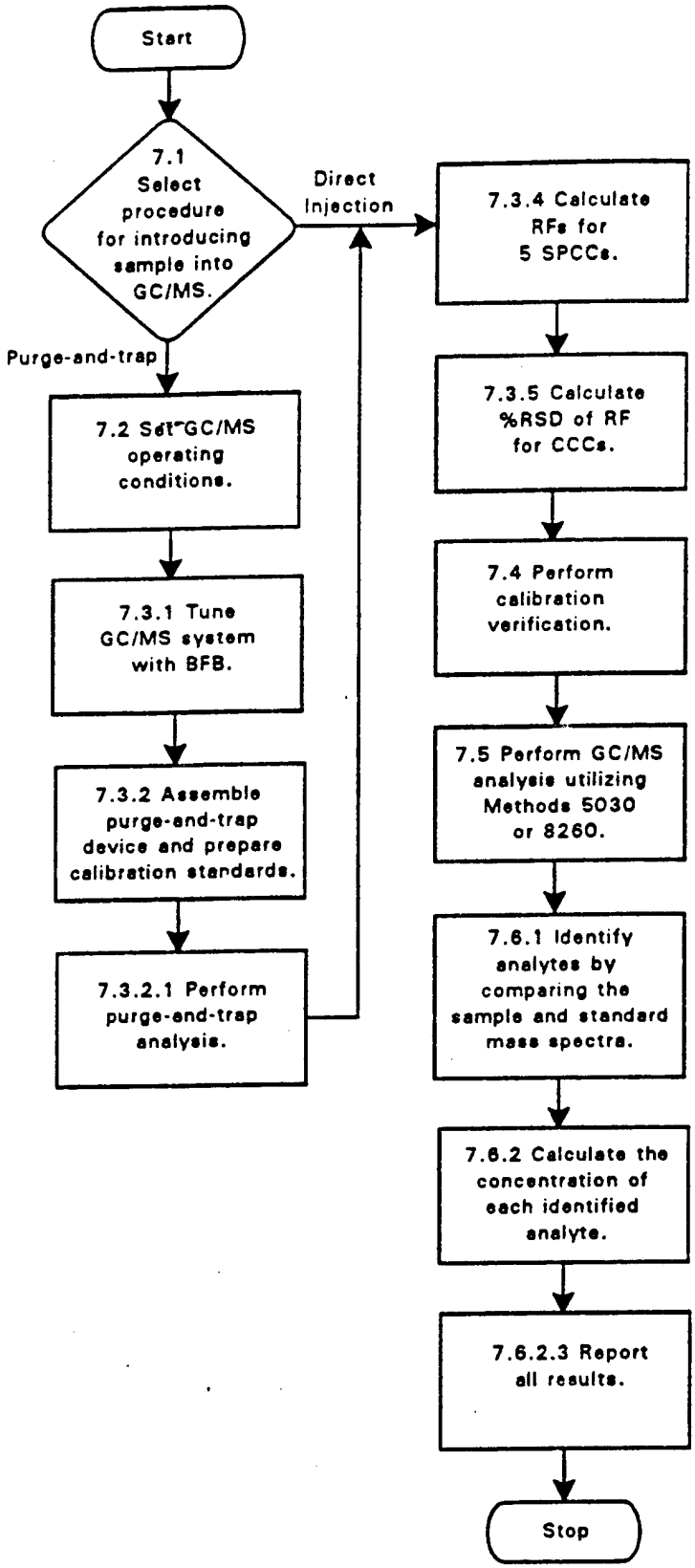


0.5 g/L PER COMPOUND

1. 1,1-DICHLOROETHYLENE
2. METHYLENE CHLORIDE
3. TRANS-1,2-DICHLOROETHYLENE
4. 1,1 DICHLOROETHANE
5. ISOPROPYLETHER
6. CHLOROFORM
7. 1,1,1-TRICHLOROETHANE
8. 1,2-DICHLORORETHYLENE
9. CARBON TETRACHLORIDE
10. BENZENE
11. FLOUROBEXZENE (INT. STD.)
12. TRICHLOROETHYENE
13. 1,2-DICHLOROPROPANE
14. BROMODICHLOROMETHANE
15. TOLUENE
16. BROMOCHLOROPROPANE INT. STD.)
17. DIBROMOCHLOROMETHANE
18. TETRACHLOROETHYLENE
19. CHLOROBENZENE
20. ETHYLBENZENE
21. 1,3-XYLENE
22. BROMOFORM
23. BROMOBENZENE
24. 1,4-DICHLOROBENZENE
25. 1,2,4-TRICHLOROBENZENE
26. NAPHTHALENE

FIGURE 9.
LOW SOILS IMPINGER




Start
7.1 Select procedure for introducing sample into GC/MS.
Direct Injection
Purge-and-trap
7.2 Set GC/MS operating conditions.
7.3.1 Tune GC/MS system with BFB.
7.3.2 Assemble purge-and-trap device and prepare calibration standards.
7.3.2.1 Perform purge-and-trap analysis.
7.3.4 Calculate RFs for 5 SPCCs.
7.3.5 Calculate %RSD of RF for CCCs.
7.4 Perform calibration verification.
7.5 Perform GC/MS analysis utilizing Methods 5030 or 8260.
7.6.1 Identify analytes by comparing the sample and standard mass spectra.
7.6.2 Calculate the concentration of each identified analyte.
7.6.2.3 Report all results.
Stop

Appendix D

Semi-Volatile Organics Compounds - Method 8270 B

METHOD 8270B

SEMIVOLATILE ORGANIC COMPOUNDS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS): CAPILLARY COLUMN TECHNIQUE

1.0 SCOPE AND APPLICATION

1.1 Method 8270 is used to determine the concentration of semivolatile organic compounds in extracts prepared from all types of solid waste matrices, soils, and ground water. Direct injection of a sample may be used in limited applications. The following compounds can be determined by this method:

		Appropriate Preparation Techniques				
Compounds	CAS No[a]	3510	3520	3540/ 3541	3550	3580
Acenaphthene	83-32-9	X	X	X	X	X
Acenaphthene-d_{10} (I.S.)		X	X	X	X	X
Acenaphthylene	208-96-8	X	X	X	X	X
Acetophenone	98-86-2	X	ND	ND	ND	X
2-Acetylaminofluorene	53-96-3	X	ND	ND	ND	X
1-Acetyl-2-thiourea	591-08-2	LR	ND	ND	ND	LR
Aldrin	309-00-2	X	X	X	X	X
2-Aminoanthraquinone	117-79-3	X	ND	ND	ND	X
Aminoazobenzene	60-09-3	X	ND	ND	ND	X
4-Aminobiphenyl	92-67-1	X	ND	ND	ND	X
3-Amino-9-ethylcarbazole	132-32-1	X	X	ND	ND	ND
Anilazine	101-05-3	X	ND	ND	ND	X
Aniline	62-53-3	X	X	ND	X	X
o-Anisidine	90-04-0	X	ND	ND	ND	X
Anthracene	120-12-7	X	X	X	X	X
Aramite	140-57-8	HS(43)	ND	ND	ND	X
Aroclor - 1016	12674-11-2	X	X	X	X	X
Aroclor - 1221	11104-28-2	X	X	X	X	X
Aroclor - 1232	11141-16-5	X	X	X	X	X
Aroclor - 1242	53469-21-9	X	X	X	X	X
Aroclor - 1248	12672-29-6	X	X	X	X	X
Aroclor - 1254	11097-69-1	X	X	X	X	X
Aroclor - 1260	11096-82-5	X	X	X	X	X
Azinphos-methyl	86-50-0	HS(62)	ND	ND	ND	X
Barban	101-27-9	LR	ND	ND	ND	LR
Benzidine	92-87-5	CP	CP	CP	CP	CP
Benzoic acid	65-85-0	X	X	ND	X	X
Benz(a)anthracene	56-55-3	X	X	X	X	X
Benzo(b)fluoranthene	205-99-2	X	X	X	X	X
Benzo(k)fluoranthene	207-08-9	X	X	X	X	X
Benzo(g,h,i)perylene	191-24-2	X	X	X	X	X
Benzo(a)pyrene	50-32-8	X	X	X	X	X

Compounds	CAS No[a]	Appropriate Preparation Techniques 3510	3520	3540/ 3541	3550	3580
p-Benzoquinone	106-51-4	OE	ND	ND	ND	X
Benzyl alcohol	100-51-6	X	X	ND	X	X
α-BHC	319-84-6	X	X	X	X	X
β-BHC	319-85-7	X	X	X	X	X
δ-BHC	319-86-8	X	X	X	X	X
γ-BHC (Lindane)	58-89-9	X	X	X	X	X
Bis(2-chloroethoxy)methane	111-91-1	X	X	X	X	X
Bis(2-chloroethyl) ether	111-44-4	X	X	X	X	X
Bis(2-chloroisopropyl) ether	108-60-1	X	X	X	X	X
Bis(2-ethylhexyl) phthalate	117-81-7	X	X	X	X	X
4-Bromophenyl phenyl ether	101-55-3	X	X	X	X	X
Bromoxynil	1689-84-5	X	ND	ND	ND	X
Butyl benzyl phthalate	85-68-7	X	X	X	X	X
2-sec-Butyl-4,6-dinitrophenol	88-85-7	X	ND	ND	ND	X
Captafol	2425-06-1	HS(55)	ND	ND	ND	X
Captan	133-06-2	HS(40)	ND	ND	ND	X
Carbaryl	63-25-2	X	ND	ND	ND	X
Carbofuran	1563-66-2	X	ND	ND	ND	X
Carbophenothion	786-19-6	X	ND	ND	ND	X
Chlordane	57-74-9	X	X	X	X	X
Chlorfenvinphos	470-90-6	X	ND	ND	ND	X
4-Chloroaniline	106-47-8	X	ND	ND	ND	X
Chlorobenzilate	510-15-6	X	ND	ND	ND	X
5-Chloro-2-methylaniline	95-79-4	X	ND	ND	ND	X
4-Chloro-3-methylphenol	59-50-7	X	X	X	X	X
3-(Chloromethyl)pyridine hydrochloride	6959-48-4	X	ND	ND	ND	X
1-Chloronaphthalene	90-13-1	X	X	X	X	X
2-Chloronaphthalene	91-58-7	X	X	X	X	X
2-Chlorophenol	95-57-8	X	X	X	X	X
4-Chloro-1,2-phenylenediamine	95-83-0	X	X	ND	ND	ND
4-Chloro-1,3-phenylenediamine	5131-60-2	X	X	ND	ND	ND
4-Chlorophenyl phenyl ether	7005-72-3	X	X	X	X	X
Chrysene	218-01-9	X	X	X	X	X
Chrysene-d_{12} (I.S.)		X	X	X	X	X
Coumaphos	56-72-4	X	ND	ND	ND	X
p-Cresidine	120-71-8	X	ND	ND	ND	X
Crotoxyphos	7700-17-6	X	ND	ND	ND	X
2-Cyclohexyl-4,6-dinitro-phenol	131-89-5	X	ND	ND	ND	LR
4,4′-DDD	72-54-8	X	X	X	X	X
4,4′-DDE	72-55-9	X	X	X	X	X
4,4′-DDT	50-29-3	X	X	X	X	X
Demeton-O	298-03-3	HS(68)	ND	ND	ND	X
Demeton-S	126-75-0	X	ND	ND	ND	X
Diallate (cis or trans)	2303-16-4	X	ND	ND	ND	X
2,4-Diaminotoluene	95-80-7	DC,OE(42)	ND	ND	ND	X

Compounds	CAS No*	Appropriate Preparation Techniques				
		3510	3520	3540/ 3541	3550	3580
Dibenz(a,j)acridine	224-42-0	X	ND	ND	ND	X
Dibenz(a,h)anthracene	53-70-3	X	X	X	X	X
Dibenzofuran	132-64-9	X	X	ND	X	X
Dibenzo(a,e)pyrene	192-65-4	ND	ND	ND	ND	X
1,2-Dibromo-3-chloropropane	96-12-8	X	X	ND	ND	ND
Di-n-butyl phthalate	84-74-2	X	X	X	X	X
Dichlone	117-80-6	OE	ND	ND	ND	X
1,2-Dichlorobenzene	95-50-1	X	X	X	X	X
1,3-Dichlorobenzene	541-73-1	X	X	X	X	X
1,4-Dichlorobenzene	106-46-7	X	X	X	X	X
1,4-Dichlorobenzene-d_4 (I.S)		X	X	X	X	X
3,3'-Dichlorobenzidine	91-94-1	X	X	X	X	X
2,4-Dichlorophenol	120-83-2	X	X	X	X	X
2,6-Dichlorophenol	87-65-0	X	ND	ND	ND	X
Dichlorovos	62-73-7	X	ND	ND	ND	X
Dicrotophos	141-66-2	X	ND	ND	ND	X
Dieldrin	60-57-1	X	X	X	X	X
Diethyl phthalate	84-66-2	X	X	X	X	X
Diethylstilbestrol	56-53-1	AW,OS(67)	ND	ND	ND	X
Diethyl sulfate	64-67-5	LR	ND	ND	ND	LR
Dihydrosaffrole	56312-13-1	ND	ND	ND	ND	ND
Dimethoate	60-51-5	HE,HS(31)	ND	ND	ND	X
3,3'-Dimethoxybenzidine	119-90-4	X	ND	ND	ND	LR
Dimethylaminoazobenzene	60-11-7	X	ND	ND	ND	X
7,12-Dimethylbenz(a)-anthracene	57-97-6	CP(45)	ND	ND	ND	CP
3,3'-Dimethylbenzidine	119-93-7	X	ND	ND	ND	X
α,α-Dimethylphenethylamine	122-09-8	ND	ND	ND	ND	X
2,4-Dimethylphenol	105-67-9	X	X	X	X	X
Dimethyl phthalate	131-11-3	X	X	X	X	X
1,2-Dinitrobenzene	528-29-0	X	ND	ND	ND	X
1,3-Dinitrobenzene	99-65-0	X	ND	ND	ND	X
1,4-Dinitrobenzene	100-25-4	HE(14)	ND	ND	ND	X
4,6-Dinitro-2-methylphenol	534-52-1	X	X	X	X	X
2,4-Dinitrophenol	51-28-5	X	X	X	X	X
2,4-Dinitrotoluene	121-14-2	X	X	X	X	X
2,6-Dinitrotoluene	606-20-2	X	X	X	X	X
Dinocap	39300-45-3	CP,HS(28)	ND	ND	ND	CP
Dinoseb	88-85-7	X	ND	ND	ND	X
Dioxathion	78-34-2	ND	ND	ND	ND	ND
Diphenylamine	122-39-4	X	X	X	X	X
5,5-Diphenylhydantoin	57-41-0	X	ND	ND	ND	X
1,2-Diphenylhydrazine	122-66-7	X	X	X	X	X
Di-n-octyl phthalate	117-84-0	X	X	X	X	X
Disulfoton	298-04-4	X	ND	ND	ND	X

Compounds	CAS No[a]	Appropriate Preparation Techniques				
		3510	3520	3540/ 3541	3550	3580
Endosulfan I	959-98-8	X	X	X	X	X
Endosulfan II	33213-65-9	X	X	X	X	X
Endosulfan sulfate	1031-07-8	X	X	X	X	X
Endrin	72-20-8	X	X	X	X	X
Endrin aldehyde	7421-93-4	X	X	X	X	X
Endrin ketone	53494-70-5	X	X	ND	X	X
EPN	2104-64-5	X	ND	ND	ND	X
Ethion	563-12-2	X	ND	ND	ND	X
Ethyl carbamate	51-79-6	DC(28)	ND	ND	ND	X
Ethyl methanesulfonate	62-50-0	X	ND	ND	ND	X
Ethyl parathion	56-38-2	X	X	ND	ND	ND
Famphur	52-85-7	X	ND	ND	ND	X
Fensulfothion	115-90-2	X	ND	ND	ND	X
Fenthion	55-38-9	X	ND	ND	ND	X
Fluchloralin	33245-39-5	X	ND	ND	ND	X
Fluoranthene	206-44-0	X	X	X	X	X
Fluorene	86-73-7	X	X	X	X	X
2-Fluorobiphenyl (surr.)	321-60-8	X	X	X	X	X
2-Fluorophenol (surr.)	367-12-4	X	X	X	X	X
Heptachlor	76-44-8	X	X	X	X	X
Heptachlor epoxide	1024-57-3	X	X	X	X	X
Hexachlorobenzene	118-74-1	X	X	X	X	X
Hexachlorobutadiene	87-68-3	X	X	X	X	X
Hexachlorocyclopentadiene	77-47-4	X	X	X	X	X
Hexachloroethane	67-72-1	X	X	X	X	X
Hexachlorophene	70-30-4	AW,CP(62)	ND	ND	ND	CP
Hexachloropropene	1888-71-7	X	ND	ND	ND	X
Hexamethylphosphoramide	680-31-9	X	ND	ND	ND	X
Hydroquinone	123-31-9	ND	ND	ND	ND	X
Indeno(1,2,3-cd)pyrene	193-39-5	X	X	X	X	X
Isodrin	465-73-6	X	ND	ND	ND	X
Isophorone	78-59-1	X	X	X	X	X
Isosafrole	120-58-1	DC(46)	ND	ND	ND	X
Kepone	143-50-0	X	ND	ND	ND	X
Leptophos	21609-90-5	X	ND	ND	ND	X
Malathion	121-75-5	HS(5)	ND	ND	ND	X
Maleic anhydride	108-31-6	HE	ND	ND	ND	X
Mestranol	72-33-3	X	ND	ND	ND	X
Methapyrilene	91-80-5	X	ND	ND	ND	X
Methoxychlor	72-43-5	X	ND	ND	ND	X
3-Methylcholanthrene	56-49-5	X	ND	ND	ND	X
4,4'-Methylenebis (2-chloroaniline)	101-14-4	OE,OS(0)	ND	ND	ND	LR
4,4'-Methylenebis (N,N-dimethylaniline)	101-61-1	X	X	ND	ND	ND

Compounds	CAS No[a]	Appropriate Preparation Techniques 3510	3520	3540/ 3541	3550	3580
Methyl methanesulfonate	66-27-3	X	ND	ND	ND	X
2-Methylnaphthalene	91-57-6	X	X	ND	X	X
2-Methyl-5-nitroaniline	99-55-8	X	X	ND	ND	ND
Methyl parathion	298-00-0	X	ND	ND	ND	X
2-Methylphenol	95-48-7	X	ND	ND	ND	X
3-Methylphenol	108-39-4	X	ND	ND	ND	X
4-Methylphenol	106-44-5	X	ND	ND	ND	X
2-Methylpyridine	109-06-8	X	X	ND	ND	ND
Mevinphos	7786-34-7	X	ND	ND	ND	X
Mexacarbate	315-18-4	HE,HS(68)	ND	ND	ND	X
Mirex	2385-85-5	X	ND	ND	ND	X
Monocrotophos	6923-22-4	HE	ND	ND	ND	X
Naled	300-76-5	X	ND	ND	ND	X
Naphthalene	91-20-3	X	X	X	X	X
Naphthalene-d_8 (I.S.)		X	X	X	X	X
1,4-Naphthoquinone	130-15-4	X	ND	ND	ND	X
1-Naphthylamine	134-32-7	OS(44)	ND	ND	ND	X
2-Naphthylamine	91-59-8	X	ND	ND	ND	X
Nicotine	54-11-5	DE(67)	ND	ND	ND	X
5-Nitroacenaphthene	602-87-9	X	ND	ND	ND	X
2-Nitroaniline	88-74-4	X	X	ND	X	X
3-Nitroaniline	99-09-2	X	X	ND	X	X
4-Nitroaniline	100-01-6	X	X	ND	X	X
5-Nitro-o-anisidine	99-59-2	X	ND	ND	ND	X
Nitrobenzene	98-95-3	X	X	X	X	X
Nitrobenzene-d_5 (surr.)		X	X	X	X	X
4-Nitrobiphenyl	92-93-3	X	ND	ND	ND	X
Nitrofen	1836-75-5	X	ND	ND	ND	X
2-Nitrophenol	88-75-5	X	X	X	X	X
4-Nitrophenol	100-02-7	X	X	X	X	X
5-Nitro-o-toluidine	99-55-8	X	ND	ND	ND	X
Nitroquinoline-1-oxide	56-57-5	X	ND	ND	ND	X
N-Nitrosodibutylamine	924-16-3	X	ND	ND	ND	X
N-Nitrosodiethylamine	55-18-5	X	ND	ND	ND	X
N-Nitrosodimethylamine	62-75-9	X	X	X	X	X
N-Nitrosomethylethylamine	10595-95-6	X	ND	ND	ND	X
N-Nitrosodiphenylamine	86-30-6	X	X	X	X	X
N-Nitrosodi-n-propylamine	621-64-7	X	X	X	X	X
N-Nitrosomorpholine	59-89-2	ND	ND	ND	ND	X
N-Nitrosopiperidine	100-75-4	X	ND	ND	ND	X
N-Nitrosopyrrolidine	930-55-2	X	ND	ND	ND	X
Octamethyl pyrophosphoramide	152-16-9	LR	ND	ND	ND	LR
4,4'-Oxydianiline	101-80-4	X	ND	ND	ND	X
Parathion	56-38-2	X	ND	ND	ND	X
Pentachlorobenzene	608-93-5	X	ND	ND	ND	X

Compounds	CAS No[a]	Appropriate Preparation Techniques 3510	3520	3540/ 3541	3550	3580
Pentachloronitrobenzene	82-68-8	X	ND	ND	ND	X
Pentachlorophenol	87-86-5	X	X	X	X	X
Perylene-d_{12} (I.S.)		X	X	X	X	X
Phenacetin	62-44-2	X	ND	ND	ND	X
Phenanthrene	85-01-8	X	X	X	X	X
Phenanthrene-d_{10} (I.S.)		X	X	X	X	X
Phenobarbital	50-06-6	X	ND	ND	ND	X
Phenol	108-95-2	DC(28)	X	X	X	X
Phenol-d_6 (surr.)		DC(28)	X	X	X	X
1,4-Phenylenediamine	106-50-3	X	ND	ND	ND	X
Phorate	298-02-2	X	ND	ND	ND	X
Phosalone	2310-17-0	HS(65)	ND	ND	ND	X
Phosmet	732-11-6	HS(15)	ND	ND	ND	X
Phosphamidon	13171-21-6	HE(63)	ND	ND	ND	X
Phthalic anhydride	85-44-9	CP,HE(1)	ND	ND	ND	CP
2-Picoline	109-06-8	ND	ND	ND	ND	ND
Piperonyl sulfoxide	120-62-7	X	ND	ND	ND	X
Pronamide	23950-58-5	X	ND	ND	ND	X
Propylthiouracil	51-52-5	LR	ND	ND	ND	LR
Pyrene	129-00-0	X	X	X	X	X
Pyridine	110-86-1	ND	ND	ND	ND	ND
Resorcinol	108-46-3	DC,OE(10)	ND	ND	ND	X
Safrole	94-59-7	X	ND	ND	ND	X
Strychnine	60-41-3	AW,OS(55)	ND	ND	ND	X
Sulfallate	95-06-7	X	ND	ND	ND	X
Terbufos	13071-79-9	X	ND	ND	ND	X
Terphenyl-d_{14}(surr.)	1718-51-0	X	X	ND	X	X
1,2,4,5-Tetrachlorobenzene	95-94-3	X	ND	ND	ND	X
2,3,4,6-Tetrachlorophenol	58-90-2	X	ND	ND	ND	X
Tetrachlorvinphos	961-11-5	X	ND	ND	ND	X
Tetraethyl dithiopyrophosphate	3689-24-5	X	X	ND	ND	ND
Tetraethyl pyrophosphate	107-49-3	X	ND	ND	ND	X
Thionazine	297-97-2	X	ND	ND	ND	X
Thiophenol (Benzenethiol)	108-98-5	X	ND	ND	ND	X
Toluene diisocyanate	584-84-9	HE(6)	ND	ND	ND	X
o-Toluidine	95-53-4	X	ND	ND	ND	X
Toxaphene	8001-35-2	X	X	X	X	X
2,4,6-Tribromophenol (surr.)		X	X	X	X	X
1,2,4-Trichlorobenzene	120-82-1	X	X	X	X	X
2,4,5-Trichlorophenol	95-95-4	X	X	ND	X	X
2,4,6-Trichlorophenol	88-06-2	X	X	X	X	X
Trifluralin	1582-09-8	X	ND	ND	ND	X
2,4,5-Trimethylaniline	137-17-7	X	ND	ND	ND	X
Trimethyl phosphate	512-56-1	HE(60)	ND	ND	ND	X

Compounds	CAS No[a]	Appropriate Preparation Techniques 3510	3520	3540/ 3541	3550	3580
1,3,5-Trinitrobenzene	99-35-4	X	ND	ND	ND	X
Tris(2,3-dibromopropyl) phosphate	126-72-7	X	ND	ND	ND	LR
Tri-p-tolyl phosphate	78-32-0	X	ND	ND	ND	X
0,0,0-Triethyl phosphorothioate	126-68-1	X	ND	ND	ND	X

a Chemical Abstract Service Registry Number.

AW = Adsorption to walls of glassware during extraction and storage.
CP = Nonreproducible chromatographic performance.
DC = Unfavorable distribution coefficient (number in parenthesis is percent recovery).
HE = Hydrolysis during extraction accelerated by acidic or basic conditions (number in parenthesis is percent recovery).
HS = Hydrolysis during storage (number in parenthesis is percent stability).
LR = Low response.
ND = Not determined.
OE = Oxidation during extraction accelerated by basic conditions (number in parenthesis is percent recovery).
OS = Oxidation during storage (number in parenthesis is percent stability).
X = Greater than 70 percent recovery by this technique.

1.2 Method 8270 can be used to quantitate most neutral, acidic, and basic organic compounds that are soluble in methylene chloride and capable of being eluted without derivatization as sharp peaks from a gas chromatographic fused-silica capillary column coated with a slightly polar silicone. Such compounds include polynuclear aromatic hydrocarbons, chlorinated hydrocarbons and pesticides, phthalate esters, organophosphate esters, nitrosamines, haloethers, aldehydes, ethers, ketones, anilines, pyridines, quinolines, aromatic nitro compounds, and phenols, including nitrophenols. See Table 1 for a list of compounds and their characteristic ions that have been evaluated on the specified GC/MS system.

1.3 The following compounds may require special treatment when being determined by this method. Benzidine can be subject to oxidative losses during solvent concentration. Also, chromatography is poor. Under the alkaline conditions of the extraction step, α-BHC, γ-BHC, Endosulfan I and II, and Endrin are subject to decomposition. Neutral extraction should be performed if these compounds are expected. Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition. N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described. N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine. Pentachlorophenol, 2,4-dinitrophenol,

4-nitrophenol, 4,6-dinitro-2-methylphenol, 4-chloro-3-methylphenol, benzoic acid, 2-nitroaniline, 3-nitroaniline, 4-chloroaniline, and benzyl alcohol are subject to erratic chromatographic behavior, especially if the GC system is contaminated with high boiling material.

1.4 The estimated quantitation limit (EQL) of Method 8270 for determining an individual compound is approximately 1 mg/kg (wet weight) for soil/sediment samples, 1-200 mg/kg for wastes (dependent on matrix and method of preparation), and 10 µg/L for ground water samples (see Table 2). EQLs will be proportionately higher for sample extracts that require dilution to avoid saturation of the detector.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Prior to using this method, the samples should be prepared for chromatography using the appropriate sample preparation and cleanup methods. This method describes chromatographic conditions that will allow for the separation of the compounds in the extract and for their qualitative and quantitative analysis by mass spectrometry.

3.0 INTERFERENCES

3.1 Raw GC/MS data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

3.2 Contamination by carryover can occur whenever high-concentration and low-concentration samples are sequentially analyzed. To reduce carryover, the sample syringe must be rinsed out between samples with solvent. Whenever an unusually concentrated sample is encountered, it should be followed by the analysis of solvent to check for cross contamination.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph/mass spectrometer system

4.1.1 Gas chromatograph - An analytical system complete with a temperature-programmable gas chromatograph suitable for splitless injection and all required accessories, including syringes, analytical columns, and gases. The capillary column should be directly coupled to the source.

4.1.2 Column - 30 m x 0.25 mm ID (or 0.32 mm ID) 1 μm film thickness silicone-coated fused-silica capillary column (J&W Scientific DB-5 or equivalent).

4.1.3 Mass spectrometer - Capable of scanning from 35 to 500 amu every 1 sec or less, using 70 volts (nominal) electron energy in the electron impact ionization mode. The mass spectrometer must be capable of producing a mass spectrum for decafluorotriphenylphosphine (DFTPP) which meets all of the criteria in Table 3 when 1 μL of the GC/MS tuning standard is injected through the GC (50 ng of DFTPP).

4.1.4 GC/MS interface - Any GC-to-MS interface that gives acceptable calibration points at 50 ng per injection for each compound of interest and achieves acceptable tuning performance criteria may be used. For a narrow-bore capillary column, the interface is usually capillary-direct into the mass spectrometer source.

4.1.5 Data system - A computer system must be interfaced to the mass spectrometer. The system must allow the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer must have software that can search any GC/MS data file for ions of a specific mass and that can plot such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. The most recent version of the EPA/NIST Mass Spectral Library should also be available.

4.1.6 Guard column (optional) (J&W Deactivated Fused Silica, 0.25 mm ID x 6 m, or equivalent) between the injection port and the analytical column joined with column joiners (Hewlett Packard No. 5062-3556, or equivalent).

4.2 Syringe - 10 μL.

4.3 Volumetric flasks, Class A - Appropriate sizes with ground glass stoppers.

4.4 Balance - Analytical, 0.0001 g.

4.5 Bottles - glass with Teflon-lined screw caps or crimp tops.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Stock standard solutions (1000 mg/L) - Standard solutions can be prepared from pure standard materials or purchased as certified solutions.

5.3.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality acetone or other suitable solvent and dilute to volume in a 10 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

5.3.2 Transfer the stock standard solutions into bottles with Teflon lined screw-caps. Store at -10°C to -20°C or less and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

5.3.3 Stock standard solutions must be replaced after 1 year or sooner if comparison with quality control check samples indicates a problem.

5.4 Internal standard solutions - The internal standards recommended are 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12} (see Table 5). Other compounds may be used as internal standards as long as the requirements given in Sec. 7.3.2 are met. Dissolve 0.200 g of each compound with a small volume of carbon disulfide. Transfer to a 50 mL volumetric flask and dilute to volume with methylene chloride so that the final solvent is approximately 20% carbon disulfide. Most of the compounds are also soluble in small volumes of methanol, acetone, or toluene, except for perylene-d_{12}. The resulting solution will contain each standard at a concentration of 4,000 ng/µL. Each 1 mL sample extract undergoing analysis should be spiked with 10 µL of the internal standard solution, resulting in a concentration of 40 ng/µL of each internal standard. Store at -10°C to -20°C or less when not being used.

5.5 GC/MS tuning standard - A methylene chloride solution containing 50 ng/µL of decafluorotriphenylphosphine (DFTPP) should be prepared. The standard should also contain 50 ng/µL each of 4,4'-DDT, pentachlorophenol, and benzidine to verify injection port inertness and GC column performance. Store at -10°C to -20°C or less when not being used.

5.6 Calibration standards - A minimum of five calibration standards should be prepared. One of the calibration standards should be at a concentration near, but above, the method detection limit; the others should correspond to the range of concentrations found in real samples but should not exceed the working range of the GC/MS system. Each standard should contain each analyte for detection by this method (e.g. some or all of the compounds listed in Table 1 may be included). Each 1 mL aliquot of calibration standard should

be spiked with 10 µL of the internal standard solution prior to analysis. All standards should be stored at -10°C to -20°C or less, and should be freshly prepared once a year, or sooner if check standards indicate a problem. The daily calibration standard should be prepared weekly and stored at 4°C.

5.7 Surrogate standards - The recommended surrogate standards are phenol-d_6, 2-fluorophenol, 2,4,6-tribromophenol, nitrobenzene-d_5, 2-fluorobiphenyl, and p-terphenyl-d_{14}. See Method 3500 for the instructions on preparing the surrogate standards. Determine what concentration should be in the blank extracts after all extraction, cleanup, and concentration steps. Inject this concentration into the GC/MS to determine recovery of surrogate standards in all blanks, spikes, and sample extracts. Take into account all dilutions of sample extracts.

5.8 Matrix spike standards - See Method 3500 for instructions on preparing the matrix spike standard. Determine what concentration should be in the blank extracts after all extraction, cleanup, and concentration steps. Inject this concentration into the GC/MS to determine recovery of surrogate standards in all matrix spikes. Take into account all dilutions of sample extracts.

5.9 Acetone, hexane, methylene chloride, isooctane, carbon disulfide, toluene, and other appropriate solvents - Pesticide quality or equivalent

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

7.0 PROCEDURE

7.1 Sample preparation - Samples must be prepared by one of the following methods prior to GC/MS analysis.

Matrix	Methods
Water	3510, 3520
Soil/sediment	3540, 3541, 3550
Waste	3540, 3541, 3550, 3580

7.1.1 Direct injection - In very limited applications direct injection of the sample into the GC/MS system with a 10 µL syringe may be appropriate. The detection limit is very high (approximately 10,000 µg/L); therefore, it is only permitted where concentrations in excess of 10,000 µg/L are expected. The system must be calibrated by direct injection.

7.2 Extract cleanup - Extracts may be cleaned up by any of the following methods prior to GC/MS analysis.

Compounds	Methods
Phenols	3630, 3640, 8040[a]
Phthalate esters	3610, 3620, 3640
Nitrosamines	3610, 3620, 3640
Organochlorine pesticides & PCBs	3620, 3660
Nitroaromatics and cyclic ketones	3620, 3640
Polynuclear aromatic hydrocarbons	3611, 3630, 3640
Haloethers	3620, 3640
Chlorinated hydrocarbons	3620, 3640
Organophosphorus pesticides	3620
Petroleum waste	3611, 3650
All priority pollutant base, neutral, and acids	3640

- Method 8040 includes a derivatization technique followed by GC/ECD analysis, if interferences are encountered on GC/FID.

7.3 Initial calibration - The recommended GC/MS operating conditions:

Mass range:	35-500 amu
Scan time:	1 sec/scan
Initial temperature:	40°C, hold for 4 minutes
Temperature program:	40-270°C at 10°C/min
Final temperature:	270°C, hold until benzo[g,h,i]perylene has eluted
Injector temperature:	250-300°C
Transfer line temperature:	250-300°C
Source temperature:	According to manufacturer's specifications
Injector:	Grob-type, splitless
Sample volume:	1-2 µL
Carrier gas:	Hydrogen at 50 cm/sec or helium at 30 cm/sec

(Split injection is allowed if the sensitivity of the mass spectrometer is sufficient).

7.3.1 Each GC/MS system must be hardware-tuned to meet the criteria in Table 3 for a 50 ng injection of DFTPP. Analyses should not begin until all these criteria are met. Background subtraction should be straightforward and designed only to eliminate column bleed or instrument background ions. The GC/MS tuning standard should also be used to assess GC column performance and injection port inertness. Degradation of DDT to DDE and DDD should not exceed 20%. (See Sec. 8.3.1 of Method 8081 for the percent breakdown calculation). Benzidine and pentachlorophenol should be present at their normal responses, and no peak tailing should be visible. If degradation is excessive and/or poor chromatography is noted, the injection port may require cleaning. It may also be necessary to break off the first 6-12 in. of the capillary column. The use of a guard column (Sec. 4.1.6) between the injection port and the analytical column may help prolong analytical column performance.

7.3.2 The internal standards selected in Sec. 5.4 should permit most of the components of interest in a chromatogram to have retention times of 0.80-1.20 relative to one of the internal standards. Use the base peak ion from the specific internal standard as the primary ion for quantitation (see Table 1). If interferences are noted, use the next most intense ion as the quantitation ion (i.e. for 1,4-dichlorobenzene-d_4, use 152 m/z for quantitation).

7.3.3 Analyze 1 μL of each calibration standard (containing internal standards) and tabulate the area of the primary characteristic ion against concentration for each compound (as indicated in Table 1). Figure 1 shows a chromatogram of a calibration standard containing base/neutral and acid analytes. Calculate response factors (RFs) for each compound relative to one of the internal standards as follows:

$$RF = (A_x C_{is})/(A_{is} C_x)$$

where:

A_x = Area of the characteristic ion for the compound being measured.

A_{is} = Area of the characteristic ion for the specific internal standard.

C_{is} = Concentration of the specific internal standard (ng/μL).

C_x = Concentration of the compound being measured (ng/μL).

7.3.4 A system performance check must be performed to ensure that minimum average RFs are met before the calibration curve is used. For semivolatiles, the System Performance Check Compounds (SPCCs) are: N-nitroso-di-n-propylamine;hexachlorocyclopentadiene;2,4-dinitro-phenol; and 4-nitrophenol. The minimum acceptable average RF for these compounds is 0.050. These SPCCs typically have very low RFs (0.1-0.2) and tend to decrease in response as the chromatographic system begins to deteriorate or the standard material begins to deteriorate. They are usually the first to show poor performance. Therefore, they must meet the minimum requirement when the system is calibrated.

7.3.4.1 The percent relative standard deviation (%RSD) should be less than 15% for each compound. However, the %RSD for each individual Calibration Check Compound (CCC) (see Table 4) must be less than 30%. The relative retention times of each compound in each calibration run should agree within 0.06 relative retention time units. Late-eluting compounds usually have much better agreement.

$$\%RSD = \frac{SD}{\overline{RF}} \times 100$$

where:

RSD = relative standard deviation.

$\overline{RF}$ = mean of 5 initial RFs for a compound.

SD = standard deviation of average RFs for a compound.

$$SD = \sqrt{\sum_{i=1}^{N} \frac{(RF_i - \overline{RF})^2}{N - 1}}$$

where:

RF_i = RF for each of the 5 calibration levels
N = Number of RF values (i.e., 5)

7.3.4.2 If the %RSD of any CCC is 30% or greater, then the chromatographic system is too reactive for analysis to begin. Clean or replace the injector liner and/or capillary column, then repeat the calibration procedure beginning with section 7.3.

7.3.5 Linearity - If the %RSD of any compound is 15% or less, then the relative response factor is assumed to be constant over the calibration range, and the average relative response factor may be used for quantitation (Sec. 7.6.2).

7.3.5.1 If the %RSD of any compound is greater than 15%, construct calibration curves of area ratio (A/A_{is}) versus concentration using first or higher order regression fit of the five calibration points. The analyst should select the regression order which introduces the least calibration error into the quantitation (Sec. 7.6.2.2 and 7.6.2.3). The use of calibration curves is a recommended alternative to average response factor calibration, and a useful diagnostic of standard preparation accuracy and absorption activity in the chromatographic system.

7.4 Daily GC/MS calibration

7.4.1 Prior to analysis of samples, the GC/MS tuning standard must be analyzed. A 50 ng injection of DFTPP must result in a mass spectrum for DFTPP which meets the criteria given in Table 3. These criteria must be demonstrated during each 12 hour shift.

7.4.2 A calibration standard(s) at mid-concentration containing all semivolatile analytes, including all required surrogates, must be analyzed every 12 hours during analysis. Compare the instrument response factor from the standards every 12 hours with the SPCC (Sec. 7.4.3) and CCC (Sec. 7.4.4) criteria.

7.4.3 System Performance Check Compounds (SPCCs): A system performance check must be made during every 12 hour shift. For each SPCC compound in the daily calibration a minimum response factor of 0.050 must be obtained. This is the same check that is applied during the initial calibration. If the minimum response factors are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. The minimum RF for semivolatile SPCCs is 0.050. Some possible problems are standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column,

and active sites in the column or chromatographic system. This check must be met before analysis begins.

7.4.4 Calibration Check Compounds (CCCs): After the system performance check is met, CCCs listed in Table 4 are used to check the validity of the initial calibration.

Calculate the percent drift using:

$$\% \text{ Drift} = \frac{C_i - C_c}{C_i} \times 100$$

where:

C_i = Calibration Check Compound standard concentration.
C_c = Measured concentration using selected quantitation method.

If the percent difference for each CCC is less than or equal to 20%, the initial calibration is assumed to be valid. If the criterion is not met (> 20% drift) for any one CCC, corrective action must be taken. Problems similar to those listed under SPCCs could affect this criterion. If no source of the problem can be determined after corrective action has been taken, a new five-point calibration must be generated. This criterion must be met before sample analysis begins. If the CCCs are not analytes required by the permit, then all required analytes must meet the 20% drift criterion.

7.4.5 The internal standard responses and retention times in the calibration check standard must be evaluated immediately after or during data acquisition. If the retention time for any internal standard changes by more than 30 seconds from the last calibration check (12 hours), the chromatographic system must be inspected for malfunctions and corrections must be made, as required. If the EICP area for any of the internal standards changes by a factor of two (-50% to +100%) from the last daily calibration check standard, the mass spectrometer must be inspected for malfunctions and corrections must be made, as appropriate. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required.

7.5 GC/MS analysis

7.5.1 It is highly recommended that the extract be screened on a GC/FID or GC/PID using the same type of capillary column. This will minimize contamination of the GC/MS system from unexpectedly high concentrations of organic compounds.

7.5.2 Spike the 1 mL extract obtained from sample preparation with 10 µL of the internal standard solution just prior to analysis.

7.5.3 Analyze the 1 mL extract by GC/MS using a 30 m x 0.25 mm (or 0.32 mm) silicone-coated fused-silica capillary column. The volume to be injected should ideally contain 100 ng of base/neutral and 200 ng of acid

surrogates (for a 1 µL injection). The recommended GC/MS operating conditions to be used are specified in Sec. 7.3.

7.5.4 If the response for any quantitation ion exceeds the initial calibration curve range of the GC/MS system, extract dilution must take place. Additional internal standard must be added to the diluted extract to maintain the required 40 ng/µL of each internal standard in the extracted volume. The diluted extract must be reanalyzed.

7.5.5 Perform all qualitative and quantitative measurements as described in Sec. 7.6. Store the extracts at 4°C, protected from light in screw-cap vials equipped with unpierced Teflon lined septa.

7.6 Data interpretation

7.6.1 Qualitative analysis

7.6.1.1 The qualitative identification of compounds determined by this method is based on retention time, and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined to be the three ions of greatest relative intensity, or any ions over 30% relative intensity if less than three such ions occur in the reference spectrum. Compounds should be identified as present when the criteria below are met.

7.6.1.1.1 The intensities of the characteristic ions of a compound maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention time will be accepted as meeting this criterion.

7.6.1.1.2 The RRT of the sample component is within ± 0.06 RRT units of the RRT of the standard component.

7.6.1.1.3 The relative intensities of the characteristic ions agree within 30% of the relative intensities of these ions in the reference spectrum. (Example: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 20% and 80%.)

7.6.1.1.4 Structural isomers that produce very similar mass spectra should be identified as individual isomers if they have sufficiently different GC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

7.6.1.1.5 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When gas chromatographic peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important. Examination of extracted ion current profiles of appropriate ions can aid in the selection of spectra, and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria can be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

7.6.1.2 For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The necessity to perform this type of identification will be determined by the purpose of the analyses being conducted. Computer generated library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other. For example, the RCRA permit or waste delisting requirements may require the reporting of nontarget analytes. Only after visual comparison of sample spectra with the nearest library searches will the mass spectral interpretation specialist assign a tentative identification. Guidelines for making tentative identification are:

(1) Relative intensities of major ions in the reference spectrum (ions > 10% of the most abundant ion) should be present in the sample spectrum.

(2) The relative intensities of the major ions should agree within ± 20%. (Example: For an ion with an abundance of 50% in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70%.)

(3) Molecular ions present in the reference spectrum should be present in the sample spectrum.

(4) Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of coeluting compounds.

(5) Ions present in the reference spectrum but not in the sample spectrum should be reviewed for possible subtraction from the sample spectrum because of background contamination or coeluting peaks. Data system library reduction programs can sometimes create these discrepancies.

7.6.2 Quantitative analysis

7.6.2.1 When a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion.

7.6.2.2 If the %RSD of a compound's relative response factor is 15% or less, then the concentration in the extract may be determined using the average response factor ($\overline{RF}$) from initial calibration data (7.4.5.2) and the following equation:.

$$C_{ex}\ (mg/L) = \frac{(A_x \times C_{is})}{(A_{is} \times \overline{RF})}$$

where C_{ex} is the concentration of the compound in the extract, and the other terms are as defined in Sec. 7.4.3.

7.6.2.3 Alternatively, the regression line fitted to the initial calibration (Sec. 7.3.5.1) may be used for determination of the extract concentration.

7.6.2.4 Compute the concentration of the analyte in the sample using the equations in Secs. 7.6.2.4.1 and 7.6.2.4.2.

7.6.2.4.1 The concentration of the analyte in the liquid phase of the sample is calculated using the concentration of the analyte in the extract and the volume of liquid extracted, as follows:

$$\text{Concentration in liquid } (\mu g/L) = \frac{(C_{ex} \times V_{ex})}{V_o}$$

where:

V_{ex} = extract volume, in mL
V_o = volume of liquid extracted, in L.

7.6.2.4.2 The concentration of the analyte in the solid phase of the sample is calculated using the concentration of the pollutant in the extract and the weight of the solids, as follows:

$$\text{Concentration in solid } (\mu g/kg) = \frac{(C_{ex} \times V_{ex})}{W_s}$$

where:

V_{ex} = extract volume, in mL
W_s = sample weight, in kg.

7.6.2.5 Where applicable, an estimate of concentration for noncalibrated components in the sample should be made. The formulae

given above should be used with the following modifications: The areas A_x and A_{is} should be from the total ion chromatograms and the RF for the compound should be assumed to be 1. The concentration obtained should be reported indicating (1) that the value is an estimate and (2) which internal standard was used to determine concentration. Use the nearest internal standard free of interferences.

7.6.2.6 Quantitation of multicomponent compounds (e.g. Aroclors) is beyond the scope of Method 8270. Normally, quantitation is performed using a GC/ECD by Method 8081.

8.0 QUALITY CONTROL

8.1 Each laboratory that uses these methods is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and an ongoing analysis of spiked samples to evaluate and document quality data. The laboratory must maintain records to document the quality of the data generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. When results of sample spikes indicate atypical method performance, a quality control reference sample (Sec. 8.5.1) must be analyzed to confirm that the measurements were performed in an in-control mode of operation.

8.2 Before processing any samples, the analyst should demonstrate, through the analysis of a method blank, that interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against chronic laboratory contamination. The blanks should be carried through all stages of sample preparation and measurement.

8.3 The experience of the analyst performing GC/MS analyses is invaluable to the success of the methods. Each day that analysis is performed, the daily calibration standard should be evaluated to determine if the chromatographic system is operating properly. Questions that should be asked are: Do the peaks look normal?; Is the response obtained comparable to the response from previous calibrations? Careful examination of the standard chromatogram can indicate whether the column is still good, the injector is leaking, the injector septum needs replacing, etc. If any changes are made to the system (e.g. column changed), recalibration of the system must take place.

8.4 Required instrument QC is found in the following sections

8.4.1 The GC/MS system must be tuned to meet the DFTPP specifications in Secs. 7.3.1 and 7.4.1.

8.4.2 There must be an initial calibration of the GC/MS system as specified in Sec. 7.3.

8.4.3 The GC/MS system must meet the SPCC criteria specified in Sec. 7.4.3 and the CCC criteria in Sec. 7.4.4, each 12 hours.

8.5 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.5.1 A quality control (QC) reference sample concentrate is required containing each base/neutral analyte at a concentration of 100 mg/L and each acid analyte at a concentration of 200 mg/L in acetone or methanol. (See Sec. 5.5.1 of Method 3500 for minimum requirements.) The QC reference sample concentrate may be prepared from pure standard materials or purchased as certified solutions. If prepared by the laboratory, the QC reference sample concentrate must be made using stock standards prepared independently from those used for calibration.

8.5.2 Using a pipet, prepare QC reference samples at a concentration of 100 µg/L by adding 1.00 mL of QC reference sample concentrate to each of four 1-L aliquots of water.

8.5.3 Analyze the well-mixed QC reference samples according to the method beginning in Sec. 7.1 with extraction of the samples.

8.5.4 Calculate the average recovery ($\bar{x}$) in µg/L, and the standard deviation of the recovery (s) in µg/L, for each analyte of interest using the four results.

8.5.5 For each analyte compare s and $\bar{x}$ with the corresponding acceptance criteria for precision and accuracy, respectively, found in Table 6. If s and $\bar{x}$ for all analytes meet the acceptance criteria, the system performance is acceptable and analysis of actual samples can begin. If any individual s exceeds the precision limit or any individual $\bar{x}$ falls outside the range for accuracy, then the system performance is unacceptable for that analyte.

NOTE: The large number of analytes in Table 6 present a substantial probability that one or more will fail at least one of the acceptance criteria when all analytes of a given method are analyzed.

8.5.6 When one or more of the analytes tested fail at least one of the acceptance criteria, the analyst must proceed according to Sec. 8.5.6.1 or 8.5.6.2.

8.5.6.1 Locate and correct the source of the problem and repeat the test for all analytes of interest beginning with Sec. 8.5.2.

8.5.6.2 Beginning with Sec. 8.5.2, repeat the test only for those analytes that failed to meet criteria. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning with Sec. 8.5.2.

8.6 The laboratory must, on an ongoing basis, analyze a method blank, a matrix spike, and a replicate for each analytical batch (up to a maximum of 20

samples/batch) to assess accuracy. For soil and waste samples where detectable amounts of organics are present, replicate samples may be appropriate in place of matrix spiked samples. For laboratories analyzing one to ten samples per month, at least one spiked sample per month is required.

8.6.1 The concentration of the spike in the sample should be determined as follows:

8.6.1.1 If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit, the spike should be at that limit or 1 to 5 times higher than the background concentration determined in Sec. 8.6.2, whichever concentration would be larger.

8.6.1.2 If the concentration of a specific analyte in a water sample is not being checked against a limit specific to that analyte, the spike should be at 100 µg/L or 1 to 5 times higher than the background concentration determined in Step 8.6.2, whichever concentration would be larger. For other matrices, recommended spiking concentration is 20 times the EQL.

8.6.1.3 If it is impractical to determine background levels before spiking (e.g. maximum holding times will be exceeded), the spike concentration should be at (1) the regulatory concentration limit, if any; or, if none (2) the larger of either 5 times higher than the expected background concentration or 100 µg/L. For other matrices, recommended spiking concentration is 20 times the EQL.

8.6.2 Analyze one sample aliquot to determine the background concentration (B) of each analyte. If necessary, prepare a new QC reference sample concentrate (Sec. 8.5.1) appropriate for the background concentration in the sample. Spike a second sample aliquot with 1.00 mL of the QC reference sample concentrate and analyze it to determine the concentration after spiking (A) of each analyte. Calculate each percent recovery (p) as 100(A-B)%/T, where T is the known true value of the spike.

8.6.3 Compare the percent recovery (p) for each analyte in a water sample with the corresponding QC acceptance criteria found in Table 6. These acceptance criteria were calculated to include an allowance for error in measurement of both the background and spike concentrations, assuming a spike to background ratio of 5:1. This error will be accounted for to the extent that the analyst's spike to background ratio approaches 5:1. If spiking was performed at a concentration lower than 100 µg/L, the analyst must use either the QC acceptance criteria presented in Table 6, or optional QC acceptance criteria calculated for the specific spike concentration. To calculate optional acceptance criteria for the recovery of an analyte: (1) Calculate accuracy (x') using the equation found in Table 7, substituting the spike concentration (T) for C; (2) calculate overall precision (S') using the equation in Table 7, substituting x' for $\bar{x}$; (3) calculate the range for recovery at the spike concentration as $(100x'/T) \pm 2.44(100S'/T)\%$.

8.6.4 If any individual p falls outside the designated range for recovery, that analyte has failed the acceptance criteria. A check standard containing each analyte that failed the criteria must be analyzed as described in Sec. 8.7.

8.7 If any analyte in a sample fails the acceptance criteria for recovery in Sec. 8.6, a QC reference sample containing each analyte that failed must be prepared and analyzed.

NOTE: The frequency for the required analysis of a QC reference sample will depend upon the number of analytes being simultaneously tested, the complexity of the sample matrix, and the performance of the laboratory. If the entire list of analytes in Table 6 must be measured in the sample in Sec. 8.6, the probability that the analysis of a QC reference sample will be required is high. In this case, the QC reference sample should be routinely analyzed with the spiked sample.

8.7.1 Prepare the QC reference sample by adding 1.0 mL of the QC reference sample concentrate (Sec. 8.5.1 or 8.6.2) to 1 L of water. The QC reference sample needs only to contain the analytes that failed criteria in the test in Sec. 8.6.

8.7.2 Analyze the QC reference sample to determine the concentration measured (A) of each analyte. Calculate each percent recovery (p_s) as $100(A/T)\%$, where T is the true value of the standard concentration.

8.7.3 Compare the percent recovery (p_s) for each analyte with the corresponding QC acceptance criteria found in Table 6. Only analytes that failed the test in Sec. 8.6 need to be compared with these criteria. If the recovery of any such analyte falls outside the designated range, the laboratory performance for that analyte is judged to be out of control, and the problem must be immediately identified and corrected. The analytical result for that analyte in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

8.8 As part of the QC program for the laboratory, method accuracy for each matrix studied must be assessed and records must be maintained. After the analysis of five spiked samples (of the same matrix) as in Sec. 8.6, calculate the average percent recovery ($\bar{p}$) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $\bar{p} - 2s_p$ to $\bar{p} + 2s_p$. If $\bar{p} = 90\%$ and $s_p = 10\%$, for example, the accuracy interval is expressed as 70-110%. Update the accuracy assessment for each analyte on a regular basis (e.g. after each five to ten new accuracy measurements).

8.9 The following procedure should be performed to determine acceptable accuracy and precision limits for surrogate standards.

8.9.1 For each sample analyzed, calculate the percent recovery of each surrogate in the sample.

8.9.2 Once a minimum of thirty samples of the same matrix have been analyzed, calculate the average percent recovery (P) and standard deviation of the percent recovery (s) for each of the surrogates.

8.9.3 For a given matrix, calculate the upper and lower control limit for method performance for each surrogate standard. This should be done as follows:

Upper Control Limit (UCL) = P + 3s
Lower Control Limit (LCL) = P - 3s

8.9.4 For aqueous and soil matrices, these laboratory-established surrogate control limits should, if applicable, be compared with the control limits listed in Table 8. The limits given in Table 8 are multi-laboratory performance-based limits for soil and aqueous samples, and therefore, the single-laboratory limits established in Sec. 8.9.3 must fall within those given in Table 8 for these matrices.

8.9.5 If recovery is not within limits, the following procedures are required.

- Check to be sure there are no errors in calculations, surrogate solutions and internal standards. Also, check instrument performance.
- Recalculate the data and/or reanalyze the extract if any of the above checks reveal a problem.
- Reextract and reanalyze the sample if none of the above are a problem or flag the data as "estimated concentration".

8.9.6 At a minimum, each laboratory should update surrogate recovery limits on a matrix-by-matrix basis, annually.

8.10 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to assess the precision of the environmental measurements. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or a mass spectrometer must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 Method 8250 (the packed column version of Method 8270) was tested by 15 laboratories using organic-free reagent water, drinking water, surface water, and industrial wastewaters spiked at six concentrations over the range 5-1,300 µg/L. Single operator accuracy and precision, and method accuracy were found to be directly related to the concentration of the analyte and essentially

independent of the sample matrix. Linear equations to describe these relationships are presented in Table 7.

9.2 Chromatograms from calibration standards analyzed with Day 0 and Day 7 samples were compared to detect possible deterioration of GC performance. These recoveries (using Method 3510 extraction) are presented in Table 9.

9.3 Method performance data (using Method 3541 Automated Soxhlet extraction) are presented in Table 10. Single laboratory accuracy and precision data were obtained for semivolatile organics in a clay soil by spiking at a concentration of 6 mg/kg for each compound. The spiking solution was mixed into the soil during addition and then allowed to equilibrate for approximately 1 hr prior to extraction. The spiked samples were then extracted by Method 3541 (Automated Soxhlet). Three determinations were performed and each extract was analyzed by gas chromatography/ mass spectrometry following Method 8270. The low recovery of the more volatile compounds is probably due to volatilization losses during equilibration. These data are listed in Table 11 and were taken from Reference 9.

10.0 REFERENCES

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5. "Interlaboratory Method Study for EPA Method 625-Base/Neutrals, Acids, and Pesticides," Final Report for EPA Contract 68-03-3102 (in preparation).

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9. Lopez-Avila, V. (W. Beckert, Project Officer); "Development of a Soxtec Extraction Procedure for Extraction of Organic Compounds from Soils and Sediments"; U.S. Environmental Protection Agency. Environmental Monitoring and Support Laboratory. Las Vegas, NV, October 1991; EPA 600/X-91/140.

TABLE 1.
CHARACTERISTIC IONS FOR SEMIVOLATILE COMPOUNDS

Compound	Retention Time (min.)	Primary Ion	Secondary Ion(s)
2-Picoline	3.75*	93	66,92
Aniline	5.68	93	66,65
Phenol	5.77	94	65,66
Bis(2-chloroethyl) ether	5.82	93	63,95
2-Chlorophenol	5.97	128	64,130
1,3-Dichlorobenzene	6.27	146	148,111
1,4-Dichlorobenzene-d_4 (I.S.)	6.35	152	150,115
1,4-Dichlorobenzene	6.40	146	148,111
Benzyl alcohol	6.78	108	79,77
1,2-Dichlorobenzene	6.85	146	148,111
N-Nitrosomethylethylamine	6.97	88	42,88,43,56
Bis(2-chloroisopropyl) ether	7.22	45	77,121
Ethyl carbamate	7.27	62	62,44,45,74
Thiophenol (Benzenethiol)	7.42	110	110,66,109,84
Methyl methanesulfonate	7.48	80	80,79,65,95
N-Nitrosodi-n-propylamine	7.55	70	42,101,130
Hexachloroethane	7.65	117	201,199
Maleic anhydride	7.65	54	54,98,53,44
Nitrobenzene	7.87	77	123,65
Isophorone	8.53	82	95,138
N-Nitrosodiethylamine	8.70	102	102,42,57,44,56
2-Nitrophenol	8.75	139	109,65
2,4-Dimethylphenol	9.03	122	107,121
p-Benzoquinone	9.13	108	54,108,82,80
Bis(2-chloroethoxy)methane	9.23	93	95,123
Benzoic acid	9.38	122	105,77
2,4-Dichlorophenol	9.48	162	164,98
Trimethyl phosphate	9.53	110	110,79,95,109,140
Ethyl methanesulfonate	9.62	79	79,109,97,45,65
1,2,4-Trichlorobenzene	9.67	180	182,145
Naphthalene-d_8 (I.S.)	9.75	136	68
Naphthalene	9.82	128	129,127
Hexachlorobutadiene	10.43	225	223,227
Tetraethyl pyrophosphate	11.07	99	99,155,127,81,109
Diethyl sulfate	11.37	139	139,45,59,99,111,125
4-Chloro-3-methylphenol	11.68	107	144,142
2-Methylnaphthalene	11.87	142	141
2-Methylphenol	12.40	107	107,108,77,79,90
Hexachloropropene	12.45	213	213,211,215,117,106,141
Hexachlorocyclopentadiene	12.60	237	235,272
N-Nitrosopyrrolidine	12.65	100	100,41,42,68,69
Acetophenone	12.67	105	71,105,51,120
4-Methylphenol	12.82	107	107,108,77,79,90
2,4,6-Trichlorophenol	12.85	196	198,200
o-Toluidine	12.87	106	106,107,77,51,79
3-Methylphenol	12.93	107	107,108,77,79,90

TABLE 1.
(Continued)

Compound	Retention Time (min.)	Primary Ion	Secondary Ion(s)
2-Chloronaphthalene	13.30	162	127,164
N-Nitrosopiperidine	13.55	114	42,114,55,56,41
1,4-Phenylenediamine	13.62	108	108,80,53,54,52
1-Chloronaphthalene	13.65[a]	162	127,164
2-Nitroaniline	13.75	65	92,138
5-Chloro-2-methylaniline	14.28	106	106,141,140,77,89
Dimethyl phthalate	14.48	163	194,164
Acenaphthylene	14.57	152	151,153
2,6-Dinitrotoluene	14.62	165	63,89
Phthalic anhydride	14.62	104	104,76,50,148
o-Anisidine	15.00	108	80,108,123,52
3-Nitroaniline	15.02	138	108,92
Acenaphthene-d_{10} (I.S.)	15.05	164	162,160
Acenaphthene	15.13	154	153,152
2,4-Dinitrophenol	15.35	184	63,154
2,6-Dinitrophenol	15.47	162	162,164,126,98,63
4-Chloroaniline	15.50	127	127,129,65,92
Isosafrole	15.60	162	162,131,104,77,51
Dibenzofuran	15.63	168	139
2,4-Diaminotoluene	15.78	121	121,122,94,77,104
2,4-Dinitrotoluene	15.80	165	63,89
4-Nitrophenol	15.80	139	109,65
2-Naphthylamine	16.00[a]	143	115,116
1,4-Naphthoquinone	16.23	158	158,104,102,76,50,130
p-Cresidine	16.45	122	122,94,137,77,93
Dichlorovos	16.48	109	109,185,79,145
Diethyl phthalate	16.70	149	177,150
Fluorene	16.70	166	165,167
2,4,5-Trimethylaniline	16.70	120	120,135,134,91,77
N-Nitrosodibutylamine	16.73	84	84,57,41,116,158
4-Chlorophenyl phenyl ether	16.78	204	206,141
Hydroquinone	16.93	110	110,81,53,55
4,6-Dinitro-2-methylphenol	17.05	198	51,105
Resorcinol	17.13	110	110,81,82,53,69
N-Nitrosodiphenylamine	17.17	169	168,167
Safrole	17.23	162	162,162,104,77,103,135
Hexamethyl phosphoramide	17.33	135	135,44,179,92,42
3-(Chloromethyl)pyridine hydrochloride	17.50	92	92,127,129,65,39
Diphenylamine	17.54[a]	169	168,167
1,2,4,5-Tetrachlorobenzene	17.97	216	216,214,179,108,143,218
1-Naphthylamine	18.20	143	143,115,89,63
1-Acetyl-2-thiourea	18.22	118	43,118,42,76
4-Bromophenyl phenyl ether	18.27	248	250,141
Toluene diisocyanate	18.42	174	174,145,173,146,132,91
2,4,5-Trichlorophenol	18.47	196	196,198,97,132,99
Hexachlorobenzene	18.65	284	142,249

TABLE 1.
(Continued)

Compound	Retention Time (min.)	Primary Ion	Secondary Ion(s)
Nicotine	18.70	84	84,133,161,162
Pentachlorophenol	19.25	266	264,268
5-Nitro-o-toluidine	19.27	152	77,152,79,106,94
Thionazine	19.35	107	96,107,97,143,79,68
4-Nitroaniline	19.37	138	138,65,108,92,80,39
Phenanthrene-d_{10}(i.s.)	19.55	188	94,80
Phenanthrene	19.62	178	179,176
Anthracene	19.77	178	176,179
1,4-Dinitrobenzene	19.83	168	168,75,50,76,92,122
Mevinphos	19.90	127	127,192,109,67,164
Naled	20.03	109	109,145,147,301,79,189
1,3-Dinitrobenzene	20.18	168	168,76,50,75,92,122
Diallate (cis or trans)	20.57	86	86,234,43,70
1,2-Dinitrobenzene	20.58	168	168,50,63,74
Diallate (trans or cis)	20.78	86	86,234,43,70
Pentachlorobenzene	21.35	250	250,252,108,248,215,254
5-Nitro-o-anisidine	21.50	168	168,79,52,138,153,77
Pentachloronitrobenzene	21.72	237	237,142,214,249,295,265
4-Nitroquinoline-1-oxide	21.73	174	174,101,128,75,116
Di-n-butyl phthalate	21.78	149	150,104
2,3,4,6-Tetrachlorophenol	21.88	232	232,131,230,166,234,168
Dihydrosaffrole	22.42	135	135,64,77
Demeton-O	22.72	88	88,89,60,61,115,171
Fluoranthene	23.33	202	101,203
1,3,5-Trinitrobenzene	23.68	75	75,74,213,120,91,63
Dicrotophos	23.82	127	127,67,72,109,193,237
Benzidine	23.87	184	92,185
Trifluralin	23.88	306	306,43,264,41,290
Bromoxynil	23.90	277	277,279,88,275,168
Pyrene	24.02	202	200,203
Monocrotophos	24.08	127	127,192,67,97,109
Phorate	24.10	75	75,121,97,93,260
Sulfallate	24.23	188	188,88,72,60,44
Demeton-S	24.30	88	88,60,81,89,114,115
Phenacetin	24.33	108	180,179,109,137,80
Dimethoate	24.70	87	87,93,125,143,229
Phenobarbital	24.70	204	204,117,232,146,161
Carbofuran	24.90	164	164,149,131,122
Octamethyl pyrophosphoramide	24.95	135	135,44,199,286,153,243
4-Aminobiphenyl	25.08	169	169,168,170,115
Dioxathion	25.25	97	97,125,270,153
Terbufos	25.35	231	231,57,97,153,103
α,α-Dimethylphenylamine	25.43	58	58,91,65,134,42
Pronamide	25.48	173	173,175,145,109,147
Aminoazobenzene	25.72	197	92,197,120,65,77
Dichlone	25.77	191	191,163,226,228,135,193

TABLE 1.
(Continued)

Compound	Retention Time (min.)	Primary Ion	Secondary Ion(s)
Dinoseb	25.83	211	211,163,147,117,240
Disulfoton	25.83	88	88,97,89,142,186
Fluchloralin	25.88	306	306,63,326,328,264,65
Mexacarbate	26.02	165	165,150,134,164,222
4,4'-Oxydianiline	26.08	200	200,108,171,80,65
Butyl benzyl phthalate	26.43	149	91,206
4-Nitrobiphenyl	26.55	199	199,152,141,169,151
Phosphamidon	26.85	127	127,264,72,109,138
2-Cyclohexyl-4,6-Dinitrophenol	26.87	231	231,185,41,193,266
Methyl parathion	27.03	109	109,125,263,79,93
Carbaryl	27.17	144	144,115,116,201
Dimethylaminoazobenzene	27.50	225	225,120,77,105,148,42
Propylthiouracil	27.68	170	170,142,114,83
Benz(a)anthracene	27.83	228	229,226
Chrysene-d_{12} (I.S.)	27.88	240	120,236
3,3'-Dichlorobenzidine	27.88	252	254,126
Chrysene	27.97	228	226,229
Malathion	28.08	173	173,125,127,93,158
Kepone	28.18	272	272,274,237,178,143,270
Fenthion	28.37	278	278,125,109,169,153
Parathion	28.40	109	109,97,291,139,155
Anilazine	28.47	239	239,241,143,178,89
Bis(2-ethylhexyl) phthalate	28.47	149	167,279
3,3'-Dimethylbenzidine	28.55	212	212,106,196,180
Carbophenothion	28.58	157	157,97,121,342,159,199
5-Nitroacenaphthene	28.73	199	199,152,169,141,115
Methapyrilene	28.77	97	97,50,191,71
Isodrin	28.95	193	193,66,195,263,265,147
Captan	29.47	79	79,149,77,119,117
Chlorfenvinphos	29.53	267	267,269,323,325,295
Crotoxyphos	29.73	127	127,105,193,166
Phosmet	30.03	160	160,77,93,317,76
EPN	30.11	157	157,169,185,141,323
Tetrachlorvinphos	30.27	329	109,329,331,79,333
Di-n-octyl phthalate	30.48	149	167,43
2-Aminoanthraquinone	30.63	223	223,167,195
Barban	30.83	222	222,51,87,224,257,153
Aramite	30.92	185	185,191,319,334,197,321
Benzo(b)fluoranthene	31.45	252	253,125
Nitrofen	31.48	283	283,285,202,139,253
Benzo(k)fluoranthene	31.55	252	253,125
Chlorobenzilate	31.77	251	251,139,253,111,141
Fensulfothion	31.87	293	293,97,308,125,292
Ethion	32.08	231	231,97,153,125,121
Diethylstilbestrol	32.15	268	268,145,107,239,121,159
Famphur	32.67	218	218,125,93,109,217

TABLE 1.
(Continued)

Compound	Retention Time (min.)	Primary Ion	Secondary Ion(s)
Tri-p-tolyl phosphate[b]	32.75	368	368,367,107,165,198
Benzo(a)pyrene	32.80	252	253,125
Perylene-d_{12} (I.S.)	33.05	264	260,265
7,12-Dimethylbenz(a)anthracene	33.25	256	256,241,239,120
5,5-Diphenylhydantoin	33.40	180	180,104,252,223,209
Captafol	33.47	79	79,77,80,107
Dinocap	33.47	69	69,41,39
Methoxychlor	33.55	227	227,228,152,114,274,212
2-Acetylaminofluorene	33.58	181	181,180,223,152
4,4'-Methylenebis(2-chloroaniline)	34.38	231	231,266,268,140,195
3,3'-Dimethoxybenzidine	34.47	244	244,201,229
3-Methylcholanthrene	35.07	268	268,252,253,126,134,113
Phosalone	35.23	182	182,184,367,121,379
Azinphos-methyl	35.25	160	160,132,93,104,105
Leptophos	35.28	171	171,377,375,77,155,379
Mirex	35.43	272	272,237,274,270,239,235
Tris(2,3-dibromopropyl) phosphate	35.68	201	137,201,119,217,219,199
Dibenz(a,j)acridine	36.40	279	279,280,277,250
Mestranol	36.48	277	277,310,174,147,242
Coumaphos	37.08	362	362,226,210,364,97,109
Indeno(1,2,3-cd)pyrene	39.52	276	138,227
Dibenz(a,h)anthracene	39.82	278	139,279
Benzo(g,h,i)perylene	41.43	276	138,277
1,2:4,5-Dibenzopyrene	41.60	302	302,151,150,300
Strychnine	45.15	334	334,335,333
Piperonyl sulfoxide	46.43	162	162,135,105,77
Hexachlorophene	47.98	196	196,198,209,211,406,408
Aldrin	--	66	263,220
Aroclor-1016	--	222	260,292
Aroclor-1221	--	190	224,260
Aroclor-1232	--	190	224,260
Aroclor-1242	--	222	256,292
Aroclor-1248	--	292	362,326
Aroclor-1254	--	292	362,326
Aroclor-1260	--	360	362,394
α-BHC	--	183	181,109
β-BHC	--	181	183,109
δ-BHC	--	183	181,109
γ-BHC (Lindane)	--	183	181,109
4,4'-DDD	--	235	237,165
4,4'-DDE	--	246	248,176
4,4'-DDT	--	235	237,165
Dieldrin	--	79	263,279
1,2-Diphenylhydrazine	--	77	105,182
Endosulfan I	--	195	339,341
Endosulfan II	--	337	339,341

TABLE 1.
(Continued)

Compound	Retention Time (min.)	Primary Ion	Secondary Ion(s)
Endosulfan sulfate	--	272	387,422
Endrin	--	263	82,81
Endrin aldehyde	--	67	345,250
Endrin ketone	--	317	67,319
2-Fluorobiphenyl (surr.)	--	172	171
2-Fluorophenol (surr.)	--	112	64
Heptachlor	--	100	272,274
Heptachlor epoxide	--	353	355,351
Nitrobenzene-d_5 (surr.)	--	82	128,54
N-Nitrosodimethylamine	--	42	74,44
Phenol-d_6 (surr.)	--	99	42,71
Terphenyl-d_{14} (surr.)	--	244	122,212
2,4,6-Tribromophenol (surr.)	--	330	332,141
Toxaphene	--	159	231,233

I.S. = internal standard.
surr. = surrogate.
[a]Estimated retention times.
[b]Substitute for the non-specific mixture, tricresyl phosphate.

TABLE 2.
ESTIMATED QUANTITATION LIMITS (EQLs) FOR SEMIVOLATILE ORGANICS

Semivolatiles	Estimated Quantitation Limits[a] Ground water µg/L	Low Soil/Sediment[b] µg/kg
Acenaphthene	10	660
Acenaphthylene	10	660
Acetophenone	10	ND
2-Acetylaminofluorene	20	ND
1-Acetyl-2-thiourea	1000	ND
2-Aminoanthraquinone	20	ND
Aminoazobenzene	10	ND
4-Aminobiphenyl	20	ND
Anilazine	100	ND
o-Anisidine	10	ND
Anthracene	10	660
Aramite	20	ND
Azinphos-methyl	100	ND
Barban	200	ND
Benz(a)anthracene	10	660
Benzo(b)fluoranthene	10	660
Benzo(k)fluoranthene	10	660
Benzoic acid	50	3300
Benzo(g,h,i)perylene	10	660
Benzo(a)pyrene	10	660
p-Benzoquinone	10	ND
Benzyl alcohol	20	1300
Bis(2-chloroethoxy)methane	10	660
Bis(2-chloroethyl) ether	10	660
Bis(2-chloroisopropyl) ether	10	660
4-bromophenyl phenyl ether	10	660
Bromoxynil	10	ND
Butyl benzyl phthalate	10	660
Captafol	20	ND
Captan	50	ND
Carbaryl	10	ND
Carbofuran	10	ND
Carbophenothion	10	ND
Chlorfenvinphos	20	ND
4-Chloroaniline	20	1300
Chlorobenzilate	10	ND
5-Chloro-2-methylaniline	10	ND
4-Chloro-3-methylphenol	20	1300
3-(Chloromethyl)pyridine hydrochloride	100	ND
2-Chloronaphthalene	10	660
2-Chlorophenol	10	660
4-Chlorophenyl phenyl ether	10	660
Chrysene	10	660
Coumaphos	40	ND

TABLE 2.
(Continued)

Semivolatiles	Estimated Quantitation Limits[a] Ground water µg/L	Low Soil/Sediment[b] µg/kg
p-Cresidine	10	ND
Crotoxyphos	20	ND
2-Cyclohexyl-4,6-dinitrophenol	100	ND
Demeton-O	10	ND
Demeton-S	10	ND
Diallate (cis or trans)	10	ND
Diallate (trans or cis)	10	ND
2,4-Diaminotoluene	20	ND
Dibenz(a,j)acridine	10	ND
Dibenz(a,h)anthracene	10	660
Dibenzofuran	10	660
Dibenzo(a,e)pyrene	10	ND
Di-n-butyl phthalate	10	ND
Dichlone	NA	ND
1,2-Dichlorobenzene	10	660
1,3-Dichlorobenzene	10	660
1,4-Dichlorobenzene	10	660
3,3′-Dichlorobenzidine	20	1300
2,4-Dichlorophenol	10	660
2,6-Dichlorophenol	10	ND
Dichlorovos	10	ND
Dicrotophos	10	ND
Diethyl phthalate	10	660
Diethylstilbestrol	20	ND
Diethyl sulfate	100	ND
Dimethoate	20	ND
3,3′-Dimethoxybenzidine	100	ND
Dimethylaminoazobenzene	10	ND
7,12-Dimethylbenz(a)anthracene	10	ND
3,3′-Dimethylbenzidine	10	ND
a,a-Dimethylphenethylamine	ND	ND
2,4-Dimethylphenol	10	660
Dimethyl phthalate	10	660
1,2-Dinitrobenzene	40	ND
1,3-Dinitrobenzene	20	ND
1,4-Dinitrobenzene	40	ND
4,6-Dinitro-2-methylphenol	50	3300
2,4-Dinitrophenol	50	3300
2,4-Dinitrotoluene	10	660
2,6-Dinitrotoluene	10	660
Dinocap	100	ND
Dinoseb	20	ND
5,5-Diphenylhydantoin	20	ND
Di-n-octyl phthalate	10	660

TABLE 2.
(Continued)

Semivolatiles	Estimated Quantitation Limits[a] Ground water µg/L	Low Soil/Sediment[b] µg/kg
Disulfoton	10	ND
EPN	10	ND
Ethion	10	ND
Ethyl carbamate	50	ND
Bis(2-ethylhexyl) phthalate	10	660
Ethyl methanesulfonate	20	ND
Famphur	20	ND
Fensulfothion	40	ND
Fenthion	10	ND
Fluchloralin	20	ND
Fluoranthene	10	660
Fluorene	10	660
Hexachlorobenzene	10	660
Hexachlorobutadiene	10	660
Hexachlorocyclopentadiene	10	660
Hexachloroethane	10	660
Hexachlorophene	50	ND
Hexachloropropene	10	ND
Hexamethylphosphoramide	20	ND
Hydroquinone	ND	ND
Indeno(1,2,3-cd)pyrene	10	660
Isodrin	20	ND
Isophorone	10	660
Isosafrole	10	ND
Kepone	20	ND
Leptophos	10	ND
Malathion	50	ND
Maleic anhydride	NA	ND
Mestranol	20	ND
Methapyrilene	100	ND
Methoxychlor	10	ND
3-Methylcholanthrene	10	ND
4,4'-Methylenebis(2-chloroaniline)	NA	ND
Methyl methanesulfonate	10	ND
2-Methylnaphthalene	10	660
Methyl parathion	10	ND
2-Methylphenol	10	660
3-Methylphenol	10	ND
4-Methylphenol	10	660
Mevinphos	10	ND
Mexacarbate	20	ND
Mirex	10	ND
Monocrotophos	40	ND
Naled	20	ND

TABLE 2.
(Continued)

Semivolatiles	Estimated Quantitation Limits[a] Ground water µg/L	Low Soil/Sediment[b] µg/kg
Naphthalene	10	660
1,4-Naphthoquinone	10	ND
1-Naphthylamine	10	ND
2-Naphthylamine	10	ND
Nicotine	20	ND
5-Nitroacenaphthene	10	ND
2-Nitroaniline	50	3300
3-Nitroaniline	50	3300
4-Nitroaniline	20	ND
5-Nitro-o-anisidine	10	ND
Nitrobenzene	10	660
4-Nitrobiphenyl	10	ND
Nitrofen	20	ND
2-Nitrophenol	10	660
4-Nitrophenol	50	3300
5-Nitro-o-toluidine	10	ND
4-Nitroquinoline-1-oxide	40	ND
N-Nitrosodibutylamine	10	ND
N-Nitrosodiethylamine	20	ND
N-Nitrosodiphenylamine	10	660
N-Nitroso-di-n-propylamine	10	660
N-Nitrosopiperidine	20	ND
N-Nitrosopyrrolidine	40	ND
Octamethyl pyrophosphoramide	200	ND
4,4'-Oxydianiline	20	ND
Parathion	10	ND
Pentachlorobenzene	10	ND
Pentachloronitrobenzene	20	ND
Pentachlorophenol	50	3300
Phenacetin	20	ND
Phenanthrene	10	660
Phenobarbital	10	ND
Phenol	10	660
1,4-Phenylenediamine	10	ND
Phorate	10	ND
Phosalone	100	ND
Phosmet	40	ND
Phosphamidon	100	ND
Phthalic anhydride	100	ND
2-Picoline	ND	ND
Piperonyl sulfoxide	100	ND
Pronamide	10	ND
Propylthiouracil	100	ND
Pyrene	10	660

TABLE 2.
(Continued)

Semivolatiles	Estimated Quantitation Limits[a] Ground water µg/L	Low Soil/Sediment[b] µg/kg
Pyridine	ND	ND
Resorcinol	100	ND
Safrole	10	ND
Strychnine	40	ND
Sulfallate	10	ND
Terbufos	20	ND
1,2,4,5-Tetrachlorobenzene	10	ND
2,3,4,6-Tetrachlorophenol	10	ND
Tetrachlorvinphos	20	ND
Tetraethyl pyrophosphate	40	ND
Thionazine	20	ND
Thiophenol (Benzenethiol)	20	ND
Toluene diisocyanate	100	ND
o-Toluidine	10	ND
1,2,4-Trichlorobenzene	10	660
2,4,5-Trichlorophenol	10	660
2,4,6-Trichlorophenol	10	660
Trifluralin	10	ND
2,4,5-Trimethylaniline	10	ND
Trimethyl phosphate	10	ND
1,3,5-Trinitrobenzene	10	ND
Tris(2,3-dibromopropyl) phosphate	200	ND
Tri-p-tolyl phosphate(h)	10	ND
0,0,0-Triethyl phosphorothioate	NT	ND

a Sample EQLs are highly matrix-dependent. The EQLs listed herein are provided for guidance and may not always be achievable.
b EQLs listed for soil/sediment are based on wet weight. Normally data are reported on a dry weight basis, therefore, EQLs will be higher based on the % dry weight of each sample. These EQLs are based on a 30 g sample and gel permeation chromatography cleanup.
ND = Not determined.
NA = Not applicable.
NT = Not tested.

Other Matrices	Factor[c]
High-concentration soil and sludges by sonicator	7.5
Non-water miscible waste	75

[c]EQL = (EQL for Low Soil/Sediment given above in Table 2) X (Factor).

TABLE 3.
DFTPP KEY IONS AND ION ABUNDANCE CRITERIA[a,b]

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	< 2% of mass 69
70	< 2% of mass 69
127	40-60% of mass 198
197	< 1% of mass 198
198	Base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	> 1% of mass 198
441	Present but less than mass 443
442	> 40% of mass 198
443	17-23% of mass 442

[a] See Reference 3.

[b] Alternate tuning criteria may be used (e.g., CLP, Method 525, or manufacturers' instructions), provided that method performance is not adversely affected.

TABLE 4.
CALIBRATION CHECK COMPOUNDS

Base/Neutral Fraction	Acid Fraction
Acenaphthene	4-Chloro-3-methylphenol
1,4-Dichlorobenzene	2,4-Dichlorophenol
Hexachlorobutadiene	2-Nitrophenol
N-Nitrosodiphenylamine	Phenol
Di-n-octyl phthalate	Pentachlorophenol
Fluoranthene	2,4,6-Trichlorophenol
Benzo(a)pyrene	

TABLE 5.
SEMIVOLATILE INTERNAL STANDARDS WITH CORRESPONDING ANALYTES ASSIGNED FOR QUANTITATION

1,4-Dichlorobenzene-d_4	Naphthalene-d_8	Acenaphthene-d_{10}
Aniline	Acetophenone	Acenaphthene
Benzyl alcohol	Benzoic acid	Acenaphthylene
Bis(2-chloroethyl) ether	Bis(2-chloroethoxy)methane	1-Chloronaphthalene
Bis(2-chloroisopropyl) ether	4-Chloroaniline	2-Chloronaphthalene
2-Chlorophenol	4-Chloro-3-methylphenol	4-Chlorophenyl phenyl ether
1,3-Dichlorobenzene	2,4-Dichlorophenol	Dibenzofuran
1,4-Dichlorobenzene	2,6-Dichlorophenol	Diethyl phthalate
1,2-Dichlorobenzene	α,α-Dimethylphenethylamine	Dimethyl phthalate
Ethyl methanesulfonate	2,4-Dimethylphenol	2,4-Dinitrophenol
2-Fluorophenol (surr.)	Hexachlorobutadiene	2,4-Dinitrotoluene
Hexachloroethane	Isophorone	2,6-Dinitrotoluene
Methyl methanesulfonate	2-Methylnaphthalene	Fluorene
2-Methylphenol	Naphthalene	2-Fluorobiphenyl (surr.)
4-Methylphenol	Nitrobenzene	Hexachlorocyclopentadiene
N-Nitrosodimethylamine	Nitrobenzene-d_8 (surr.)	1-Naphthylamine
N-Nitroso-di-n-propylamine	2-Nitrophenol	2-Naphthylamine
Phenol	N-Nitrosodibutylamine	2-Nitroaniline
Phenol-d_6 (surr.)	N-Nitrosopiperidine	3-Nitroaniline
2-Picoline	1,2,4-Trichlorobenzene	4-Nitroaniline
		4-Nitrophenol
		Pentachlorobenzene
		1,2,4,5-Tetrachlorobenzene
		2,3,4,6-Tetrachlorophenol
		2,4,6-Tribromophenol (surr.)
		2,4,6-Trichlorophenol
		2,4,5-Trichlorophenol

(surr.) = surrogate

TABLE 5.
(Continued)

Phenanthrene-d_{10}	Chrysene-d_{12}	Perylene-d_{12}
4-Aminobiphenyl	Benzidine	Benzo(b)fluor-anthene
Anthracene	Benzo(a)anthracene	Benzo(k)fluor-anthene
4-Bromophenyl phenyl ether	Bis(2-ethylhexyl) phthalate	Benzo(g,h,i)-perylene
Di-n-butyl phthalate	Butyl benzyl phthalate	Benzo(a)pyrene
4,6-Dinitro-2-methyl-phenol	Chrysene	Dibenz(a,j)acridine
Diphenylamine	3,3'-Dichlorobenzidine	Dibenz(a,h)-anthracene
Fluoranthene	p-Dimethylaminoazobenzene	7,12-Dimethylbenz-(a)anthracene
Hexachlorobenzene	Pyrene	Di-n-octyl phthalate
N-Nitrosodiphenylamine	Terphenyl-d_{14} (surr.)	Indeno(1,2,3-cd) pyrene
Pentachlorophenol		3-Methylchol-anthrene
Pentachloronitrobenzene		
Phenacetin		
Phenanthrene		
Pronamide		

(surr.) = surrogate

TABLE 6.
QC ACCEPTANCE CRITERIA*

Compound	Test conc. (µg/L)	Limit for s (µg/L)	Range for x (µg/L)	Range p, p_s (%)
Acenaphthene	100	27.6	60.1-132.3	47-145
Acenaphthylene	100	40.2	53.5-126.0	33-145
Aldrin	100	39.0	7.2-152.2	D-166
Anthracene	100	32.0	43.4-118.0	27-133
Benz(a)anthracene	100	27.6	41.8-133.0	33-143
Benzo(b)fluoranthene	100	38.8	42.0-140.4	24-159
Benzo(k)fluoranthene	100	32.3	25.2-145.7	11-162
Benzo(a)pyrene	100	39.0	31.7-148.0	17-163
Benzo(ghi)perylene	100	58.9	D-195.0	D-219
Benzyl butyl phthalate	100	23.4	D-139.9	D-152
β-BHC	100	31.5	41.5-130.6	24-149
δ-BHC	100	21.6	D-100.0	D-110
Bis(2-chloroethyl) ether	100	55.0	42.9-126.0	12-158
Bis(2-chloroethoxy)methane	100	34.5	49.2-164.7	33-184
Bis(2-chloroisopropyl) ether	100	46.3	62.8-138.6	36-166
Bis(2-ethylhexyl) phthalate	100	41.1	28.9-136.8	8-158
4-Bromophenyl phenyl ether	100	23.0	64.9-114.4	53-127
2-Chloronaphthalene	100	13.0	64.5-113.5	60-118
4-Chlorophenyl phenyl ether	100	33.4	38.4-144.7	25-158
Chrysene	100	48.3	44.1-139.9	17-168
4,4'-DDD	100	31.0	D-134.5	D-145
4,4'-DDE	100	32.0	19.2-119.7	4-136
4,4'-DDT	100	61.6	D-170.6	D-203
Dibenzo(a,h)anthracene	100	70.0	D-199.7	D-227
Di-n-butyl phthalate	100	16.7	8.4-111.0	1-118
1,2-Dichlorobenzene	100	30.9	48.6-112.0	32-129
1,3-Dichlorobenzene	100	41.7	16.7-153.9	D-172
1,4-Dichlorobenzene	100	32.1	37.3-105.7	20-124
3,3'-Dichlorobenzidine	100	71.4	8.2-212.5	D-262
Dieldrin	100	30.7	44.3-119.3	29-136
Diethyl phthalate	100	26.5	D-100.0	D-114
Dimethyl phthalate	100	23.2	D-100.0	D-112
2,4-Dinitrotoluene	100	21.8	47.5-126.9	39-139
2,6-Dinitrotoluene	100	29.6	68.1-136.7	50-158
Di-n-octyl phthalate	100	31.4	18.6-131.8	4-146
Endosulfan sulfate	100	16.7	D-103.5	D-107
Endrin aldehyde	100	32.5	D-188.8	D-209
Fluoranthene	100	32.8	42.9-121.3	26-137
Fluorene	100	20.7	71.6-108.4	59-121
Heptachlor	100	37.2	D-172.2	D-192
Heptachlor epoxide	100	54.7	70.9-109.4	26.155
Hexachlorobenzene	100	24.9	7.8-141.5	D-152
Hexachlorobutadiene	100	26.3	37.8-102.2	24-116

TABLE 6.
(Continued)

Compound	Test conc. (µg/L)	Limit for s (µg/L)	Range for $\bar{x}$ (µg/L)	Range p, p_s (%)
Hexachloroethane	100	24.5	55.2-100.0	40-113
Indeno(1,2,3-cd)pyrene	100	44.6	D-150.9	D-171
Isophorone	100	63.3	46.6-180.2	21-196
Naphthalene	100	30.1	35.6-119.6	21-133
Nitrobenzene	100	39.3	54.3-157.6	35-180
N-Nitrosodi-n-propylamine	100	55.4	13.6-197.9	D-230
PCB-1260	100	54.2	19.3-121.0	D-164
Phenanthrene	100	20.6	65.2-108.7	54-120
Pyrene	100	25.2	69.6-100.0	52-115
1,2,4-Trichlorobenzene	100	28.1	57.3-129.2	44-142
4-Chloro-3-methylphenol	100	37.2	40.8-127.9	22-147
2-Chlorophenol	100	28.7	36.2-120.4	23-134
2,4-Chlorophenol	100	26.4	52.5-121.7	39-135
2,4-Dimethylphenol	100	26.1	41.8-109.0	32-119
2,4-Dinitrophenol	100	49.8	D-172.9	D-191
2-Methyl-4,6-dinitrophenol	100	93.2	53.0-100.0	D-181
2-Nitrophenol	100	35.2	45.0-166.7	29-182
4-Nitrophenol	100	47.2	13.0-106.5	D-132
Pentachlorophenol	100	48.9	38.1-151.8	14-176
Phenol	100	22.6	16.6-100.0	5-112
2,4,6-Trichlorophenol	100	31.7	52.4-129.2	37-144

s = Standard deviation of four recovery measurements, in µg/L.

$\bar{x}$ = Average recovery for four recovery measurements, in µg/L.

p, p_s = Percent recovery measured.

D = Detected; result must be greater than zero.

a Criteria from 40 CFR Part 136 for Method 625. These criteria are based directly on the method performance data in Table 7. Where necessary, the limits for recovery have been broadened to assure applicability of the limits to concentrations below those used to develop Table 7.

TABLE 7.
METHOD ACCURACY AND PRECISION AS FUNCTIONS OF CONCENTRATION[a]

Compound	Accuracy, as recovery, x′ (μg/L)	Single analyst precision, s_r' (μg/L)	Overall precision, S′ (μg/L)
Acenaphthene	0.96C+0.19	0.15$\bar{x}$-0.12	0.21$\bar{x}$-0.67
Acenaphthylene	0.89C+0.74	0.24$\bar{x}$-1.06	0.26$\bar{x}$-0.54
Aldrin	0.78C+1.66	0.27$\bar{x}$-1.28	0.43$\bar{x}$+1.13
Anthracene	0.80C+0.68	0.21$\bar{x}$-0.32	0.27$\bar{x}$-0.64
Benz(a)anthracene	0.88C-0.60	0.15$\bar{x}$+0.93	0.26$\bar{x}$-0.21
Chloroethane	0.99C-1.53	0.14$\bar{x}$-0.13	0.17$\bar{x}$-0.28
Benzo(b)fluoranthene	0.93C-1.80	0.22$\bar{x}$+0.43	0.29$\bar{x}$+0.96
Benzo(k)fluoranthene	0.87C-1.56	0.19$\bar{x}$+1.03	0.35$\bar{x}$+0.40
Benzo(a)pyrene	0.90C-0.13	0.22$\bar{x}$+0.48	0.32$\bar{x}$+1.35
Benzo(ghi)perylene	0.98C-0.86	0.29$\bar{x}$+2.40	0.51$\bar{x}$-0.44
Benzyl butyl phthalate	0.66C-1.68	0.18$\bar{x}$+0.94	0.53$\bar{x}$+0.92
β-BHC	0.87C-0.94	0.20$\bar{x}$-0.58	0.30$\bar{x}$+1.94
δ-BHC	0.29C-1.09	0.34$\bar{x}$+0.86	0.93$\bar{x}$-0.17
Bis(2-chloroethyl) ether	0.86C-1.54	0.35$\bar{x}$-0.99	0.35$\bar{x}$+0.10
Bis(2-chloroethoxy)methane	1.12C-5.04	0.16$\bar{x}$+1.34	0.26$\bar{x}$+2.01
Bis(2-chloroisopropyl) ether	1.03C-2.31	0.24$\bar{x}$+0.28	0.25$\bar{x}$+1.04
Bis(2-ethylhexyl) phthalate	0.84C-1.18	0.26$\bar{x}$+0.73	0.36$\bar{x}$+0.67
4-Bromophenyl phenyl ether	0.91C-1.34	0.13$\bar{x}$+0.66	0.16$\bar{x}$+0.66
2-Chloronaphthalene	0.89C+0.01	0.07$\bar{x}$+0.52	0.13$\bar{x}$+0.34
4-Chlorophenyl phenyl ether	0.91C+0.53	0.20$\bar{x}$-0.94	0.30$\bar{x}$-0.46
Chrysene	0.93C-1.00	0.28$\bar{x}$+0.13	0.33$\bar{x}$-0.09
4,4′-DDD	0.56C-0.40	0.29$\bar{x}$-0.32	0.66$\bar{x}$-0.96
4,4′-DDE	0.70C-0.54	0.26$\bar{x}$-1.17	0.39$\bar{x}$-1.04
4,4′-DDT	0.79C-3.28	0.42$\bar{x}$+0.19	0.65$\bar{x}$-0.58
Dibenzo(a,h)anthracene	0.88C+4.72	0.30$\bar{x}$+8.51	0.59$\bar{x}$+0.25
Di-n-butyl phthalate	0.59C+0.71	0.13$\bar{x}$+1.16	0.39$\bar{x}$+0.60
1,2-Dichlorobenzene	0.80C+0.28	0.20$\bar{x}$+0.47	0.24$\bar{x}$+0.39
1,3-Dichlorobenzene	0.86C-0.70	0.25$\bar{x}$+0.68	0.41$\bar{x}$+0.11
1,4-Dichlorobenzene	0.73C-1.47	0.24$\bar{x}$+0.23	0.29$\bar{x}$+0.36
3,3′-Dichlorobenzidine	1.23C-12.65	0.28$\bar{x}$+7.33	0.47$\bar{x}$+3.45
Dieldrin	0.82C-0.16	0.20$\bar{x}$-0.16	0.26$\bar{x}$-0.07
Diethyl phthalate	0.43C+1.00	0.28$\bar{x}$+1.44	0.52$\bar{x}$+0.22
Dimethyl phthalate	0.20C+1.03	0.54$\bar{x}$+0.19	1.05$\bar{x}$-0.92
2,4-Dinitrotoluene	0.92C-4.81	0.12$\bar{x}$+1.06	0.21$\bar{x}$+1.50
2,6-Dinitrotoluene	1.06C-3.60	0.14$\bar{x}$+1.26	0.19$\bar{x}$+0.35
Di-n-octyl phthalate	0.76C-0.79	0.21$\bar{x}$+1.19	0.37$\bar{x}$+1.19
Endosulfan sulfate	0.39C+0.41	0.12$\bar{x}$+2.47	0.63$\bar{x}$-1.03
Endrin aldehyde	0.76C-3.86	0.18$\bar{x}$+3.91	0.73$\bar{x}$-0.62
Fluoranthene	0.81C+1.10	0.22$\bar{x}$-0.73	0.28$\bar{x}$-0.60

TABLE 7.
(Continued)

Compound	Accuracy, as recovery, x′ (μg/L)	Single analyst precision, s_r' (μg/L)	Overall precision, S′ (μg/L)
Fluorene	0.90C-0.00	0.12$\bar{x}$+0.26	0.13$\bar{x}$+0.61
Heptachlor	0.87C-2.97	0.24$\bar{x}$-0.56	0.50$\bar{x}$-0.23
Heptachlor epoxide	0.92C-1.87	0.33$\bar{x}$-0.46	0.28$\bar{x}$+0.64
Hexachlorobenzene	0.74C+0.66	0.18$\bar{x}$-0.10	0.43$\bar{x}$-0.52
Hexachlorobutadiene	0.71C-1.01	0.19$\bar{x}$+0.92	0.26$\bar{x}$+0.49
Hexachloroethane	0.73C-0.83	0.17$\bar{x}$+0.67	0.17$\bar{x}$+0.80
Indeno(1,2,3-cd)pyrene	0.78C-3.10	0.29$\bar{x}$+1.46	0.50$\bar{x}$-0.44
Isophorone	1.12C+1.41	0.27$\bar{x}$+0.77	0.33$\bar{x}$+0.26
Naphthalene	0.76C+1.58	0.21$\bar{x}$-0.41	0.30$\bar{x}$-0.68
Nitrobenzene	1.09C-3.05	0.19$\bar{x}$+0.92	0.27$\bar{x}$+0.21
N-Nitrosodi-n-propylamine	1.12C-6.22	0.27$\bar{x}$+0.68	0.44$\bar{x}$+0.47
PCB-1260	0.81C-10.86	0.35$\bar{x}$+3.61	0.43$\bar{x}$+1.82
Phenanthrene	0.87C+0.06	0.12$\bar{x}$+0.57	0.15$\bar{x}$+0.25
Pyrene	0.84C-0.16	0.16$\bar{x}$+0.06	0.15$\bar{x}$+0.31
1,2,4-Trichlorobenzene	0.94C-0.79	0.15$\bar{x}$+0.85	0.21$\bar{x}$+0.39
4-Chloro-3-methylphenol	0.84C+0.35	0.23$\bar{x}$+0.75	0.29$\bar{x}$+1.31
2-Chlorophenol	0.78C+0.29	0.18$\bar{x}$+1.46	0.28$\bar{x}$+0.97
2,4-Dichlorophenol	0.87C-0.13	0.15$\bar{x}$+1.25	0.21$\bar{x}$+1.28
2,4-Dimethylphenol	0.71C+4.41	0.16$\bar{x}$+1.21	0.22$\bar{x}$+1.31
2,4-Dinitrophenol	0.81C-18.04	0.38$\bar{x}$+2.36	0.42$\bar{x}$+26.29
2-Methyl-4,6-dinitrophenol	1.04C-28.04	0.10$\bar{x}$+42.29	0.26$\bar{x}$+23.10
2-Nitrophenol	0.07C-1.15	0.16$\bar{x}$+1.94	0.27$\bar{x}$+2.60
4-Nitrophenol	0.61C-1.22	0.38$\bar{x}$+2.57	0.44$\bar{x}$+3.24
Pentachlorophenol	0.93C+1.99	0.24$\bar{x}$+3.03	0.30$\bar{x}$+4.33
Phenol	0.43C+1.26	0.26$\bar{x}$+0.73	0.35$\bar{x}$+0.58
2,4,6-Trichlorophenol	0.91C-0.18	0.16$\bar{x}$+2.22	0.22$\bar{x}$+1.81

x′ = Expected recovery for one or more measurements of a sample containing a concentration of C, in μg/L.

s_r' = Expected single analyst standard deviation of measurements at an average concentration of $\bar{x}$, in μg/L.

S′ = Expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{x}$, in μg/L.

C = True value for the concentration, in μg/L.

$\bar{x}$ = Average recovery found for measurements of samples containing a concentration of C, in μg/L.

TABLE 8.
SURROGATE SPIKE RECOVERY LIMITS FOR WATER AND SOIL/SEDIMENT SAMPLES

Surrogate Compound	Low/High Water	Low/High Soil/Sediment
Nitrobenzene-d_5	35-114	23-120
2-Fluorobiphenyl	43-116	30-115
Terphenyl-d_{14}	33-141	18-137
Phenol-d_6	10-94	24-113
2-Fluorophenol	21-100	25-121
2,4,6-Tribromophenol	10-123	19-122

TABLE 9.
EXTRACTION EFFICIENCY AND AQUEOUS STABILITY RESULTS

COMPOUND	PERCENT RECOVERY ON DAY 0		PERCENT RECOVERY ON DAY 7	
	AVG.	RSD	AVG.	RSD
3-Amino-9-ethylcarbazole	80	8	73	3
4-Chloro-1,2-phenylenediamine	91	1	108	4
4-Chloro-1,3-phenylenediamine	84	3	70	3
1,2-Dibromo-3-chloropropane	97	2	98	5
2-sec-Butyl-4,6-dinitrophenol	99	3	97	6
Ethyl parathion	100	2	103	4
4,4'-Methylenebis(N,N-dimethylaniline)	108	4	90	4
2-Methyl-5-nitroaniline	99	10	93	4
2-Methylpyridine	80	4	83	4
Tetraethyl dithiopyrophosphate	92	7	70	1

Data from Reference 8.

TABLE 10.
AVERAGE PERCENT RECOVERIES AND PERCENT RSDs FOR THE TARGET COMPOUNDS FROM SPIKED CLAY SOIL AND TOPSOIL BY AUTOMATED SOXHLET EXTRACTION WITH HEXANE-ACETONE (1:1)[a]

Compound name	Clay Soil		Topsoil	
	Average percent recovery	Percent RSD	Average percent recovery	Percent RSD
1,3-Dichlorobenzene	0	--	0	--
1,2-Dichlorobenzene	0	--	0	--
Nitrobenzene	0	--	0	--
Benzal chloride	0	--	0	--
Benzotrichloride	0	--	0	--
4-Chloro-2-nitrotoluene	0	--	0	--
Hexachlorocyclopentadiene	4.1	15	7.8	23
2,4-Dichloronitrobenzene	35.2	7.6	21.2	15
3,4-Dichloronitrobenzene	34.9	15	20.4	11
Pentachlorobenzene	13.7	7.3	14.8	13
2,3,4,5-Tetrachloronitrobenzene	55.9	6.7	50.4	6.0
Benefin	62.6	4.8	62.7	2.9
alpha-BHC	58.2	7.3	54.8	4.8
Hexachlorobenzene	26.9	13	25.1	5.7
delta-BHC	95.8	4.6	99.2	1.3
Heptachlor	46.9	9.2	49.1	6.3
Aldrin	97.7	12	102	7.4
Isopropalin	102	4.3	105	2.3
Heptachlor epoxide	90.4	4.4	93.6	2.4
trans-Chlordane	90.1	4.5	95.0	2.3
Endosulfan I	96.3	4.4	101	2.2
Dieldrin	129	4.7	104	1.9
2,5-Dichlorophenyl-4-nitrophenyl ether	110	4.1	112	2.1
Endrin	102	4.5	106	3.7
Endosulfan II	104	4.1	105	0.4
p,p'-DDT	134	2.1	111	2.0
2,3,6-Trichlorophenyl-4'-nitrophenyl ether	110	4.8	110	2.8
2,3,4-Trichlorophenyl-4'-nitrophenyl ether	112	4.4	112	3.3
Mirex	104	5.3	108	2.2

[a] The operating conditions for the Soxtec apparatus were as follows: immersion time 45 min; extraction time 45 min; the sample size was 10 g; the spiking concentration was 500 ng/g, except for the surrogate compounds at 1000 ng/g, compounds 23, 27, and 28 at 1500 ng/g, compound 3 at 2000 ng/g, and compounds 1 and 2 at 5000 ng/g.

TABLE 11.
SINGLE LABORATORY ACCURACY AND PRECISION DATA FOR THE EXTRACTION OF SEMIVOLATILE ORGANICS FROM SPIKED CLAY BY METHOD 3541 (AUTOMATED SOXHLET)[a]

Compound name	Average percent recovery	Percent RSD
Phenol	47.8	5.6
Bis(2-chloroethyl)ether	25.4	13
2-Chlorophenol	42.7	4.3
Benzyl alcohol	55.9	7.2
2-Methylphenol	17.6	6.6
Bis(2-chloroisopropyl)ether	15.0	15
4-Methylphenol	23.4	6.7
N-Nitroso-di-n-propylamine	41.4	6.2
Nitrobenzene	28.2	7.7
Isophorone	56.1	4.2
2-Nitrophenol	36.0	6.5
2,4-Dimethylphenol	50.1	5.7
Benzoic acid	40.6	7.7
Bis(2-chloroethoxy)methane	44.1	3.0
2,4-Dichlorophenol	55.6	4.6
1,2,4-Trichlorobenzene	18.1	31
Naphthalene	26.2	15
4-Chloroaniline	55.7	12
4-Chloro-3-methylphenol	65.1	5.1
2-Methylnaphthalene	47.0	8.6
Hexachlorocyclopentadiene	19.3	19
2,4,6-Trichlorophenol	70.2	6.3
2,4,5-Trichlorophenol	26.8	2.9
2-Chloronaphthalene	61.2	6.0
2-Nitroaniline	73.8	6.0
Dimethyl phthalate	74.6	5.2
Acenaphthylene	71.6	5.7
3-Nitroaniline	77.6	5.3
Acenaphthene	79.2	4.0
2,4-Dinitrophenol	91.9	8.9
4-Nitrophenol	62.9	16
Dibenzofuran	82.1	5.9
2,4-Dinitrotoluene	84.2	5.4
2,6-Dinitrotoluene	68.3	5.8
Diethyl phthalate	74.9	5.4
4-Chlorophenyl-phenyl ether	67.2	3.2
Fluorene	82.1	3.4
4-Nitroaniline	79.0	7.9
4,6-Dinitro-2-methylphenol	63.4	6.8
N-Nitrosodiphenylamine	77.0	3.4
4-Bromophenyl-phenyl ether	62.4	3.0

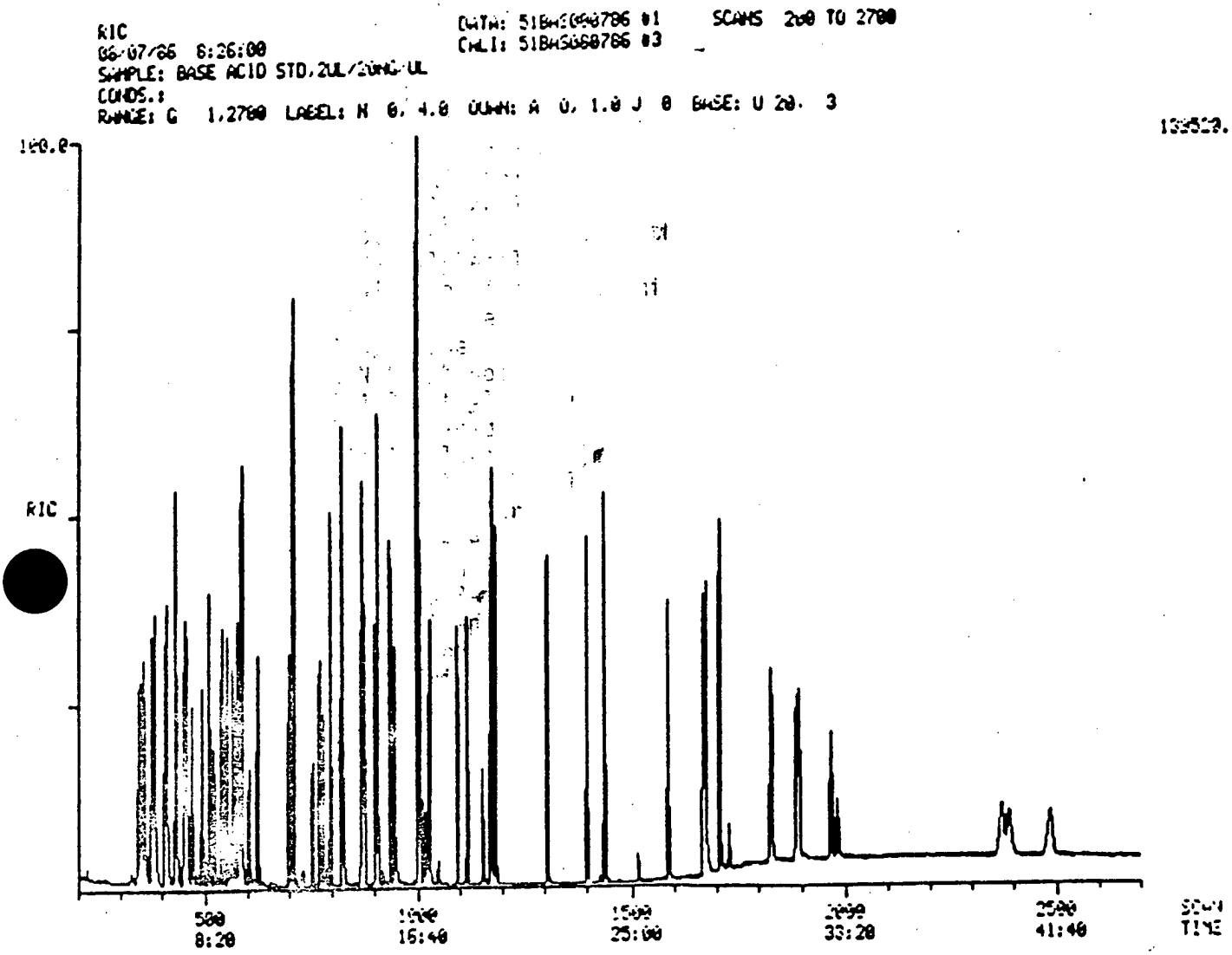
Table 11. (Continued)

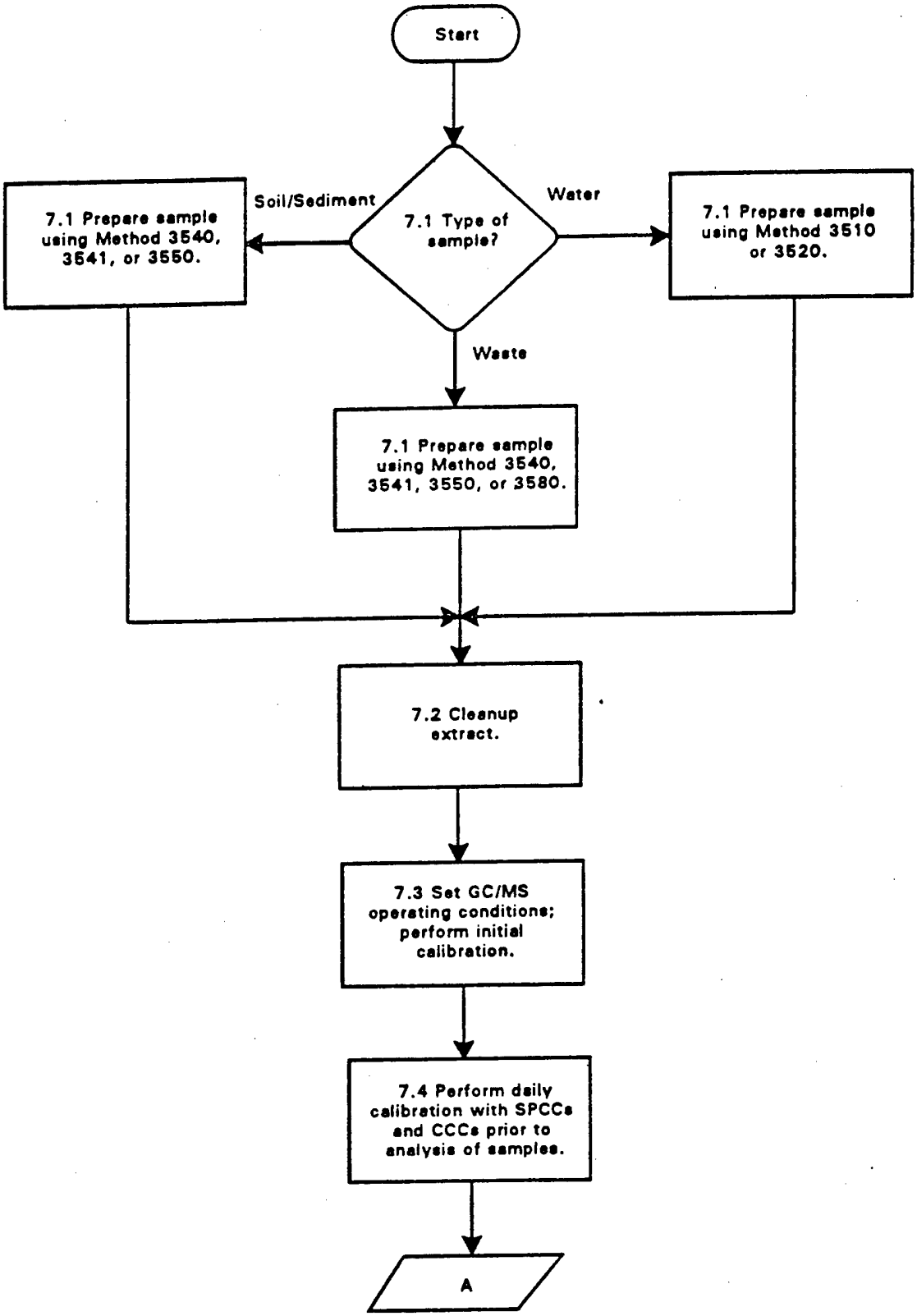
Compound name	Average percent recovery	Percent RSD
Hexachlorobenzene	72.6	3.7
Pentachlorophenol	62.7	6.1
Phenanthrene	83.9	5.4
Anthracene	96.3	3.9
Di-n-butyl phthalate	78.3	40
Fluoranthene	87.7	6.9
Pyrene	102	0.8
Butyl benzyl phthalate	66.3	5.2
3,3'-Dichlorobenzidine	25.2	11
Benzo(a)anthracene	73.4	3.8
Bis(2-ethylhexyl) phthalate	77.2	4.8
Chrysene	76.2	4.4
Di-n-octyl phthalate	83.1	4.8
Benzo(b)fluoranthene	82.7	5.0
Benzo(k)fluoranthene	71.7	4.1
Benzo(a)pyrene	71.7	4.1
Indeno(1,2,3-cd)pyrene	72.2	4.3
Dibenzo(a,h)anthracene	66.7	6.3
Benzo(g,h,i)perylene	63.9	8.0
1,2-Dichlorobenzene	0	--
1,3-Dichlorobenzene	0	--
1,4-Dichlorobenzene	0	--
Hexachloroethane	0	--
Hexachlorobutadiene	0	--

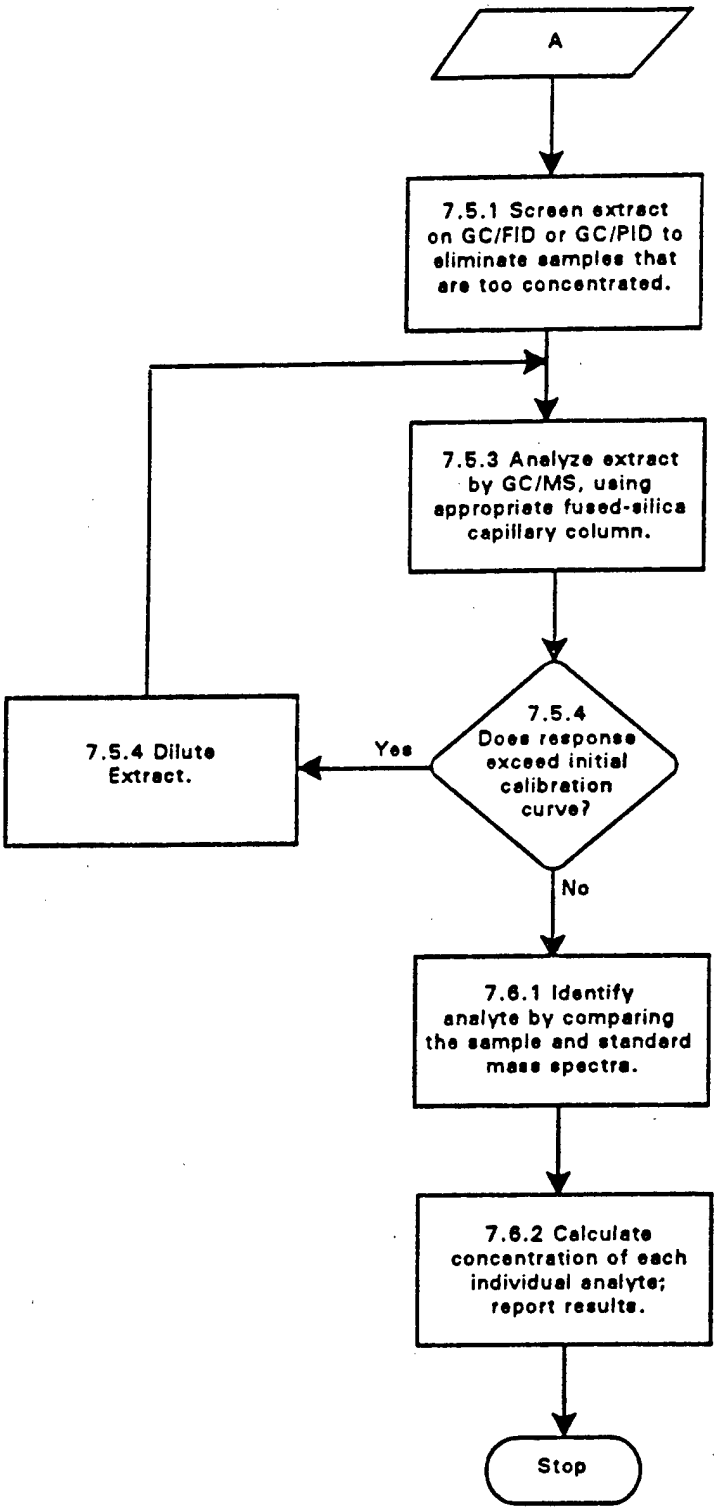
• Number of determinations was three. The operating conditions for the Soxtec apparatus were as follows: immersion time 45 min; extraction time 45 min; the sample size was 10 g clay soil; the spike concentration was 6 mg/kg per compound. The sample was allowed to equilibrate 1 hour after spiking.

Data taken from Reference 9.

FIGURE 1.
GAS CHROMATOGRAM OF BASE/NEUTRAL AND ACID CALIBRATION STANDARD




Start
7.1 Type of sample?
Soil/Sediment
Water
Waste
7.1 Prepare sample using Method 3540, 3541, or 3550.
7.1 Prepare sample using Method 3510 or 3520.
7.1 Prepare sample using Method 3540, 3541, 3550, or 3580.
7.2 Cleanup extract.
7.3 Set GC/MS operating conditions; perform initial calibration.
7.4 Perform daily calibration with SPCCs and CCCs prior to analysis of samples.
A


A
7.5.1 Screen extract on GC/FID or GC/PID to eliminate samples that are too concentrated.
7.5.3 Analyze extract by GC/MS, using appropriate fused-silica capillary column.
7.5.4 Does response exceed initial calibration curve?
Yes
7.5.4 Dilute Extract.
No
7.6.1 Identify analyte by comparing the sample and standard mass spectra.
7.6.2 Calculate concentration of each individual analyte; report results.
Stop

APPENDIX D

SCREENING OF WETLAND EMERGENT SPECIES FOR REMEDIATION OF EXPLOSIVES-CONTAMINATED GROUNDWATER: TVA REPORT, DECEMBER 12, 1995

SCREENING OF WETLAND EMERGENT SPECIES FOR REMEDIATION OF EXPLOSIVES-CONTAMINATED GROUNDWATER

TVA Report, December 12, 1995

Ten species of wetland emergent plants were screened for effectiveness in remediating explosives-contaminated groundwater (Table 1). Plant roots were suspended in 0.25 strength Hoagland's solution, and allowed to acclimate for two and a half weeks after transplanting. The Hoagland's solution was then exchanged for explosives-contaminated groundwater from Milan well MI146. Water in the pots was sampled at 1 day, 2 days, 3 days, 6 days, and 10 days, and analyzed for TNT, RDX, HMX, 2-aminodinitrotoluene (2-A-DNT), 4-aminodinitrotoluene (4-A-DNT), and 1,3,5-trinitrobenzene (1,3,5-TNB). Samples were taken at day 10 for nutrient analysis. Oxidation-reduction potentials, electrical conductivity, pH, chemical oxygen demand (COD), and dissolved oxygen (DO) were also measured on day 10.

Species were put into pots containing 3 L of groundwater at biomass densities of 45 g (low biomass) and 90 g (high biomass) plant fresh weight per liter of groundwater, for a total fresh weight of 135 g and 270 g per pot. In addition to the biomass treatment, 50 mg L^{-1} of NO_3-N was added to another set of low biomass pots to give three treatments for the complete experiment consisting of 1) high biomass density, 2) low biomass, and 3) low biomass + N. Each treatment was replicated three times, for a total of 90 pots. A completely randomized design was used for statistical analysis. For analysis of variance, the experiment was treated as two separate factorial experiments, with one having species and biomass as main factors, and the other having species and N level as main factors.

The decrease in RDX and HMX over time was modeled using the first order kinetics equation

$$\ln(C/C_0) = -KT$$

where C_0 is the initial concentration, C is the concentration at time T, and K is the rate constant determined as the slope of $\ln(C/C_0)$ plotted against time, with units for K as $days^{-1}$. A higher K indicates a more rapid degradation of explosive. The rate constant K can be standardized on a plant weight basis so that

$$k=K/P$$

where P is plant weight in units of g fresh weight/L water, so units for k are L g^{-1} $days^{-1}$. Use of k allows comparison of rate constants for plants at different biomasses.

RESULTS

TNT Remediation

Concentrations of TNT decreased from an initial concentration of 2.19 mg L^{-1} to below 0.06 mg L^{-1} in one day with high plant biomass for all species except parrots feather, smartweed, and water hyacinth (Fig.1). TNT had disappeared from the water by 3 days,

except for wool grass, which showed an increase in TNT from day 2 to day 3, with complete degradation of TNT by day 6. This response of increasing concentration followed by degradation was also observed in RDX and HMX remediation, and may be due to adsorption of the explosive to the root surface, or uptake into the roots, with subsequent release back into solution. Remediation with low plant biomass took slightly longer (Fig. 2), with TNT completely degraded by 6 days, except for 0.05 ppm remaining for smart weed and water primrose. Addition of 50 mg L^{-1} NO_3-N caused slightly higher concentrations of TNT on days 1, 2, and 3, except for water hyacinth and water primrose, which had lower TNT concentrations on these days. Complete TNT degradation still occurred by day 6, similar to low plant biomass without added N.

RDX Remediation

All species decreased RDX concentration to 2.0 mg L^{-1} or below in the high biomass pots after ten days, from an initial concentration of 3.23 mg L^{-1} (Fig. 4). Degradation of RDX occurred most rapidly with canary grass, which decreased RDX to 0.49 mg L^{-1} after ten days. Wool grass was the next most effective species, degrading RDX to a final concentration of 0.72 mg L^{-1}. Wool grass again exhibited an increase in explosives concentration at day 3, with subsequent decrease in concentrations, similar to that observed with TNT degradation (Fig. 1). Sweet flag, the third most efficient species, degraded RDX to 1.15 mg L^{-1}.

Plants were not as effective in degrading RDX at low biomass (Fig. 5). Canary grass again degraded RDX to the lowest concentration, 1.14 mg L^{-1}, with wool grass decreasing RDX to 1.65 mg L^{-1}. The remaining species decreased RDX to concentrations between 2.0 and 2.8 mg L^{-1}. Analysis of variance of RDX concentrations on day 10 shows a difference between means for high and low biomass effects (Table 2). RDX concentrations were significantly lower for canary grass and wool grass compared to the other species.

Addition of NO_3-N generally caused slightly higher RDX concentrations throughout the 10-day remediation period, with RDX concentrations of 1.55 and 2.20 mg L^{-1} for canary grass and wool grass, respectively, after 10 days remediation (Fig. 6), and these differences were statistically significant from concentrations without added N (Table 3). However, cattail performed better with added N, significantly lowering RDX concentration by 0.35 mg L^{-1} with added N.

First order rate constants indicate that canary grass and wool grass were most effective at RDX degradation (Fig. 7), similar to the results of plots of RDX concentrations over time. Sweet flag and cane grass were the next most effective species. The rate constants for these species are much smaller at the low biomass than at the high biomass, suggesting that high density planting in a constructed wetlands would be more effective at remediating explosives in groundwater. For canary grass and wool grass, addition of N at the low biomass decreased the K value, indicating that added N may inhibit the degradation of RDX. Adjusting K to account for the species differences in root biomass shows that at high biomass canary grass has the highest k value, followed by wool grass,

water hyacinth, and water primrose, which all have similar k values (Fig. 8). However, at the low biomass, water hyacinth has the highest k value. This is due to the low root biomass of water hyacinth relative to canary grass and wool grass (Fig. 9), which used as a divisor of K to determine k, gives a large value for k. This suggests that water hyacinth may be more effective at remediating explosives than other species under low biomass conditions. However, from the results of this study, for a constructed wetlands the remediation of explosives would most likely be accomplished by accumulation of as large amount of root biomass as possible, which would favor use of species such as canary grass and wool grass, which accumulate a large proportion of their biomass in the roots (Fig. 9).

HMX Remediation

Results of screening species for HMX degradation at high biomass are similar to results for screening for RDX degradation. From an initial concentration of 0.178 mg L^{-1}, canary grass reduced HMX to the lowest concentration of 0.070 mg L^{-1}, followed by concentrations of 0.090 and 0.098 mg L^{-1} for wool grass and sweet flag, respectively, after 10 days of remediation (Fig. 10). Wool grass exhibited an increase in HMX concentration at day 3, with subsequent concentration decrease, as occurred with TNT and RDX concentrations. HMX concentrations were significantly higher at low root biomass than high biomass at day 10 for seven of the ten species (Fig. 11 and Table 4). Addition of N to the groundwater did not significantly alter HMX degradation rates (Fig. 12), as shown by the non-significant main effect for N in the analysis of variance (Table 5).

Rate constants for HMX degradation at high plant biomass were highest for canary grass, followed by wool grass and sweet flag (Fig. 13). Remediation with low biomass decreased the K value for these species, whereas addition of N only slightly affected K values. Adjustment of K values with plant biomass shows that the species performed about the same (Fig. 14), with the exception of water hyacinth, which shows a high k value for low biomass, most likely due to the low root biomass of water hyacinth, as discussed above for RMX remediation.

Remediation of Breakdown Products

2-A-DNT disappeared from high biomass system within three days for all species except wool grass, which remediated 2-A-DNT after 6 days, and water hyacinth, which reduced 2-A-DNT to 0.06 mg L^{-1} at 10 days (Fig. 15). 4-A-DNT took 6 to 10 days to completely remediate for most species, with canary grass most effective in reducing 4-A-DNT concentrations (Fig 16). Parrots feather, smart weed, and water primrose did not reduce 4-A-DNT concentrations to zero in 10 days. Remediation of 1,3,5-TNB occurred rapidly with all species, with almost all TNB degraded within one day (Fig. 17).

Nutrient Concentrations

All species with high plant biomass completely depleted the available NO_3-N in the groundwater by the end of the 10-day remediation period (Fig. 18). With low plant biomass, NO_3-N was also completely depleted in all species except cattail, smartweed,

and sweet flag, and significant concentrations remained only with cattail. Addition of 50 mg L^{-1} prevented complete NO_3-N depletion in all species except canary grass and water hyacinth. These results indicate that addition of NO_3 will be required in constructed wetlands using plant species, such as canary grass, which take up large amounts of NO_3. No visual symptoms of nitrogen deficiency were observed at the end of the remediation period. However, assuming a 10-day retention time in a constructed wetlands, similar to the time period of this screening experiment, plants may be exposed to depleted nitrogen over the growing season unless sufficient nitrogen is added.

The concentration of NH_3-N in the water at the end of 10 days had increased for six of the ten species with high plant biomass, with the greatest increase for canary grass and sweet flag (Fig. 18). With low plant biomass, addition of 50 mg L^{-1} N greatly increased NH_3 concentrations with sweet flag and cattail, with the highest concentration at 0.23 mg L^{-1} for sweet flag. Canary grass and wool grass, the species most efficient in explosives remediation, showed only a very slight response in NH_3 concentration to added NO_3.

Correlation of explosives degradation with water parameters

Correlation of a species' ability to remediate explosives with a water parameter that is easily measured would allow rapid screening of a large number of species. Dissolved oxygen was the parameter most highly correlated with RDX and HMX concentrations on day 10 of the experiment (Table 6). Oxidation-reduction potential was also correlated with RDX concentration, but was poorly correlated with HMX concentration. The low correlation of the remaining parameters may preclude their use as screening parameters.

SUMMARY

The species canary grass, wool grass, and sweet flag are most effective in remediating explosives contaminated groundwater. High plant biomass provided the greatest remediation effect, and addition of NO_3-N significantly inhibited the remediation of RDX by canary grass and wool grass. However, even at low biomass and addition of 50 mg/L NO_3 at the beginning of the remediation period, canary grass depleted all nitrogen in 10 days. Addition experiments with added nitrogen at high biomass densities would be useful in determining the amount and form of nitrogen needed to provide maximum plant growth while limiting the effect of added nitrogen on RDX remediation. More detailed experiments on the correlation of explosives remediation with water parameters such as dissolved oxygen and oxidation-reduction potential would also be useful as a tool to rapidly screen a large number of emergent species for use in constructed wetlands.

Table 1. Wetland emergent plant species screened for use in remediation of explosives- contaminated groundwater.

canary grass	*Phalaris arundinacea*
cane grass	*Phragmites communis*
cattail	*Typha spp.*
horsetail	*Equisetum hyemale*
parrots feather	*Myriophyllum aquaticum*
smartweed	*Polygonum spp.*
sweet flag	*Acorus calamus*
water hyacinth	*Eichhornia crassipes*
water primrose	*Ludwigia spp.*
wool grass	*Scirpus cyperinus*

Table 2. Analysis of variance and mean comparisons for RDX concentrations on day 10 for species and biomass main factors and interactions.

Source	F Value	P>F
Species	12.14	.0001
Biomass	50.35	.0001
Species*Biomass	1.90	.0793[a]

Species	Mean[b]	
canary grass	0.82	a
cane grass	1.86	b
cattail	2.33	c
horsetail	1.90	b
parrots feather	2.12	bc
smartweed	2.17	bc
sweet flag	1.80	b
water hyacinth	1.87	b
water primrose	2.09	bc
wool grass	1.19	a

[a] Species*biomass is not significant at P=.05; mean comparisons done for main efffect species only

[b] Means with different letter are significantly different at P=.05

Table 3 . Analysis of variance and mean comparisons for RDX concentrations on day 10 for species and N level main factors and interactions.

Source	F Value	P>F
Species	19.41	.0001
N level	6.92	.0121
Species*N level	2.83	.0114

	Means[a]			
Species	no added N		+N	
canary grass	1.14	a	1.55	b
cane grass	2.27	def	2.52	fg
cattail	2.76	g	2.42	f
horsetail	2.04	cde	2.44	fg
parrots feather	2.23	def	2.41	f
smartweed	2.39	f	2.50	fg
sweet flag	2.45	fg	2.37	ef
water hyacinth	1.98	cd	1.87	bc
water primrose	2.22	def	2.23	def
wool grass	1.65	b	2.20	def

[a] Means with different letter are significantly different at P=.05

Table 4 . Analysis of variance and mean comparisons for HMX concentrations on day 10 for species and biomass main factors and interactions.

Source	F Value	P>F
Species	27.25	.0001
Biomass	52.80	.0001
Species*Biomass	2.31	.0338

	Means[a]			
Species	low biomass		high biomass	
canary grass	0.090	b	0.070	a
cane grass	0.141	ghi	0.123	cde
cattail	0.147	i	0.126	cdef
horsetail	0.133	defgh	0.137	efghi
parrots feather	0.144	hi	0.138	fghi
smartweed	0.137	efghi	0.122	cd
sweet flag	0.129	cdefg	0.098	b
water hyacinth	0.127	cdef	0.118	c
water primrose	0.133	defghi	0.118	c
wool grass	0.119	cd	0.090	b

[a] Means with different letter are significantly different at P=.05

Table 5. Analysis of variance and mean comparisons for HMX concentrations on day 10 for species and N level main factors and interactions.

Source	F Value	P>F
Species	12.14	.0001
N level	0.00	.9786
Species*N level	1.39	.2232[a]

Species	Mean[b]	
canary grass	0.098	a
cane grass	0.138	cd
cattail	0.140	cd
horsetail	0.131	bc
parrots feather	0.147	d
smartweed	0.139	cd
sweet flag	0.127	b
water hyacinth	0.123	b
water primrose	0.132	bc
wool grass	0.126	b

[a] Species*N level is not significant at P=.05; mean comparisons done for main effect species only

[b] Means with different letter are significantly different at P=.05

Table 6. Correlation coefficients for RDX and HMX concentrations and measured water parameters on day 10.

	RDX Concentration	HMX Concentration
Dissolved Oxygen	0.598	0.459
Oxidation-Reduction Potential[a]	0.501	0.246
NO_3 Concentration	0.436	0.299
Chemical Oxygen Demand	-0.414	-0.337
Conductivity	0.372	0.255
pH	0.244	0.224

[a]for high biomass only

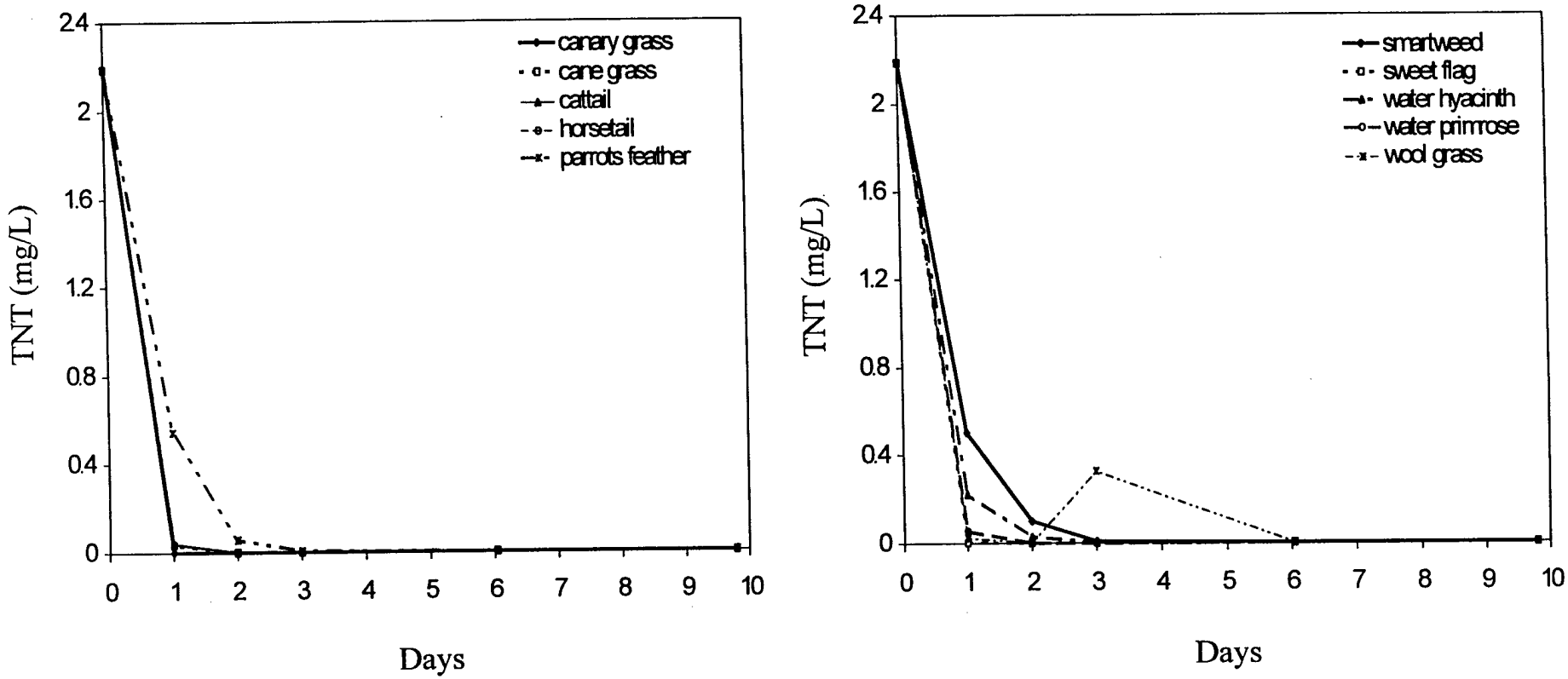


Fig. 1. Remediation of TNT with high plant biomass.

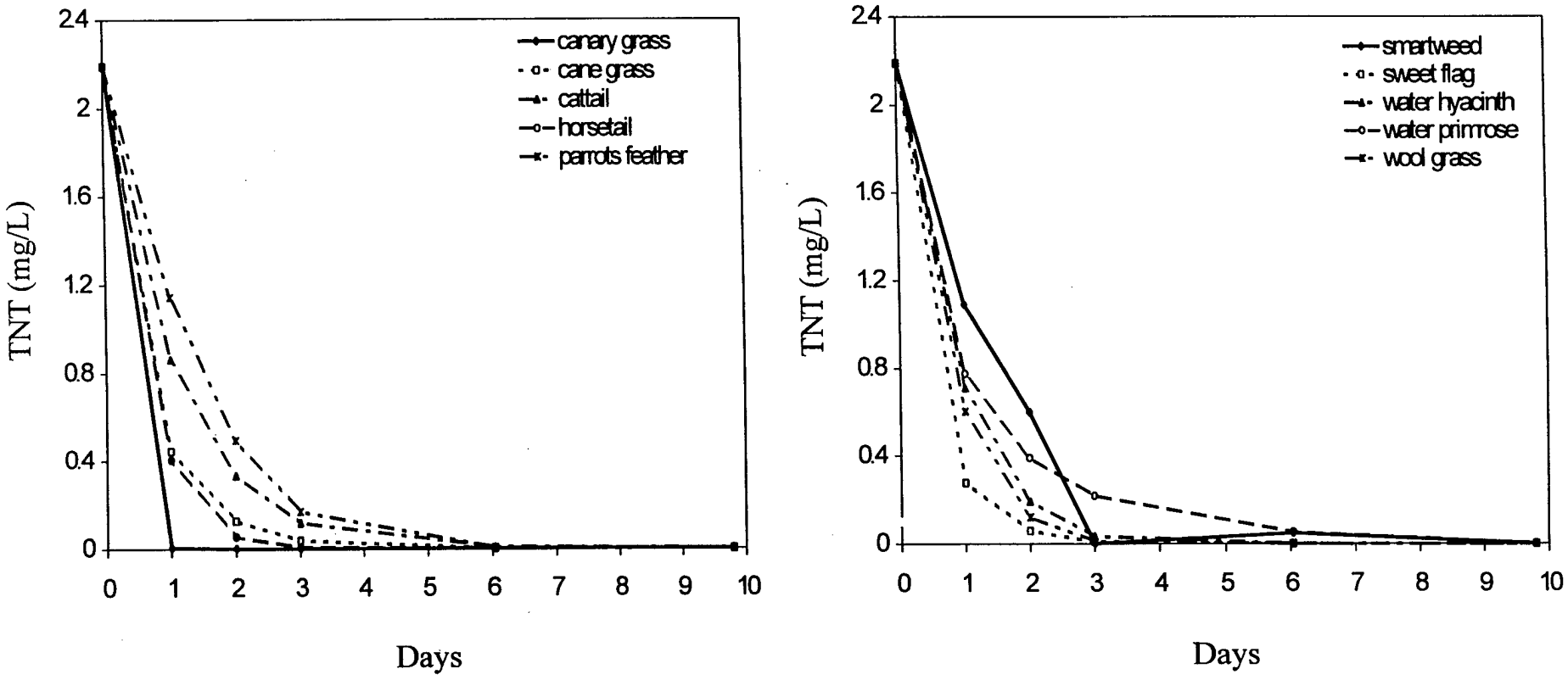


Fig. 2. Remediation of TNT with low plant biomass

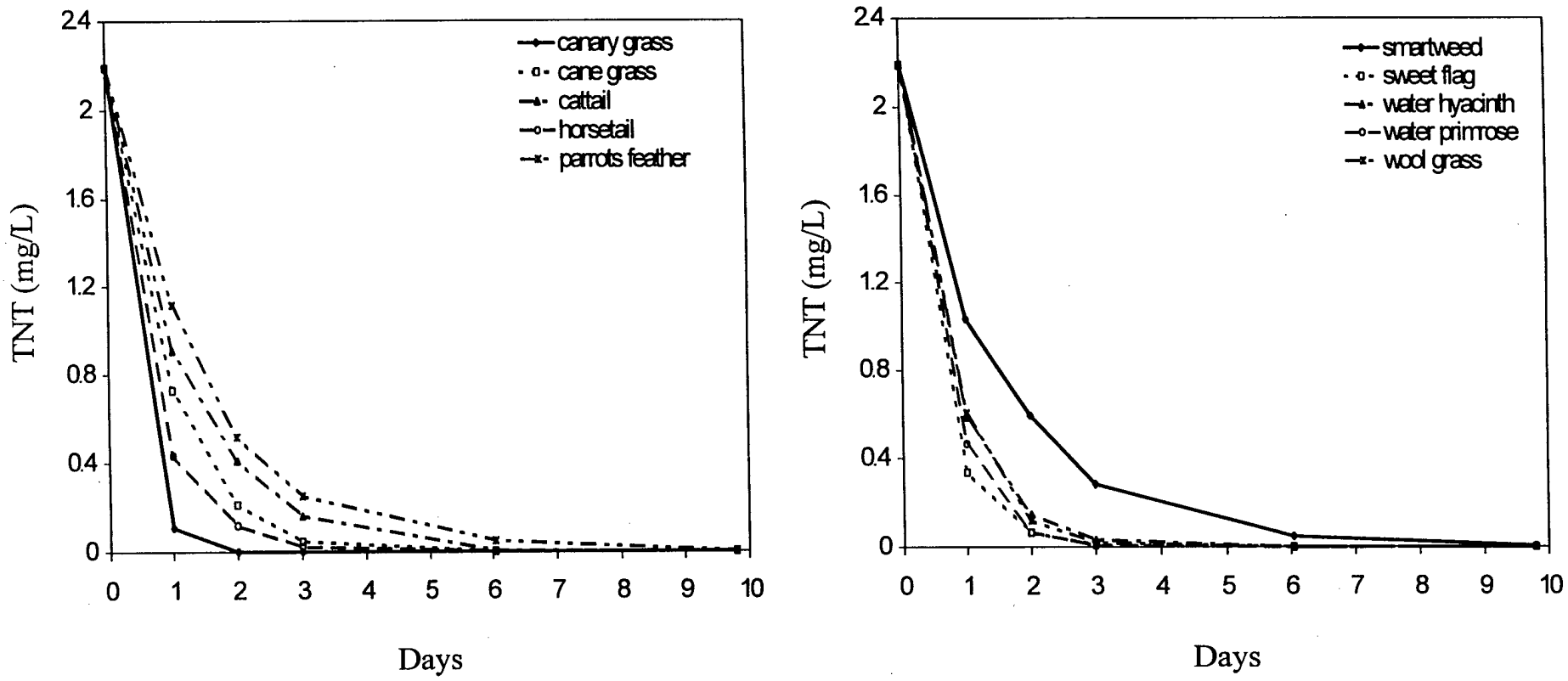


Fig. 3. Remediation of TNT with low plant biomass, and addition of 50 mg L^{-1} N to water.

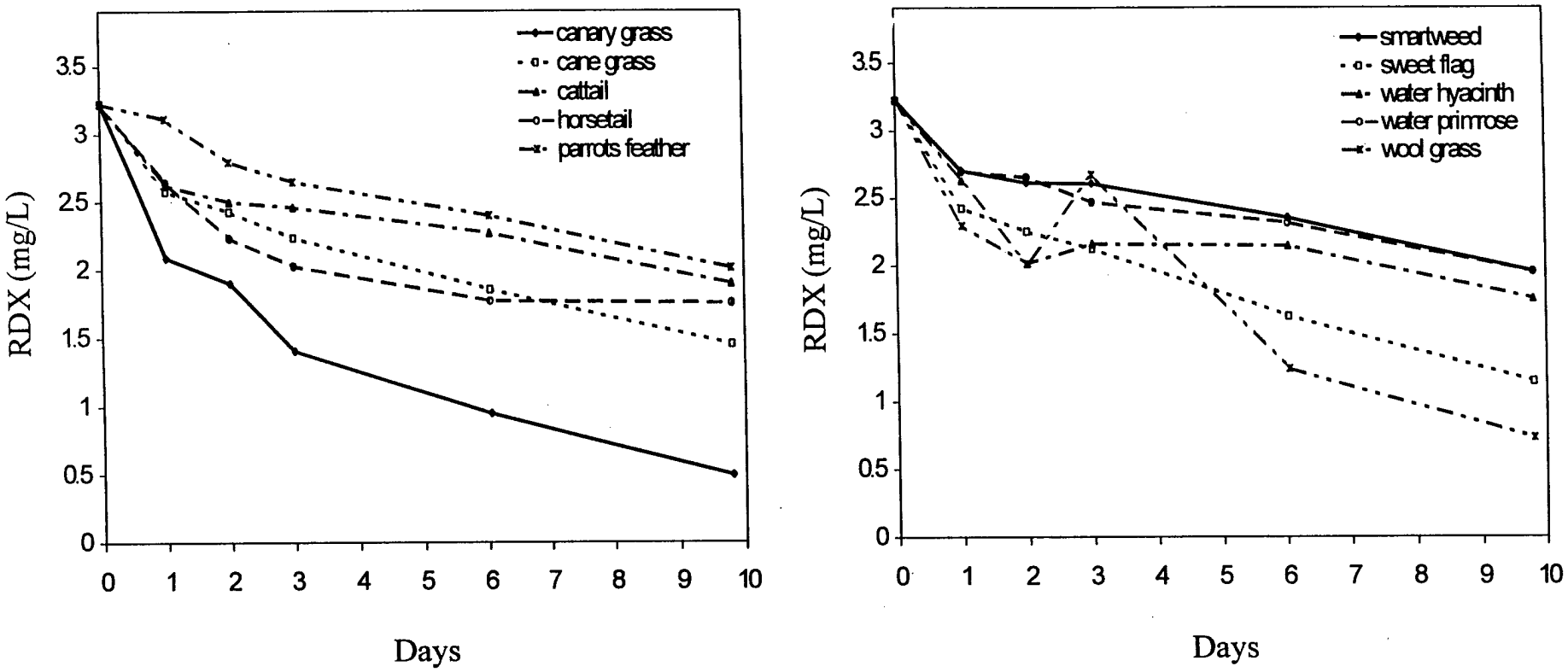


Fig. 4. Remediation of RDX with high plant biomass.

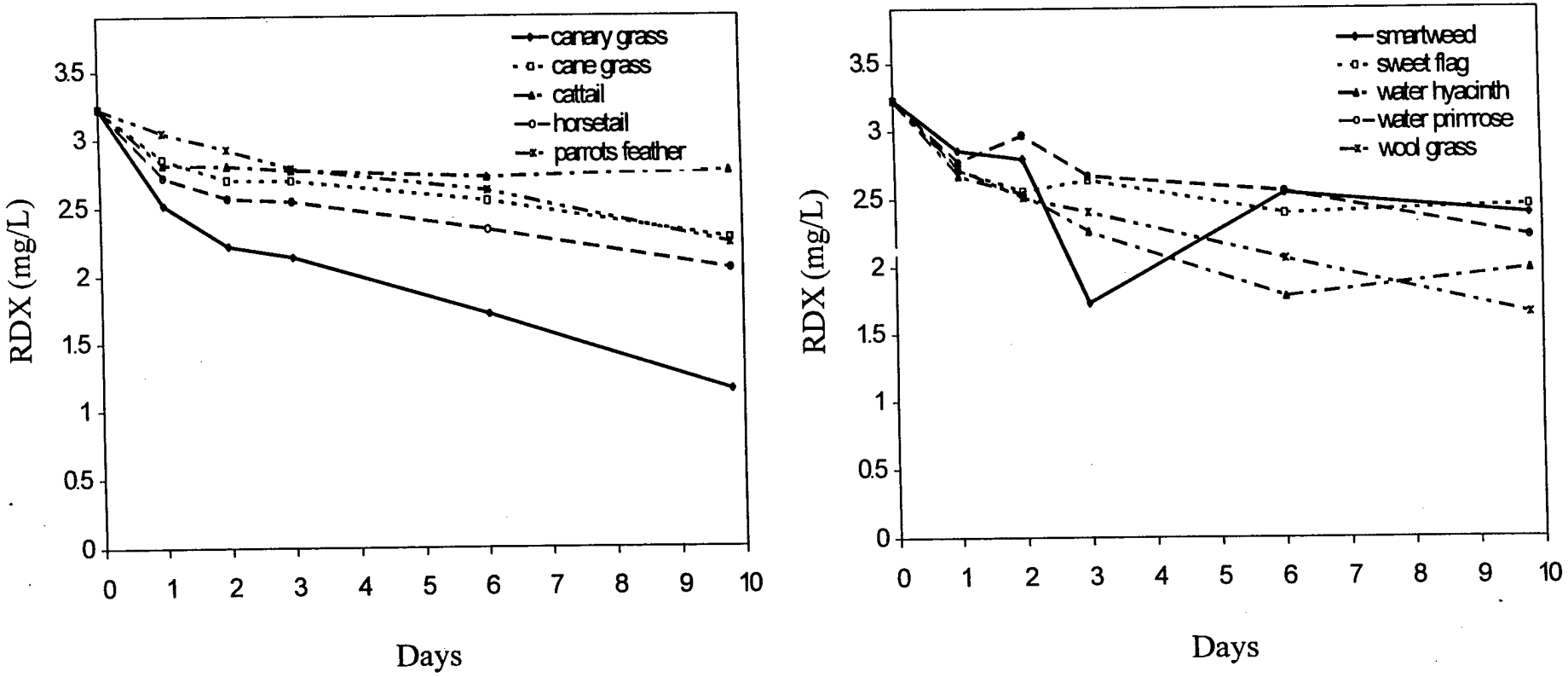


Fig. 5. Remediation of RDX with low plant biomass.

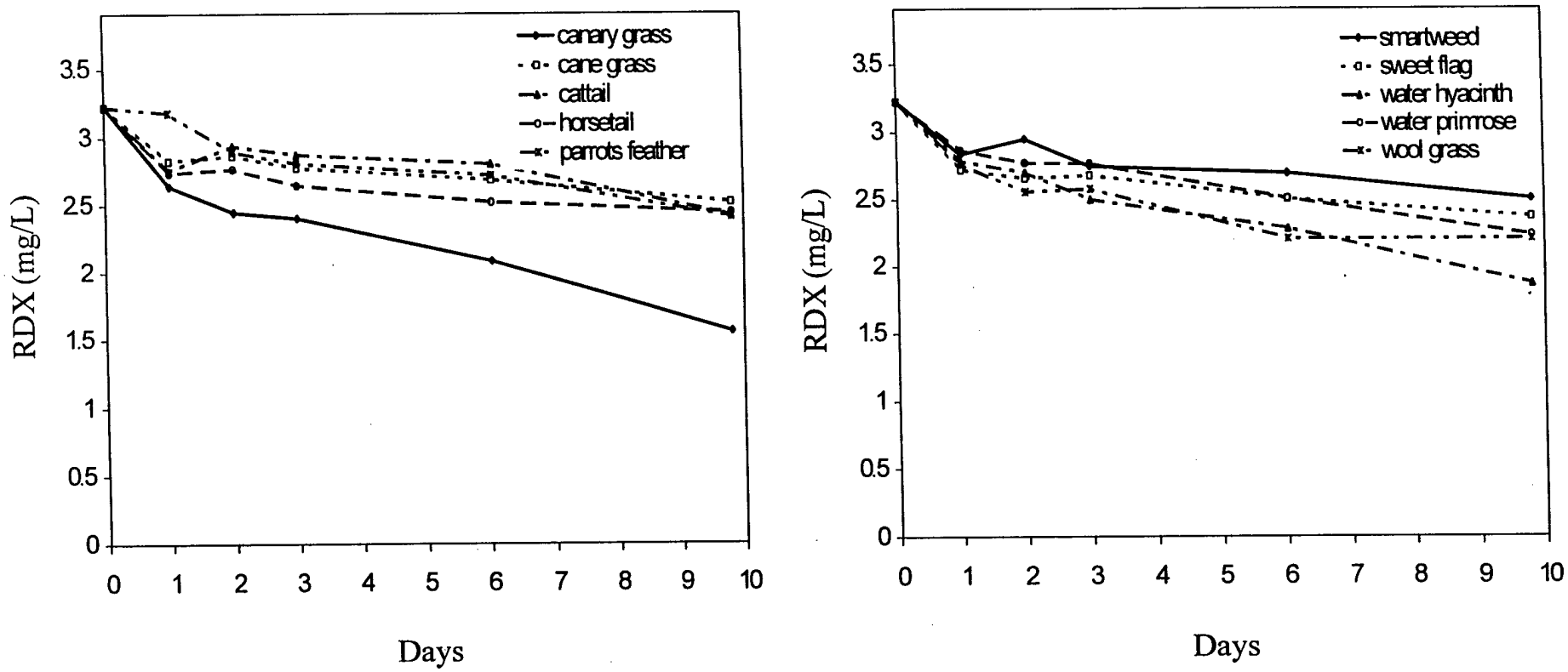


Fig 6. Remediation of RDX with low plant biomass, and addition of 50 mg L^{-1} to water.

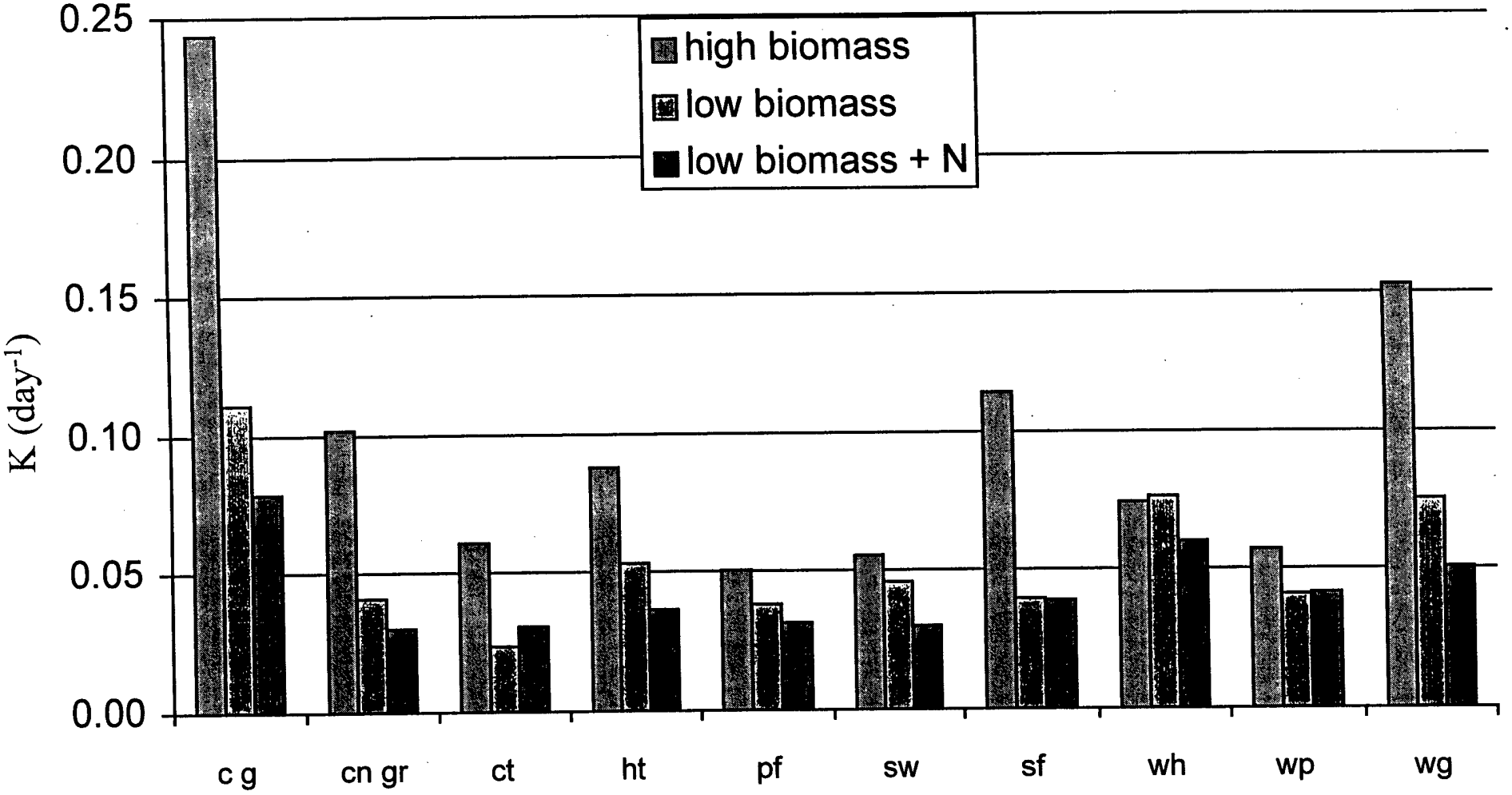


Fig. 7. First order rate constants for RDX degradation. c g canary grass, cn gr cane grass, ct cattail, ht horsetail, pf parrots feather, sw smart weed, sf sweet flag, wh water hyacinth, wp water primrose,wg wool grass.

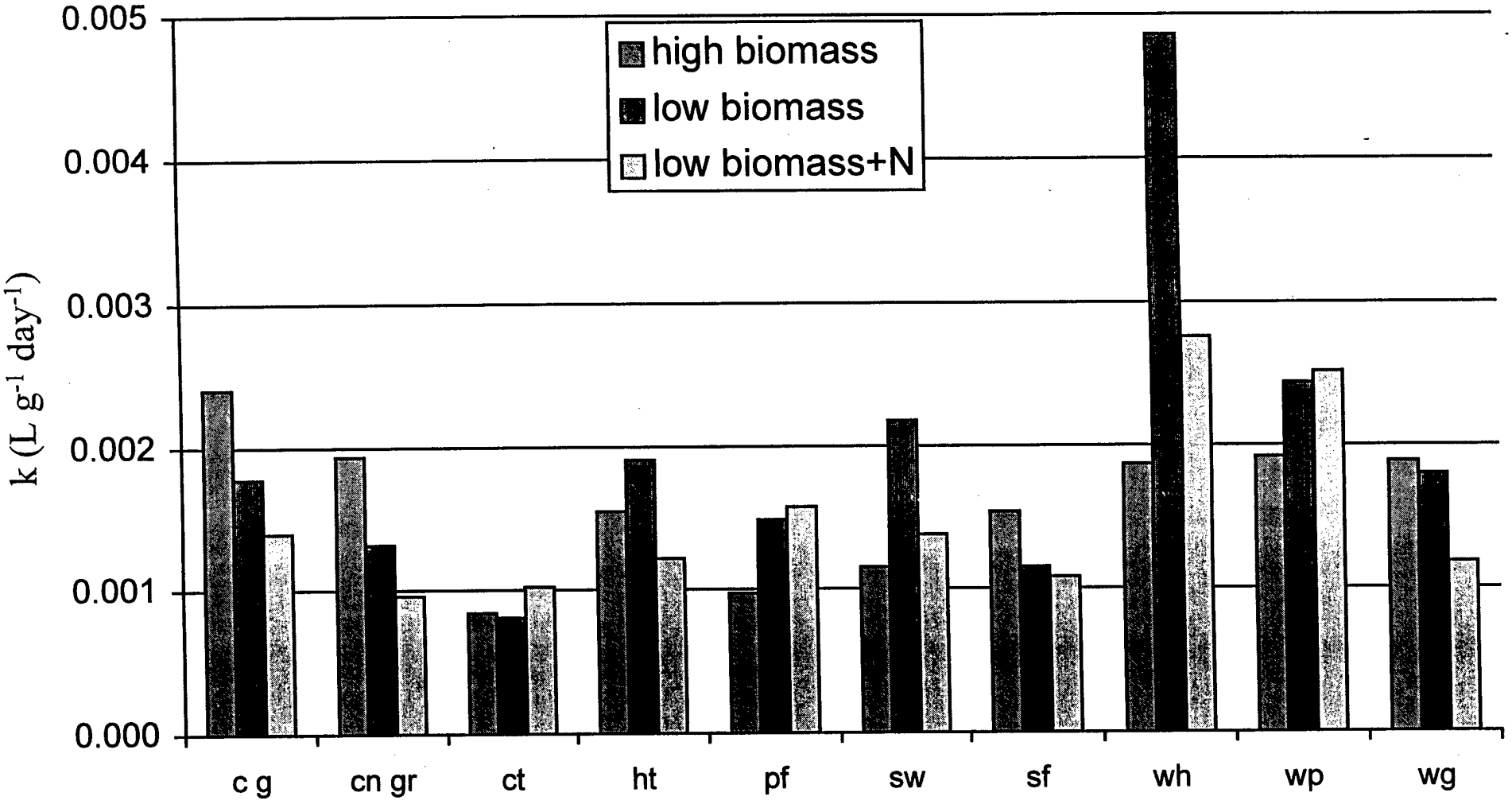


Fig 8. First order rate constants for RDX degradation adjusted for root biomass. Species abbreviations are the same as in Fig. 7.

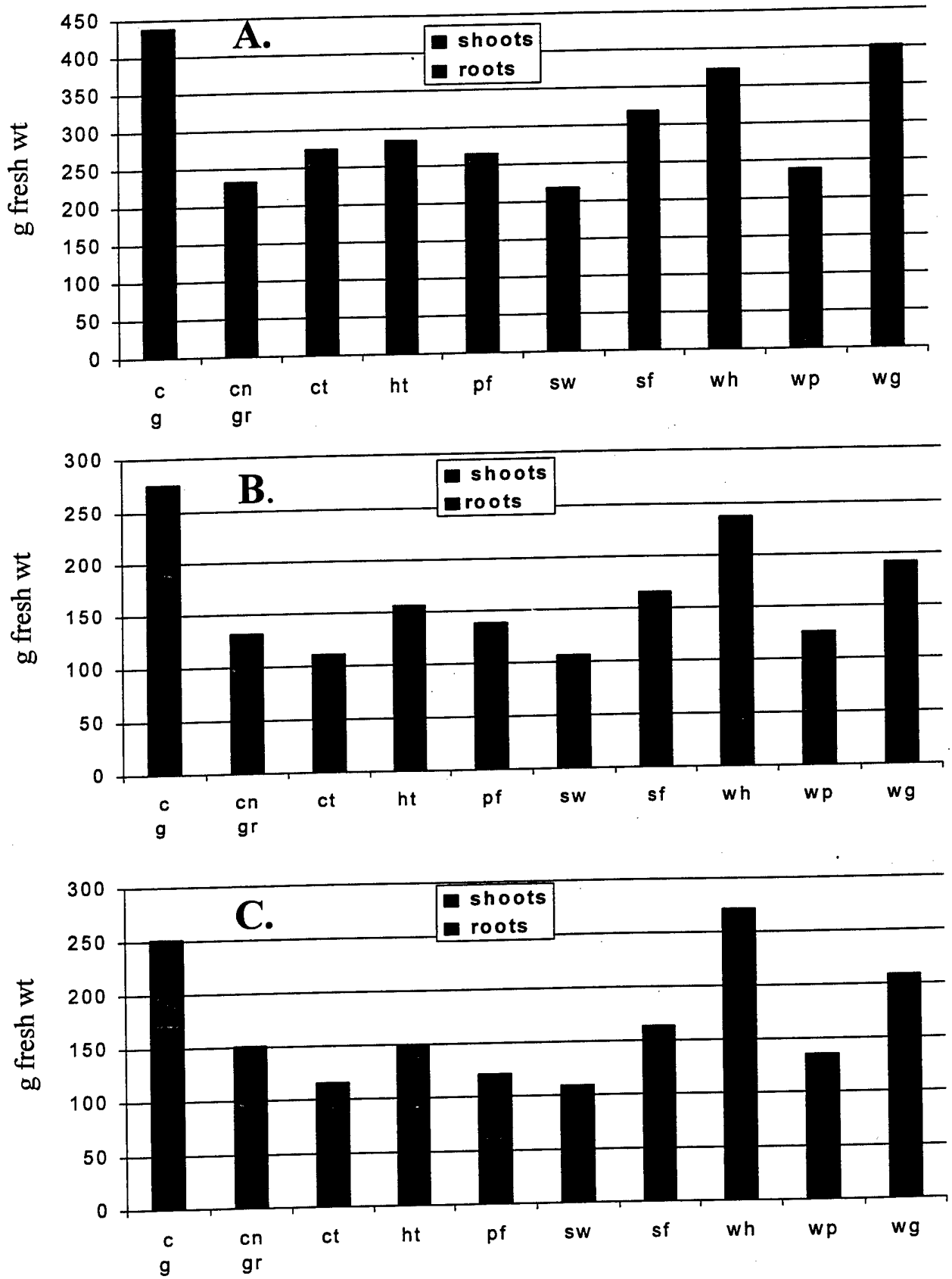


Fig. 9. Root and shoot fresh weights for: A. high biomass, B. low biomass, and C. low biomass + N treatments at end of 10 day sampling period. Species abbreviations are the same as in Fig. 7.

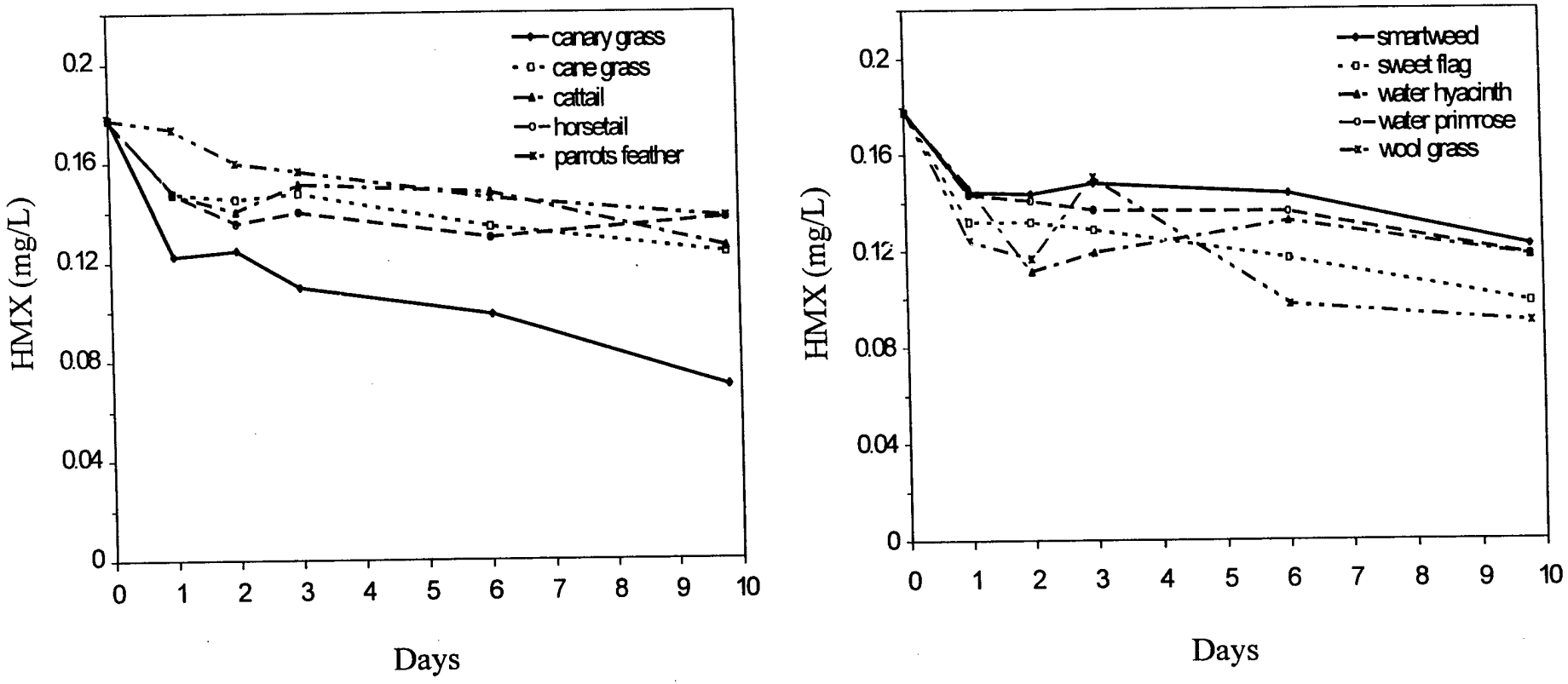


Fig. 10. Remediation of HMX with high plant biomass.

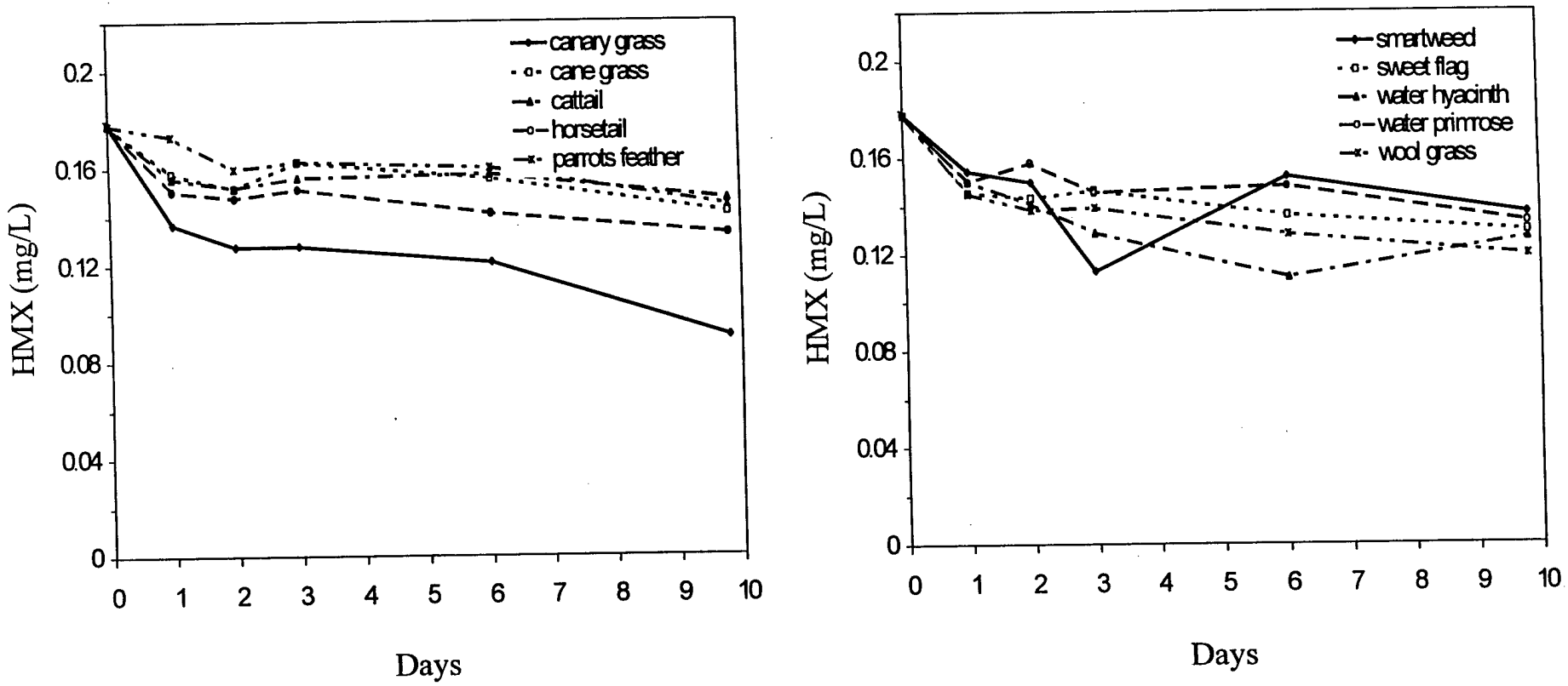


Fig. 11. Remediation of HMX with low plant biomass.

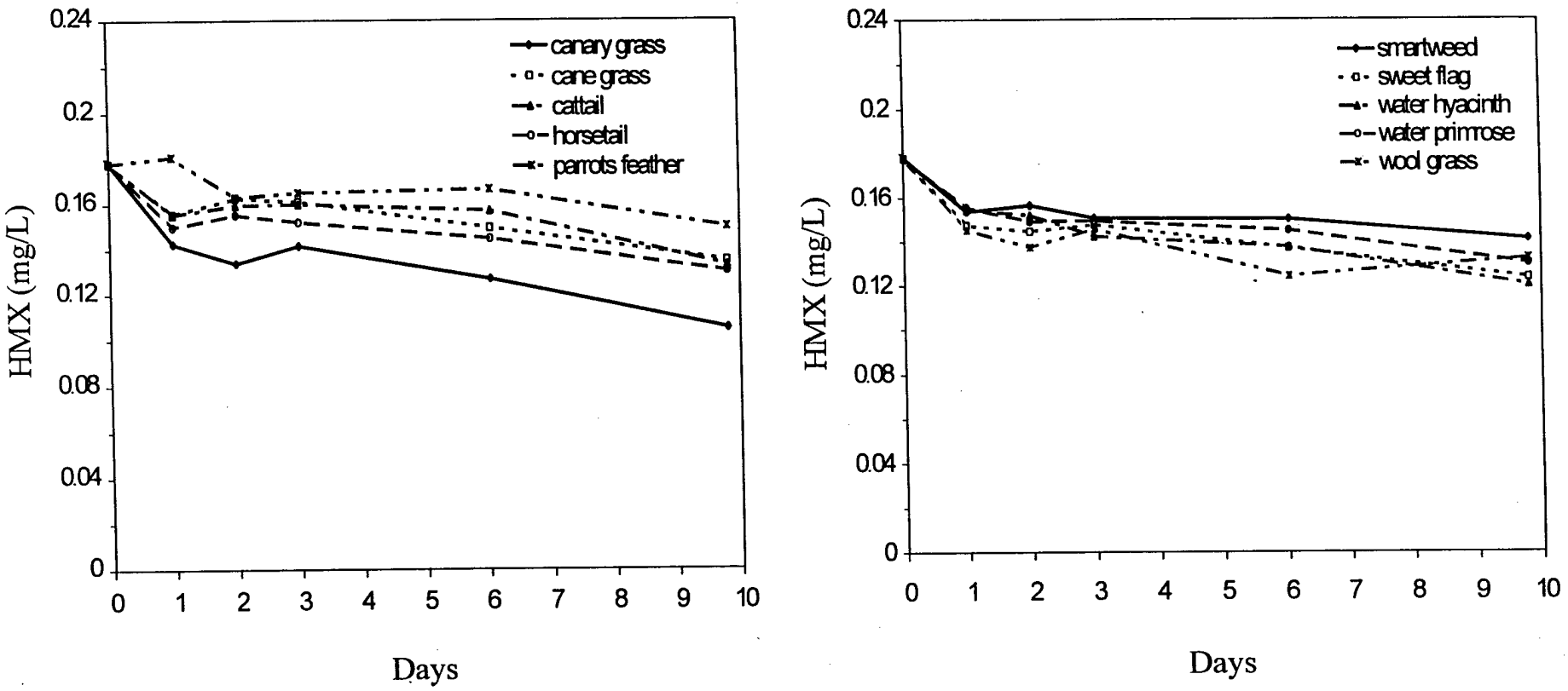


Fig. 12. Remediation of HMX with low plant biomass, and addition of 50 mg L^{-1} N to water.

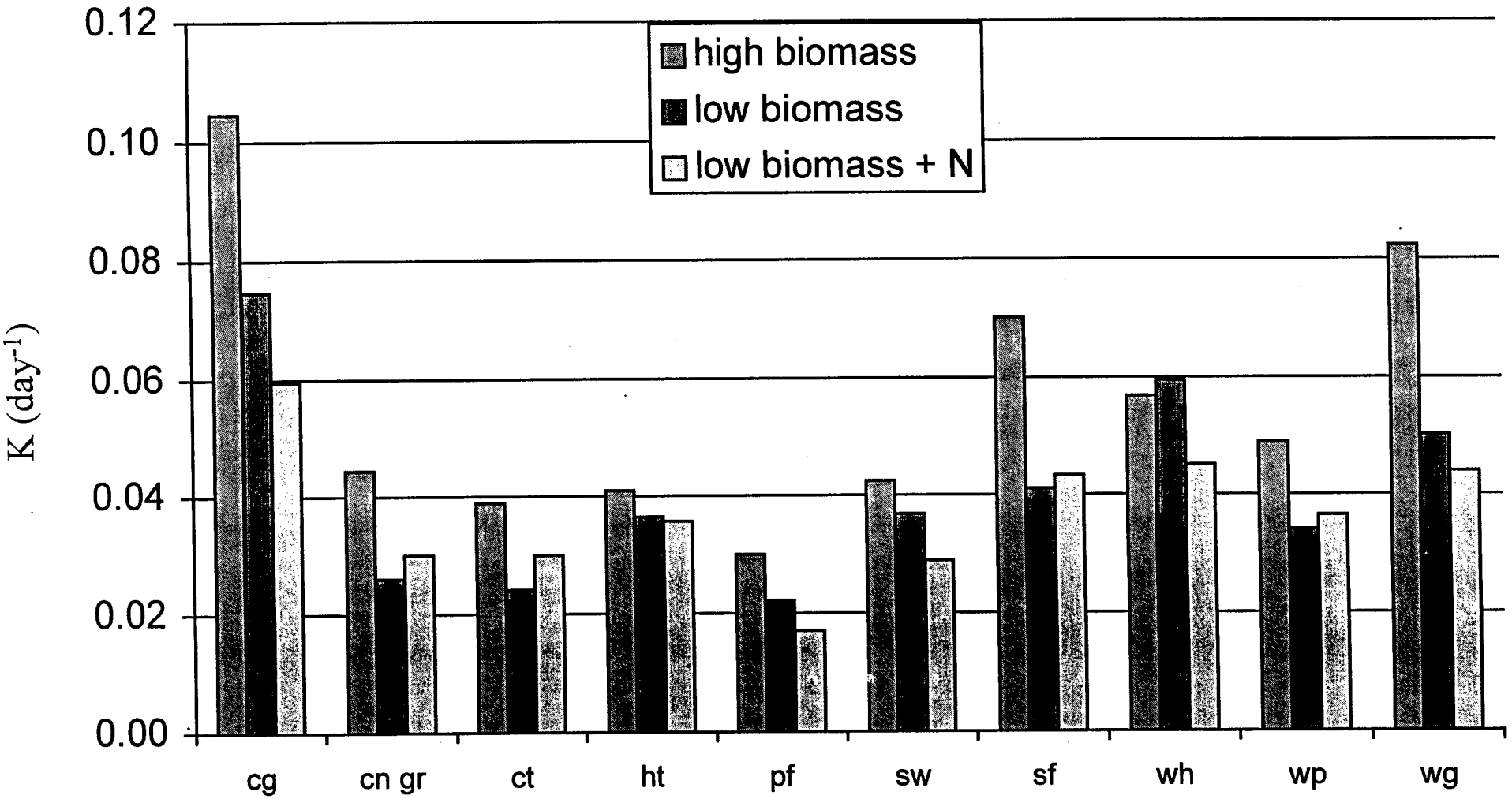


Fig. 13. First order rate constants for HMX degradation. Species abbreviations are the same as in Fig. 7.

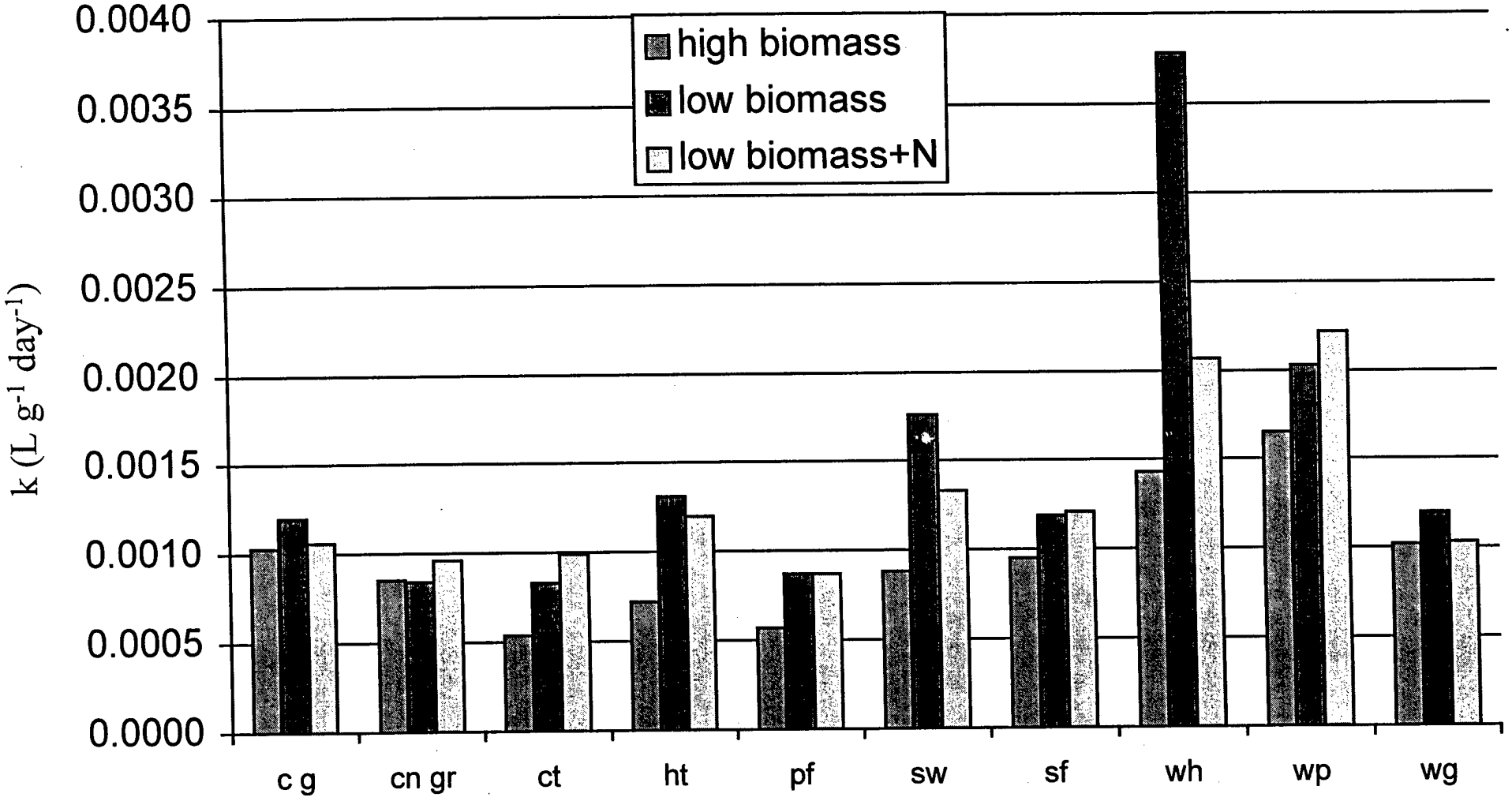


Fig. 14. First order rate constants for HMX degradation adjusted for root biomass. Species abbreviations are the same as in Fig. 7.

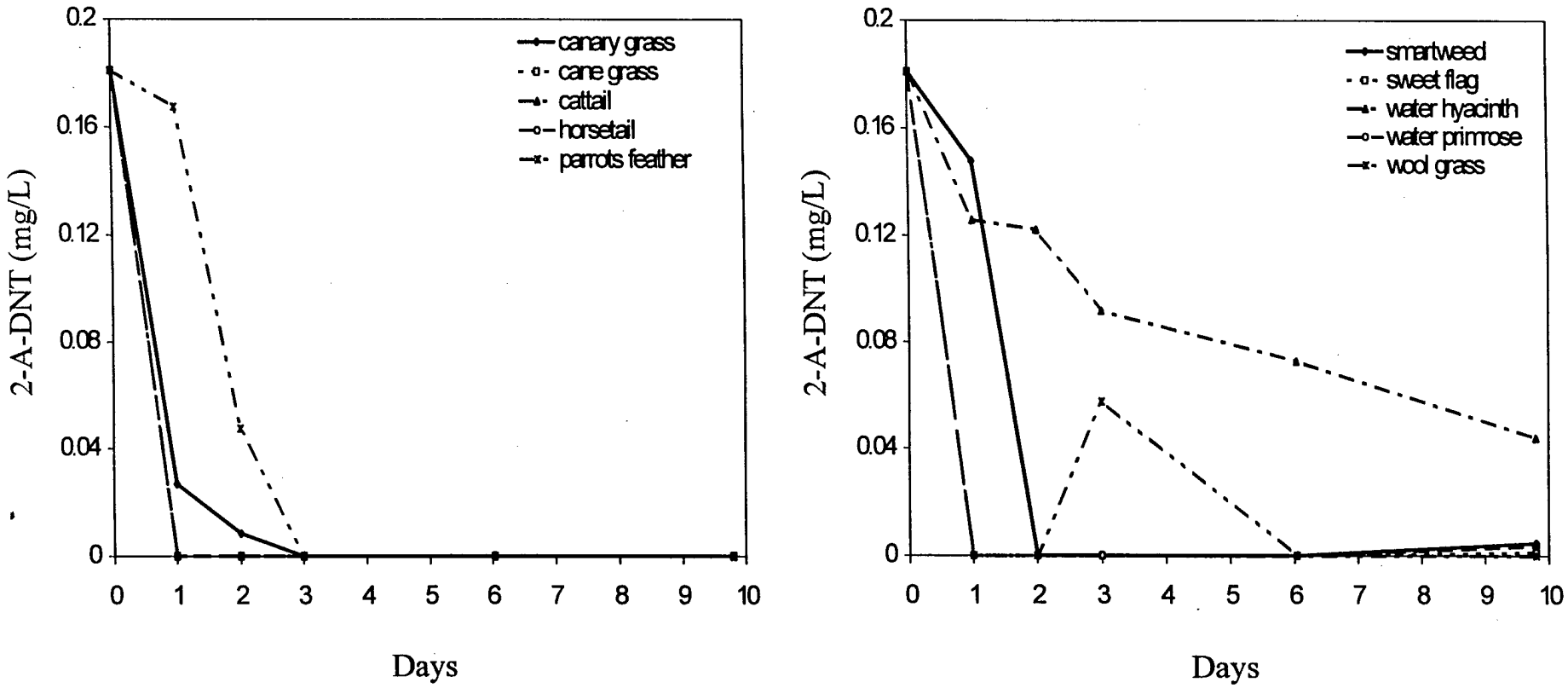


Fig. 15. Remediation of 2-A-DNT with high plant biomass.

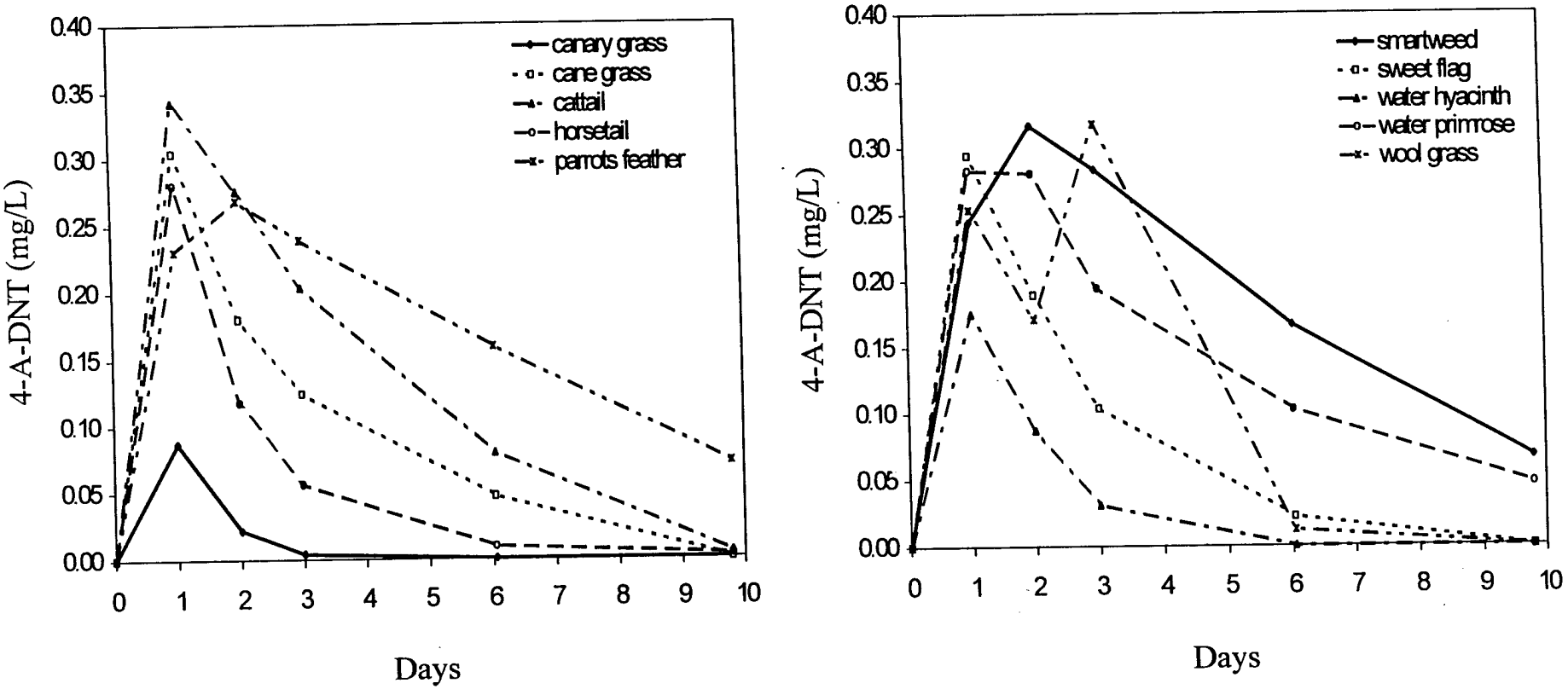


Fig. 16. Remediation of 4-A-DNT with high plant biomass.

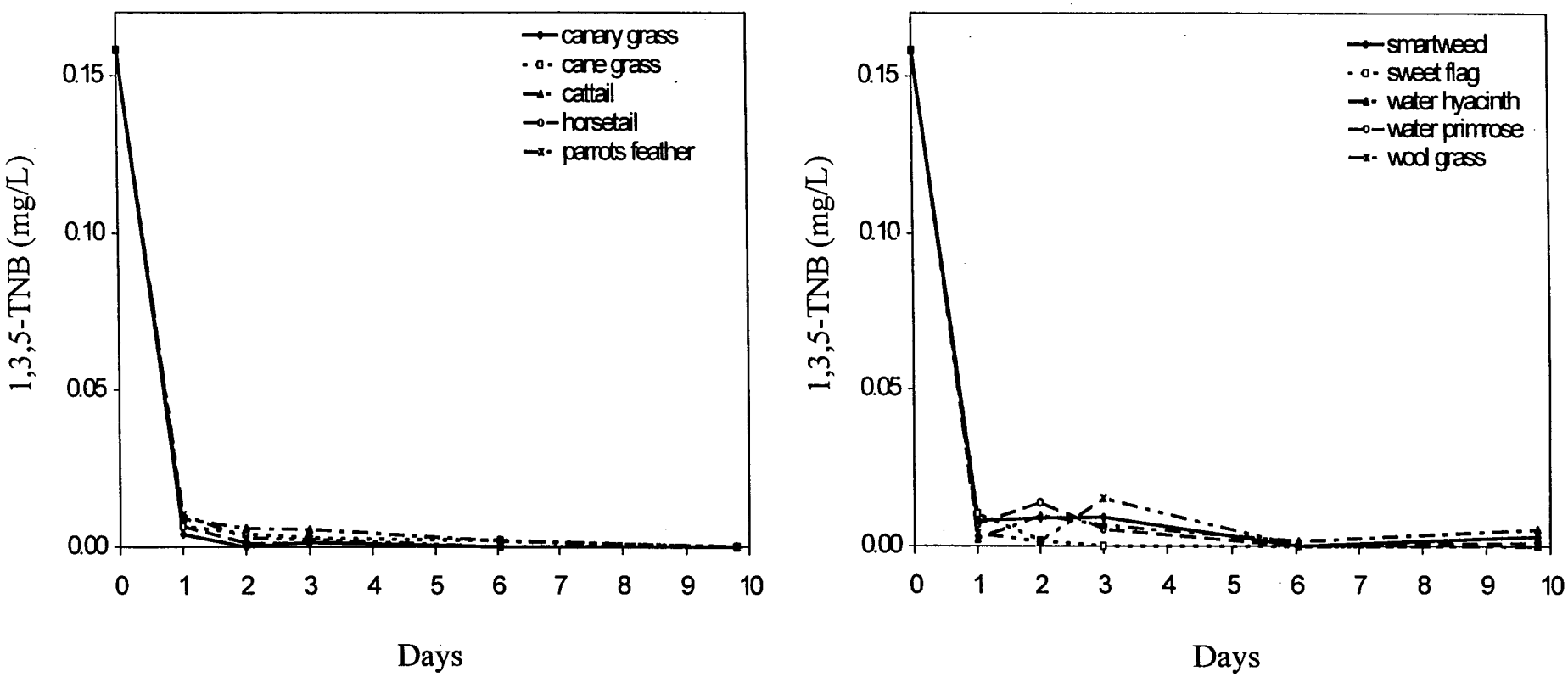


Fig. 17. Remediation of 1,3,5-TNB with high plant biomass.

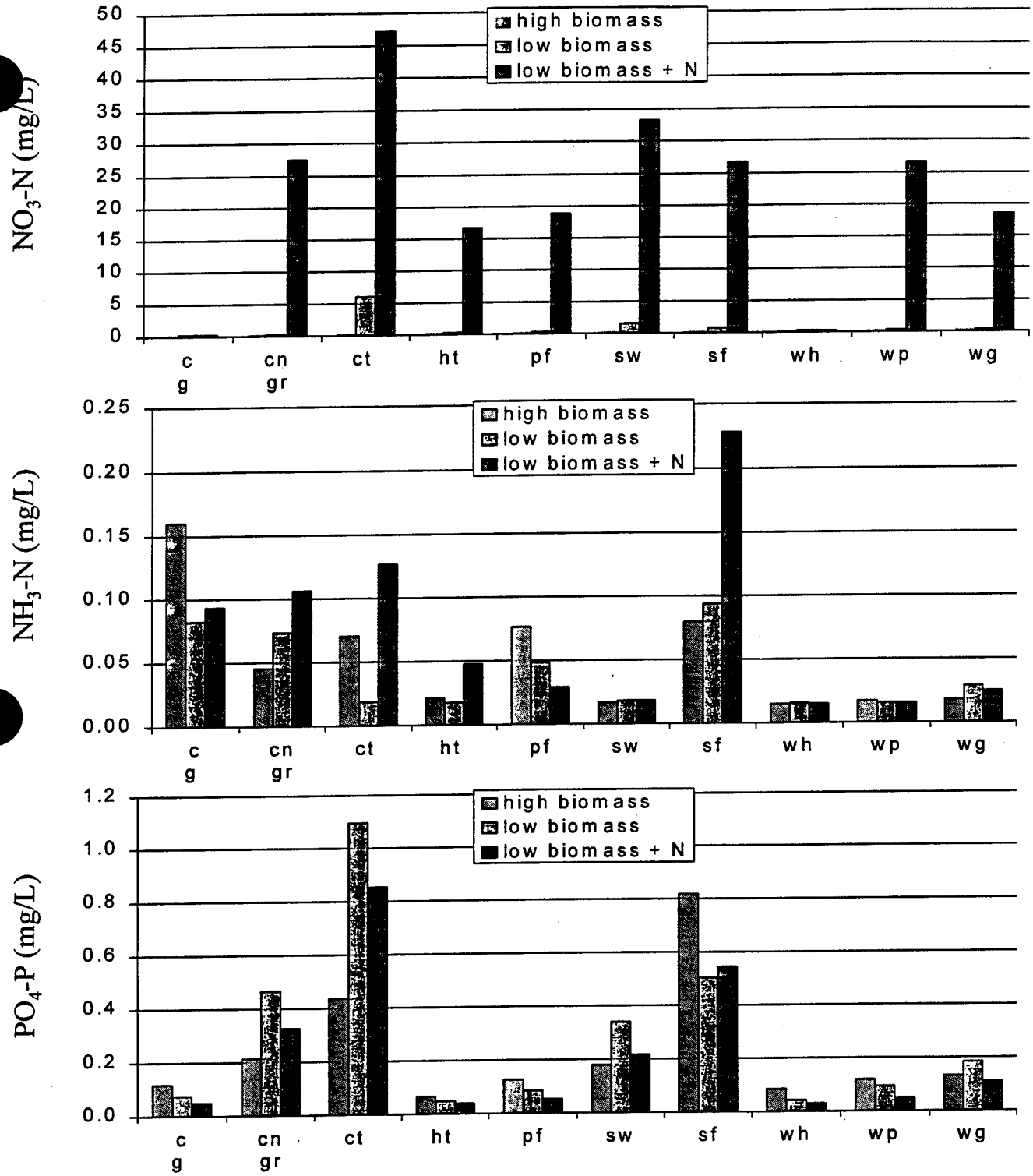


Figure 18. Nutrient concentrations of groundwater at end of 10-day remediation period. Initial nutrient concentrations for groundwater were 9.61 mg/L NO_3-N, <0.02 mg/L detection limit for NH_3-N, and 0.36 mg/L PO_4-P, with an additional 50 mg/L NO_3-N for the low biomass + N treatment. Species abbreviations are the same as in Fig. 7.

APPENDIX E

PLANT SCREENING STUDY—SUBMERGED PLANT SPECIES: USACE WATERWAYS EXPERIMENT STATION REPORT NO. EL-97-24; November 1997

PHYTOREMEDIATION OF EXPLOSIVES-CONTAMINATED GROUNDWATER USING CONSTRUCTED WETLANDS

PHASE 1 REPORT: PLANT SCREENING STUDY--SUBMERGED PLANT SPECIES

prepared by

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Environmental Laboratory
USAE Waterways Experiment Station
Vicksburg, MS

for

Army Environmental Center

February 1996

SUMMARY

This study evaluated ten submerged aquatic plant species for relative ability to decrease levels of TNT and RDX explosives and related nitrobodies in contaminated Milan Army Ammunition Plant groundwater, under controlled conditions involving factors of plant density and nutrient amendment. It was carried out in conjunction with the TVA, which examined emergent species in a similar procedure. Submerged species tested were: Eurasian watermilfoil, egeria, elodea, vallisneria, curlyleaf pondweed, sago pondweed, water stargrass, dwarf spikerush and stonewort. Parrotfeather was included in both tests as a comparison species. From this work, the submersed plant species recommended for phytoremediation of explosives are elodea, sago pondweed, water stargrass, and curlyleaf pondweed, along with parrotfeather.

During the 10-day incubation with groundwater, all plant species significantly increased amount and rate of TNT removal over that in water alone. The most effective species were elodea, sago pondweed, Eurasian watermilfoil, stonewort, curlyleaf pondweed and waterstargrass; they were equally active. Fertilization of plants with nitrogen enhanced TNT removal only slightly. Extrapolation of removal curves to the EPA-mandated potable water level of 2 $\mu g\ L^{-1}$ indicated the following residence time requirements for TNT clean-up: 56 days for water without plants or sediment, 16 to 56 days for water with sediment, and 4 to 5 days for incubation with submersed plants at a density of 9 g fr wt L^{-1}. Twice this density of plant material decreased residence time by approximately a day. Correlation analysis indicated that TNT removal from water is plant-mediated, and that required residence time decreases with increasing plant biomass.

Effects associated with RDX were highly variable and an order of magnitude slower than with TNT. Estimates of predicted residence times for clean-up to 2 μg RDX L^{-1} were 627 days for water without plants or sediment, and 50 to 297 days for water incubated with plants at the lower density. N-fertilization did not have a consistent effect. At the higher density, removal was significantly enhanced under incubation with sago pondweed, Eurasian watermilfoil, parrotfeather, and curlyleaf pondweed. Correlation analysis indicated that RDX removal from water was not directly plant-mediated; however, the required residence time for this explosive increased with oxygen concentration in water. This suggests the potential involvement of facultative and/or obligate anaerobic microorganisms in RDX removal, or the existence of non-aerotolerant enzymes.

A principal component analysis of the amount and type of explosives and TNT metabolites in water following incubation with plants indicated that two different physiological pathways for the chemical reduction of nitro-groups in TNT occur among the aquatic species tested. Removal of nitro-groups did not take place during plant-mediated degradation of TNT. The small increase in plant biomass observed may have inhibited overall nutrient uptake and interfered with response to nitrogen. Azoxy compounds were not identified in incubation water, and products attributable to photo-transformations were minor.

I: INTRODUCTION

Concerns about the environmental fate of explosive residues and transformation products present in soil and groundwater have compelled a Department of Defense (DoD) focus on cost-effective remediation technologies at military installations and ammunition plants. Aquatic plant-mediated degradation of 2,4,6-trinitritoluene (TNT) has been proposed as a promising treatment process by the US Environmental Protection Agency (EPA), Athens, GA. Under a partnering agreement by the US Army Environmental Center (AEC), the US Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, the Tennessee Valley Authority (TVA), Muscle Shoals, AL, and the EPA, a demonstration project on phytoremediation of explosives-contaminated groundwater has been funded by the DoD's Environmental Security Technology Certification Program (ESTCP). The AEC, as lead agency with other groups providing technical support, has selected Milan Army Ammunition Plant (MAAP), located near Milan, TN (longitude 88° 50'W, latitude 35° 45'N), as a demonstration site. MAAP was selected because groundwater at this site contains high concentrations of TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX, 'royal demolition explosive'), significantly above EPA-mandated potable water levels of 2 $\mu g\ L^{-1}$ (2 ppb).

Phase I of this project provides for laboratory scale screenings and field phytoremediation treatability studies using aquatic and wetland plants to support Phase II, a field-scale demonstration at MAAP. The primary objective of the screening studies is to evaluate the ability of locally-adapted plants to diminish levels of TNT and RDX explosives and their by-products in MAAP groundwater, relative to the emergent aquatic species parrotfeather. The studies also assess factors of plant density and fertilization on the extent and rate of contaminant removal by these species. WES evaluated submerged plants and TVA tested emergent and wetland species, under common conditions formulated in a standard protocol.

This report presents the results of WES' evaluation of *Myriophyllum spicatum* L. (Eurasian watermilfoil), *Egeria densa* Planch. (egeria), *Elodea canadensis* Rich. in Michx. (elodea), *Vallisneria americana* Michx. (vallisneria), *Potamogeton crispus* L. (curlyleaf pondweed), *Potamogeton pectinatus* L. (sago pondweed), *Heteranthera dubia* (Jacq.) MacM.

(water stargrass), *Eleocharis parvula* (R. & S.) Link (dwarf spikerush), and *Chara vulgaris* L. (stonewort). Parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.) was included to provide a comparison between the screening tests: project members have observed TNT-degrading activity in this species during previous studies.

II: MATERIAL AND METHODS

This study was carried out under the general conditions described in a standard treatability protocol for phytoremediation screening formulated by WES and TVA (Anonymous 1995).

1. Plant material

Species selection The nine aquatic plant species evaluated for ability to degrade explosives in MAAP groundwater were selected on the basis of several criteria. Physiological parameters included presence of nitroreductase enzyme activity (as shown by EPA immunoassay testing), explosive-degrading activity under aerobic or anaerobic conditions (from EPA, TVA, and Georgia Institute of Technology), or ability to remove aromatic hydrocarbons. Ecological traits important in field deployment were considered, including perenniality, high biomass production, extensive root/rhizome systems and year-round biomass. Attention was given to the native habitat of the species, their suitability to MAAP climate conditions, and whether they were considered as noxious weeds in Tennessee. The species chosen are listed in Table 1.

Source and acclimation of planting material Parrotfeather was obtained through TVA from a population growing in a fish pond near Muscle Shoals, AL; plants from the same source were used by TVA in screening emergents. All other species except stonewort were provided from outdoor ponds at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville TX. Stonewort ws obtained from a commercial nursery (Southern Tier Consulting, West Clarkville, NY).

Plants except for stonewort were acquired during the last two weeks of September 1995 and held in hydroponic monocultures in a WES greenhouse, using a 0.25x Hoagland's nutrient culture medium (Hoagland and Arnon, 1938) as modified by TVA. Stonewort had previously been planted into sediment in a low-alkalinity solution (Smart and Barko 1985).

Cultures were aerated to enhance mixing and air/water CO_2 exchange.

The majority of plant material was received as unrooted apical shoots; vallisneria and dwarf spike rush were received as whole plants originating from rooted crowns. Rooting of apical shoots during the acclimation period was minimal.

2. Water

Contaminated groundwater used for screening by both WES and TVA originated from a single batch collected into a tank truck by TVA from MAAP Well MI 146. A sub-sample of 833 L (220 gallons) was brought to WES in four stainless steel 208-L (55-gallon) drums at the end of September, and stored at room temperature before use. The chemical composition of this batch of groundwater is given in Table 2.

3. Sediment

Sediment used as a control in the experiment originated from soil collected in a low-lying grassland area, which had not been fertilized for the last five years, near the 'X' production line at MAAP. Soil was excavated at the end of September, placed in polypropylene 19-L (5-gallon) buckets, transported to WES, and stored in a cold room (5 C). It was prepared for the experiment by wetting with tap-water and fully blending the contents of one bucket in a mechanical mixer. Dry weight was determined from a 34 g wet weight sample. A portion of this sediment was autoclaved (1 hr at 120 C and 15 psi; mixed; 30 min at 120 C and 15 psi) before use, to inactivate soil organisms and enzymes and provide both autoclaved and non-autoclaved sediment controls.

4. Experimental design

The experimental set-up was a randomized complete block design, in which each of three blocks was a full replicate containing every treatment level evaluated. This allowed statistical testing of the effects of all species at two biomass densities and of all species at the lower density with and without fertilizer amendment, without requiring an inordinate number of experimental units. Controls were groundwater without plants and without sediment, and autoclaved and non-autoclaved MAAP soil with groundwater without plants (hereafter

generally referred to as water and non/autoclaved sediment controls).

Thus, each block contained a full set of factors in 36 experimental units: 10 plant species, each at two densities and at one fertilizer amendment of the lower density; water; and non/autoclaved sediment. The experiment contained a total of 108 units.

5. Experimental conditions

The screening was carried out over a 10-day incubation period, 3 to 13 October 1995, in a large walk-in controlled environment growth chamber. Experimental units were glass aquaria, 15 x 15 x 37.5 cm, constructed with silicone sealant. After test materials were placed in them, they were filled with groundwater to a final depth of 15 cm, giving a uniform total test volume (rather than water volume) of 3.375 L. Plants were incubated without mechanical support as approximately 15 cm apical shoots or as whole plant crowns at two densities, 9 g fresh weight (FW) L^{-1} and 18 g FW L^{-1}, giving 30.4 or 60.8 g plant material per aquarium. As an emergent aquatic, parrotfeather was expected to have approximately half its biomass above the water surface, and therefore twice as much material was incubated (60.8 g and 121.6 g). Weighed portions of sediment (255 to 270 g) were placed in aluminum foil trays into aquaria. Filled aquaria were covered with glass lids (except in the case of parrotfeather) to minimize evapotranspiration.

To test effect of nitrogen (N) fertilization on explosives removal, groundwater was amended with 50 mg NO_3-N L^{-1}. This was applied as 1.22 g KNO_3 to the applicable aquaria and dissolved in water before plants were added.

High pressure sodium and metal halide lamps provided a full photosynthetic spectrum at a level of 400 to 500 $\mu E\ m^{-2}\ s^{-1}$ at 22.5 cm above the water surface. Each block of experimental units was positioned within an area of similar irradiance. Sides of aquaria were covered with black curtains to exclude incident light. An automatic timer provided a daylength of 12 hours, and temperature was set at 25 C.

Culture solutions were not aerated in order to 1) mimic expected slow water flow in field studies, and 2) produce the low oxygen (O_2) concentrations under which RDX removal was shown to be enhanced in a recent TVA study (unpubl. comm.)

6. Experimental procedures and sampling

At the beginning of the incubation period groundwater was pumped into each aquarium, and pre-determined weights of fresh plant material, sediment, or nitrogen were added as required. It was found at the beginning of the experiment that the small amount of stonewort available prevented testing this species at the higher density.

The following sampling procedures were applied to each experimental unit. Water samples were taken 1, 4, 12, 24 and 240 hours (10 days) after incubation began. Prior to sampling, the contents of the aquarium were mixed using a glass rod; 100 ml of water was then collected into a glass cylinder and decanted into a glass bottle with a teflon-lined cap. Water samples were refrigerated in the dark until further processing, usually within 24 hours of collection.

After the final sampling (240 hours), plant materials were removed and weighed. A dry weight:fresh weight (DW:FW) ratio was determined for each species by drying a weighed portion of material in a ventilated oven at 70 C until constant weight was attained, and then re-weighing. Relative growth rates were calculated by dividing the natural log (ln) transform of final plant DW by initial DW, and dividing by the 10 days of incubation. Sediment was removed, weighed, placed in glass jars, and kept refrigerated until analysis. A 1-L sample of water was placed in a plastic bottle, pH was measured, and the bottle was placed in a freezer (-20 C) to await nutrient analysis. Oxygen concentration was measured within the aquarium using a YSI O_2 electrode.

7. Chemical Analyses

Analytical specifications, calibration compounds, and method references are included in the Appendix.

Explosives in water Levels of explosives and metabolic/degradation products of TNT were determined in the Ecosystem Processes and Effects Branch (EPEB) at WES, in all water samples. Due to the lengthy procedure required for analysis of azoxy compounds, they were measured only in the 10-day water samples of one block.

The 100-ml water samples were concentrated using a solid phase extraction. Explosives were eluted in acetonitrile, and analyzed using high pressure liquid

chromatography (HPLC). Levels of compounds found by separate WES and TVA analyses in initial samples of the common batch of MAAP groundwater used for testing are listed in the Appendix.

Alkalinity, macronutrients and calcium in water Analyses carried out in the laboratory at LAERF used a variety of methods to determine pH, alkalinity, NH_4-N, NO_3-N, available phosphate phosphorous (P-PO_4, as soluble reactive phosphorous: SRP) sulphate (SO_4), and total calcium (Ca).

Macronutrients, bulk density and organic matter in sediment These determinations were made in the EPEB at WES. Total Kjeldahl-N and phosphorus (P) were determined in soil digests, and measured colorimetrically. Exchangeable ammonium and SRP were determined with standard methods.

8. Data analysis

Final summary and analysis of explosives concentrations were carried out on TNT and RDX separately, using STATGRAPHICS Plus (Version 7; Statistical Graphics Corporation, Bitstream Inc., Cambridge, MA) to perform analysis of variance (ANOVA), regression analysis, multiple range tests, and principal component analysis (PCA). Significance was tested at the 95% confidence level, $P \leq 0.05$. PCA was performed to associate those plant species that exhibited similar concentrations of explosives and types of degradation products following incubation.

III: RESULTS AND DISCUSSION

1. Data handling

Data from HPLC analysis of all water samples were initially screened for outliers using a method based on Hotelling's T-square; however, this excluded whole species and/or classes of samples and was not informative. Subsequently, only those samples thought to have been incorrectly prepared for analysis, misinjected during HPLC, or improperly integrated, were excluded. These amounted to 22 samples, out of a total of 540, or 4.1%. Data on stonewort at the higher density were unavailable, as noted above.

Initial ANOVA of TNT and RDX data as randomized complete block experiments

showed that inter-block differences were not statistically significant (P = .576 and .207, respectively). Therefore, the data sets were subsequently analyzed as completely randomized designs with three replications, and without subtracting block effects.

2. TNT concentration in water: effects of plant species, density and fertilization

TNT concentration in groundwater incubated with plant material at the lower density decreased significantly from initial levels of 2197 $\mu g\ L^{-1}$ (Table 2) over a ten-day period; all species except egeria and parrotfeather had reached levels below detection ($\leq$ 0.1 $\mu g\ L^{-1}$) by 10 days (Figure 1). TNT decreased less rapidly in aquaria with water alone or with non/autoclaved sediment. Using data across sampling times, all species significantly enhanced TNT removal compared to water alone ($P < .001$, Tables 3 and 5). Elodea, sago pondweed, Eurasian watermilfoil, stonewort, curlyleaf pondweed and waterstargrass were most, and equally, effective. Egeria was the least active.

TNT removal from water was accelerated under incubation at the higher plant density (P = .054). All species produced readings below detection limits by 10 days; sago pondweed was at this level by 12 hr, and elodea and water stargrass by 24 hr. However, using data across all sampling times, differences among species were not significant (P = .956). The effect of N-fertilization on TNT removal over the course of sampling was not significant at the 95% level (P = .081; Table 4).

An exponential regression model, $Y = \ln(a + bX)$, where Y = concentration and X = time, and with a negative slope, gave the best fit to TNT decrease over time (Tables 6 and 7). R^2 values were highest and most consistent for plant species at the lower density (mean = 94.6% $\pm$2.7 (s.e.)). Greater variability in data for the higher density (mean R^2 = 60.4% $\pm$7.7) may have been produced by nutrient limitation and low O_2 concentrations resulting from the larger amount of plant material incubated.

Regression statistics were used to calculate half-life in hours, $t_{½} = -\ln 2/b$, and to extrapolate retention time required to reach a TNT clean-up level of 2 $\mu g\ L^{-1}$, $t_{2\mu g} = (\ln 2\ \mu g\ L^{-1} - a)/b$ (Tables 6 and 7). While half-life statistics are independent of concentration and intercept, time to target concentration does involve these data; therefore, different species with the same half-life do not necessarily provide the same clean-up kinetic.

Half-lives for TNT removal with plants present ranged from 10.1 to 53.3 hr. These were associated with approximate retention times requirements of 3 to 5 days for most plants at the lower density with or without fertilizer, and 1 to 5 days at the higher density. Egeria required 18 to 19 days for clean-up under the conditions tested. These data compared to 56 and 94 days, respectively, for unamended and N-fertilized water alone; 56 days for non-autoclaved unfertilized sediment, and 20 days with N; 16 days for autoclaved sediment alone, and 4 days when this sediment was N-amended. The significant decrease in required retention time in sterile, fertilized sediment may result from the ability of explosives-adapted microbial populations, assumed to be present in the contaminated groundwater, to function without competition from populations in the native sediment and to respond to nutrient availability.

Initial DW plant mass incubated was correlated to retention time required for clean-up, in order to assess whether TNT disappearance from water was associated with factors such as adsorption to plant surfaces, metabolic activity, etc. (Table 8). This correlation was highly significant (P-value 0.008) with a negative coefficient, showing that TNT removal from water is plant-mediated and that retention time decreases with increasing plant DW.

3. RDX concentration in water: effects of plant species, density and fertilization

RDX concentrations in groundwater incubated with plant material decreased from initial levels of 3002 $\mu g\ L^{-1}$ (Table 2) only gradually during the ten-day period, and remained high in controls (Figure 2). RDX transformation is known to require longer than that of TNT, and the relatively short incubation, the lack of sampling between 24 and 240 hr, and the small size of the initial plant effects are expected to have contributed to the higher variability in RDX data.

ANOVA and multiple range tests of plants and water controls showed that at the lower density no species contributed significantly to RDX removal ($P = .067$, Tables 9 and 11). At the higher density, only sago pondweed was significantly more effective than water alone ($P < .001$). N-amendment did not enhance RDX removal ($P = .918$, Table 10).

A linear regression model, $Y = a + bX$, where Y = concentration, X = time, and b is negative with decrease over time, gave the best fit to change in RDX during incubation.

However, slopes were not uniformly negative and R^2 values were generally low, attesting to the high variability in this data. In particular, the low R^2 for controls, (0.22 to 14.6%) makes comparisons between plant and control effects difficult. Autoclaved sediment reached clean-up levels most quickly, at 136 days. At the higher plant density, R^2 's were appreciably higher and all regression slopes were negative. Here, regression statistics were used to calculate half-life in hours, $t_{½} = ((3002\ \mu g\ L^{-1} \times .5) - a)/b$, and to extrapolate residence time required to reach RDX clean-up levels, $t_{2\mu g} = (2\ \mu g\ L^{-1} - a)/b$ (Tables 12 and 13). The resulting clean-up periods required were 10 days for sago pondweed, 24 days for milfoil, and 42 days for curlyleaf pondweed and parrotfeather.

Correlation of initial DW plant mass to required retention times (Table 14) was not significant ($P = .646$), suggesting that RDX removal from water is much less affected by plants than that of TNT. However, correlation between RDX retention time and final O_2 concentration in incubation water was highly significant with a positive coefficient ($P < 0.001$, Table 14), indicating that RDX clean-up period increases with increasing O_2. This suggests the involvement of facultative and/or obligate anaerobic microorganisms in RDX removal.

4. Separation of plant species effects on explosives and their metabolites and degradation products, using PCA

PCA was performed on concentrations of RDX, TNT, TNT-metabolites and TNT-degradation products present in initial water and at 10 days, for each plant species and control (Table 15). This statistical analysis transformed the data with the result that 44% of the variability they represented could be summarized on the first two axes of a PCA scatterplot (Figure 3). Spatial relationships among treatments plotted in this way indicate how similar they are in type and quantity of break-down products released during incubation, and by inference, how similar they are in physiological pathways of TNT metabolism. Species producing similar metabolites in water cluster more closely on the graph than those with differing out-puts.

Figure 3 shows how greatly the composition of the initial water sample (W_o) differs from all incubated samples, possibly due to high levels of trinitrobenzene (TNB: Table 15).

In some cases species belonging to the same family clustered together: in the Haloragaceae, parrotfeather (MA) and Eurasian watermilfoil (MS); in the Potamogetonaceae, curlyleaf pondweed (PC) and sago pondweed (PP). In others, species within families were widely separated: in Hydrocharitaceae, egeria (ED), elodea (EC), and vallisneria (VA).

Several points may be noted in the metabolite data (Table 15): 1) The high concentrations of 4-amino-2,6-dinitrotoluene (ORED2) in parrotfeather, egeria, vallisneria and dwarf spikerush. This metabolite is rare in plants exposed to explosives. 2) The absence of 2-amino-dinitrotoluene (ORED1) in Eurasian watermilfoil, elodea, sago pondweed and stonewort. This is the most common reduction product in plants exposed to explosives. 3) The uniform absence of products derived from TNT by nitro-group removal (NITs) in data from all plant species. This suggests that NITs are not produced during plant-mediated processes. These differences and the spatial arrangement of the PCA indicate that among the aquatic plants tested here there are two different pathways to reduce nitro-groups of TNT, with no removal of nitro-groups.

5. Azoxy compounds

No azoxy compounds were found in the ten-day water samples analyzed from a single block despite the water pH range, 5.3 to 8.4, in which they usually are present.

6. Plant health and growth

Plant health was assessed by visual inspection twice during the incubation period and by calculation of relative growth rates. Inspection indicated that most plants had not recuperated from transplant shock by seven days, and only started to do so by 10 days. Almost all growth rates were negative (Figure 4), and only parrotfeather at the higher density and with N-fertilizer showed net weight gain. This nutrient amendment was associated with less negative growth rates in all species except vallisneria, sago pondweed and stonewort. The lack of growth and limited response to N may indicate that: 1) the time required for recovery was too long for plants to take advantage of N amendment, 2) another major nutrient was more limiting than N, or 3) the plants' potential for growth was low late in the growing season (October).

The likelihood of growth limitation by nutrients was assessed from pH and macronutrient concentrations in the water at the end of the incubation period (Table 16). Alkalinity was generally low (initial value 1.25 mM) and in the range to produce carbon limitation for submerged plants, as indicated by Van et al. (1976). Final pH (5.3 to 8.4) may have inhibited carbon availability even more in species preferring bicarbonate-carbon for photosynthesis: Eurasian watermilfoil, curlyleaf pondweed, sago pondweed and vallisneria (Spence and Maberly, 1985). Lack of water mixing, inferred from low O_2 values at the end of the incubation period (Figure 5), may have limited carbon transport to diffusion alone, and would have affected all plant species, irrespective of CO_2 or bicarbonate-carbon source use (Walker, 1985). Only parrotfeather had access to CO_2 in air, where diffusion is far higher than in water.

Low light is not expected to have limited growth, as light levels in the growth chambers were close to the range in which photosynthesis of submerged plants is saturated (600 to 800 $\mu E\ m^{-1}\ s^{-1}$; Van et al., 1976).

NH_4-N and PO_4-P were often higher following incubation with plants at the lower density or sediments, although NO_3-N was usually depleted by plants at the higher density (Table 16). Increased levels may have resulted from leaching from plants and microorganisms. There is no indication that growth was limited by N or P availability.

While the tested groundwater was relatively rich in plant-available N and P, the MAAP soil used for sediment controls was low in organic matter and NH_4-N and PO_4-P (Table 17). Although MAAP groundwater has adequate levels of N and P, amendment of the local soil with organic matter may be advisable to prevent growth limitation of plants by other elements and to enlarge the organic carbon source for microorganisms.

IV: CONCLUSIONS

This study showed that all ten plant species tested enhanced removal of TNT from MAAP groundwater, and that plant-mediated removal of TNT increased with increasing biomass. Residence times required for clean-up were reduced from 56 days in water alone to 4 to 19 days when plants were included in the incubation at a density of 9 g FW L^{-1}. There was no significant response in TNT removal to nitrogen fertilization, possibly due to an

overall lack of growth resulting from carbon limitation.

Plant effects on RDX removal were extremely variable, probably due to the long residence time required to lower levels of this compound ($t_{1/2} \geq 12$ days has been reported). While nitrogen fertilization did not have an effect, higher density of plant biomass did enhance the process. RDX removal from water was correlated to decreasing O_2, indicating potential involvement of facultative and/or obligate anaerobic microorganisms, or alternative plant enzymes.

Based predominantly on TNT results, the species recommended for explosives removal from MAAP groundwater in a constructed wetland are elodea, sago pondweed, water stargrass, and curlyleaf pondweed, along with parrotfeather.

PCA results suggest that two different metabolic pathways, both of which chemically reduce nitro-groups of TNT, exist in these aquatic species: removal of one or more nitro-groups from the ring structure does not occur.

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Table 1. Submerged aquatic plant species used in factorial screening for explosives removal, USACE Waterways Experiment Station, October 1995. Common names used in the text in parentheses.

Group	Family	Plant species	
		Latin name	Common name
ANGIOSPERMS			
Monocotyledons	Hydrocharitaceae	*Egeria densa* Planch	egeria
	.	*Elodea canadensis* Rich. in Michx.	elodea
	.	*Vallisneria americana* Michx.	vallisneria
	Potamogetonaceae	*Potamogeton crispus* L.	curlyleaf pondweed
	.	*Potamogeton pectinatus* L.	sago pondweed
	Pontederiaceae	*Heteranthera dubia* (Jacq.) MacM.	water stargrass ('stargrass')
	Cyperaceae	*Eleocharis parvula* (R.&S.) Link	dwarf spikerush ('spikerush')
Dicotyledons	Haloragaceae	*Myriophyllum aquaticum* (Vell.) Verdc*	parrotfeather
	.	*Myriophyllum spicatum* L.	Eurasian watermilfoil ('milfoil')
ALGAE	Characeae	*Chara vulgaris* L.	stonewort

* Baseline species

Table 2. Chemical characteristics of the MAAP groundwater from initial characterization at WES, EPEB. Mean values and standard deviations (N=3). NA. not analyzed.

Characteristic	Concentration
pH	8.3 ± 0.1
Macro-, micronutrients (mg L^{-1})	
Alkalinity	15 ± 3
NO_3-N	5.8 ± 1.7
NH_4-N	0.08 ± 0.08
SRP	0.179 ± 0.034
SO_4	1.53 ± 0.16
Ca	5.9 ± 1.3
Explosives (μg L^{-1})	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	NA
2,6-Diamino-,4-nitro-toluene	73.9 ± 2.6
2,4-Diamino-,6-nitrotoluene	6.6 ± 1.5
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	3002.2 ± 82.0
1,3,5-Trinitro-benzene	308.2 ± 16.6
1,4-Dinitro-benzene	0
1,3-Dinitro-benzene	29.2 ± 14.2
Nitrobenzene	0
2, 4, 6-Trinitrotoluene (TNT)	2196.7 ± 68.1
2-Amino-dinitrotoluene	43.2 ± 0.6
4-Amino-, 2, 6-dinitrotoluene	35.7 ± 0.9
2,4-Dinitrotoluene	0
2,6-Dinitrotoluene	0
2-Nitrotoluene	0
4-Nitrotoluene	0
3-Nitrotoluene	0

Table 3. Plant species effects on **TN T** concentration in groundwater over ten-day incubation; multifactorial ANOVA on groundwater + plants, and groundwater control.

Source of variation	Degrees of freedom	F-ratio	Significance level
Main effects			
Plant species/ groundwater	10	21.973	<0.001
Time	5	412.942	<0.001

Table 4. Plant species and N fertilization effects on TNT concentration in groundwater over ten-day incubation; multifactorial ANOVA on groundwater + plants, and groundwater control.

Source of variation	Degrees of freedom	F-ratio	Significance level
Main effects			
Plant species	10	48.387	<0.001
Time	5	915.639	<0.001
Fertilization	1	3.058	0.081

Table 5. Plant species effects on **TNT** concentration in groundwater over ten-day incubation; multiple range analysis of species at the lower density and groundwater control.

Sample	LS Mean	Homogeneous groups
E.canadensis	634.99	a
P.pectinatus	686.42	ab
M.spicatum	714.96	abc
C.vulgaris	715.61	abc
P.crispus	741.29	abc
H.dubia	769.45	abc
M.aquaticum	811.47	bc
E.parvula	838.21	c
V.americana	987.91	d
E.densa	1010.89	d
Groundwater	1512.38	e

Table 6. Curve fit statistics for **TNT** concentrations in groundwater over ten-day incubations with plant species at two densities. and controls. Data not normalized for plant dry weight. Initial concentration TNT in groundwater: 2197 $\mu g\ L^{-1}$.

Pseudospecies	Y = ln (a+bX)			Half-life (hr)	Da to 2 $\mu g\ L^{-1}$	Total mass incubated (g DW)
	Intercept a	Slope b	R^2 (%)			Mean ± SD
Plant species: 9 g fr wt L^{-1}						
M.aquaticum	6.664	-0.048	71.12	14.4	5.2	3.99 ± 0.06
M.spicatum	6.472	-0.066	97.13	10.5	3.6	3.04 ± 0.06
E.densa	7.091	-0.015	91.02	46.2	18	3.32 ± 0.03
E.canadensis	6.174	-0.064	96.21	10.8	3.6	2.16 ± 0.02
V.americana	7.404	-0.069	99.76	10.1	4.1	1.40 ± 0.00
P.crispus	6.767	-0.067	98.49	10.3	3.8	2.20 ± 0.01
P.pectinatus	6.406	-0.065	96.71	10.7	3.7	2.21 ± 0.02
H.dubia	6.804	-0.067	98.44	10.3	3.8	1.70 ± 0.01
E.parvula	7.040	-0.068	99.07	10.2	3.9	2.59 ± 0.03
C.vulgaris	6.639	-0.066	98.41	10.5	3.8	3.13 ± 0.06
Plant species: 18 g fr wt L^{-1}						
M.aquaticum	4.368	-0.059	57.27	11.8	2.6	7.91 ± 0.02
M.spicatum	5.016	-0.061	71.64	11.4	3.0	6.00 ± 0.00
E.densa	6.779	-0.051	78.38	13.6	5.0	6.58 ± 0.01
E.canadensis	1.461	-0.049	31.99	14.1	0.7	4.32 ± 0.02
V.americana	7.245	-0.068	99.76	10.2	4.0	2.78 ± 0.03
P.crispus	4.383	-0.059	57.13	11.8	2.6	3.90 ± 0.07
P.pectinatus	0.841	-0.047	26.92	14.8	0.1	4.34 ± 0.03
H.dubia	3.507	-0.057	47.63	12.2	2.1	3.41 ± 0.04
E.parvula	5.602	-0.063	72.60	11.0	3.3	5.15 ± 0.03
Controls						
Groundwater	7.464	-0.005	77.87	139	56	0
Sediment	7.340	-0.005	68.18	139	56	261.84 ± 0.95
Autoclaved sediment	7.413	-0.018	95.64	38.5	16	256.43 ± 8.39

Table 7. Curve fit statistics for TNT concentrations in groundwater over ten-day incubations with plant species at the lower density with N fertilization, and controls. Data not normalized for plant dry weight. Initial concentration TNT in groundwater: 2197 μg L^{-1}.

Pseudospecies	Y = ln (a+bX)			Half-life (hr)	Da to 2 μg L^{-1}	Total mass incubated (g DW)
	Intercept a	Slope b	R^2 (%)			Mean ± SD
Plant species: 9 g fr wt L^{-1}						
M.aquaticum	5.825	-0.064	73.32	10.8	3.3	4.00 ± 0.06
M.spicatum	6.420	-0.065	97.40	10.7	3.7	3.03 ± 0.10
E.densa	6.776	-0.013	73.19	53.3	19	3.32 ± 0.04
E.canadensis	5.459	-0.063	72.93	11.0	3.2	2.17 ± 0.03
V.americana	7.245	-0.068	99.76	10.2	4.0	1.42 ± 0.01
P.crispus	6.834	-0.067	98.85	10.4	3.8	2.20 ± 0.03
P.pectinatus	5.632	-0.063	72.99	11.0	3.3	2.17 ± 0.03
H.dubia	6.684	-0.066	97.99	10.5	3.8	1.69 ± 0.02
E.parvula	6.799	-0.067	98.86	10.4	3.8	2.61 ± 0.03
C.vulgaris	5.740	-0.064	73.49	10.8	3.3	3.16 ± 0.08
Controls						
Groundwater	7.440	-0.003	66.54	231	94	0
Sediment	7.507	-0.014	96.59	50	20	270.78 ± 0.72
Autoclaved sediment	7.735	-0.070	99.55	10	4.2	255.51 ± 10.64

Table 8. Correlation of TNT disappearance rate, measured as clean-up period, to plant biomass, measured as initial g DW.

Components	Sample size	Coefficient	Significance level
TNT			
Clean-up period/ plant mass	31	-0.468	0.008

Table 9. Plant species effects on **RDX** concentration in groundwater over ten-day incubation; multifactorial ANOVA on groundwater + plants, and groundwater control.

Source of variation	Degrees of freedom	F-ratio	Significance level
Main effects			
Plant species/ groundwater	10	1.776	0.067
Time	5	4.217	0.001

Table 10. Plant species and N fertilization effects on **RDX** concentration in groundwater over ten-day incubation; multifactorial ANOVA on groundwater + plants, and groundwater control.

Source of variation	Degrees of freedom	F-ratio	Significance level
Main effects			
Plant species	10	2.103	0.023
Time	5	8.277	<0.001
Fertilization	1	0.011	0.918

Table 11. Plant species effects on **RDX** concentration in groundwater over ten-day incubation; multiple range analysis of species at the lower density and groundwater control.

Sample	LS Mean	Homogeneous groups
P.pectinatus	2695.23	a
M.aquaticum	2710.51	ab
P.crispus	2717.35	ab
E.densa	2719.95	ab
E.parvula	2738.40	ab
H.dubia	2800.76	abc
C.vulgaris	2801.96	abc
M.spicatum	2822.76	abc
Groundwater	2871.49	abc
V.americana	2888.87	bc
E.canadensis	2974.98	c

Table 12. Curve fit statistics for **RDX** concentrations in groundwater over ten-day incubations with plant species at two densities, and controls. Data not normalized for plant dry weight. Initial concentration RDX in groundwater: 3002 $\mu g\ L^{-1}$.

Pseudospecies	Y = a+bX			Half-life (hr)	Da to 2 $\mu g\ L^{-1}$	Total mass incubated (g DW)
	Intercept a	Slope b	R^2 (%)			Mean ± SD
Plant species: 9 g fr wt L^{-1}						
M.aquaticum	2820	-2.357	31.21	560	50	3.99 ± 0.06
M.spicatum	2822	0.186	2.68	-	-	3.04 ± 0.06
E.densa	2741	-0.458	7.88	2707	249	3.32 ± 0.03
E.canadensis	3018	-0.920	1.37	1649	137	2.16 ± 0.02
V.americana	2844	0.943	46.14	-	-	1.40 ± 0.00
P.crispus	2702	0.309	1.09	-	-	2.20 ± 0.01
P.pectinatus	2727	-0.590	7.31	2078	192	2.21 ± 0.02
H.dubia	2765	0.758	15.34	-	-	1.70 ± 0.01
E.parvula	2722	0.393	1.38	-	-	2.59 ± 0.03
C.vulgaris	2826	-0.395	14.87	3354	298	3.13 ± 0.06
Plant species: 18 g fr wt L^{-1}						
M.aquaticum	2755	-2.740	57.45	458	42	7.91 ± 0.02
M.spicatum	2804	-4.796	88.42	272	24	6.00 ± 0.00
E.densa	2805	-0.272	5.27	4794	429	6.58 ± 0.01
E.canadensis	2743	-0.926	10.25	1341	123	4.32 ± 0.02
V.americana	2747	-0.672	12.89	1939	170	2.78 ± 0.03
P.crispus	2794	-2.798	72.37	562	42	3.90 ± 0.07
P.pectinatus	2719	-10.985	95.72	111	10	4.34 ± 0.03
H.dubia	2785	-0.289	4.35	4442	401	3.41 ± 0.04
E.parvula	2765	-0.577	12.94	2191	200	5.15 ± 0.03
Controls						
Groundwater	2880	-0.191	2.18	7220	628	0
Sediment	2763	-0.099	0.22	545	1162	261.84 ± 0.95
Autoclaved sediment	2796	-0.853	14.63	65	136	256.43 ± 8.39

Table 13. Curve fit statistics for **RDX** concentrations in groundwater over ten-day incubations with plant species at the lower density with N fertilization, and controls. Data not normalized for plant dry weight. Initial concentration RDX in groundwater: 3002 μg L^{-1}.

Pseudospecies	Y = a+bX			Half-life (hr)	Da to 2 μg L^{-1}	Total mass incubated (g DW)
	Intercept a	Slope b	R^2 (%)			Mean ± SD
Plant species: 9 g fr wt L^{-1}						
M.aquaticum	2831	-1.043	37.47	1275	113	4.00 ± 0.06
M.spicatum	2858	-0.852	43.05	1593	140	3.03 ± 0.10
E.densa	2793	-1.274	34.69	1014	91	3.32 ± 0.04
E.canadensis	2885	-2.946	72.80	470	41	2.17 ± 0.03
V.americana	2804	-0.861	10.66	1513	136	1.42 ± 0.01
P.crispus	2823	-2.410	29.02	549	49	2.20 ± 0.03
P.pectinatus	2797	-0.929	32.86	1395	125	2.17 ± 0.03
H.dubia	2834	0.848	31.86	-	-	1.69 ± 0.02
E.parvula	2797	0.293	4.54	-	-	2.61 ± 0.03
C.vulgaris	2858	-0.023	0.02	5900	5174	3.16 ± 0.08
Controls						
Groundwater	2837	1.049	41.69	-	-	0
Sediment	2836	-4.368	80.35	13	27	270.78 ± 0.72
Autoclaved sediment	2750	-5.722	79.66	9	20	255.51 ± 10.64

Table 14. Correlation analyses of: 1) RDX disappearance rate, for which the required clean-up period was taken as measure (in days), and plant mass (in initial g DW); and 2) RDX clean-up period and final oxygen concentration in the water (mg O_2 L^{-1}).

Components	Sample size	Coefficient	Significance level
RDX			
Clean-up period/ plant mass	31	0.086	0.646
Clean-up period/ oxygen concentration	31	0.086	<0.001

Table 15. Concentrations of RDX and TNT, TNT-metabolites and TNT-photolysis products in groundwater incubated with/without plants, and with non/autoclaved sediment for ten days. Concentrations freshly collected groundwater also given. Concentrations in $\mu g\ L^{-1}$; mean values (N=3).
Abbreviations:
ORED: one nitro-group reduced; 1: 2-amino-dinitrotoluene; 2: 4-amino-2,6-dinitrotoluene;
TRED: two nitro-groups reduced; 1: 2,6-diamino-4-nitrotoluene; 2: 2,4-diamino-6-nitrotoluene;
NIT: nitro-group(s) removed; 2: 2,6-dinitrotoluene; 3: 2-nitrotoluene; 5: 3-nitrotoluene.
PHOT1: trinitrobenzene; PHOT2: 1,4-dinitrobenzene; PHOT3: 1,3-dinitrobenzene; PHOT4: nitrobenzene.

Pseudospecies	RDX	TNT	TNT-metabolites							TNT-photolysis products			
			ORED1	ORED2	TRED1	TRED2	NIT2	NIT3	NIT5	PHOT1	PHOT2	PHOT3	PHOT4
Plant species													
M.aquaticum	2257	16	12	101	23	0	0	1	0	1	0	0	0
M.spicatum	2878	0	0	0	21	0	0	0	0	23	0	0	0
E.densa	2638	34	21	109	23	38	6	0	0	72	0	0	
E.canadensis	2836	0	0	2	22	0	0	0	0	29	4		
V.americana	3082	0	36	241	23	43	0	0	0	86	0	0	0
P.crispus	2789	0	16	77	0	0	0	0	0	0	0	0	0
P.pectinatus	2612	0	0	40	0	4	0	0	0	17	0	0	0
H dubia	2966	0	13	82	20	4	0	0	0	78	0	0	0
E.parvula	2832	0	37	155	0	33	0	0	0	88	13	0	0
C.vulgaris	2740	0	0	0	24	5	0	0	6	61	0	0	0
Controls													
Groundwater	2840	471	91	93	0	2	0	0	0	169	0	10	7
Sediment	2759	450	130	162	25	4	0	0	0	135	23	9	0
Autocl.sediment	2600	25	103	312	0	39	0	0	0	59	0	3	0
Initial groundwater	2934	2123	42	36	72	6	0	0	0	294	0	18	0

*, Reference plant.

Table 16. Chemical characteristics of initial groundwater and after ten-day incubation with plants, alone, or with non/autoclaved sediment. Alkalinity expressed as mg $CaCO_3$ L^{-1}. Mean values and standard deviations (N=3).

Pseudospecies	pH	Alkalinity (mg L^{-1})	NO_3-N (mg L^{-1})	NH_4-N (mg L^{-1})	SRP (mg L^{-1})	SO_4 (mg L^{-1})	Ca (mg L^{-1})
INITIAL							
Groundwater	8.3 ± 0.1	15 ± 3	5.8 ± 1.7	0.08 ± 0.08	0.179 ± 0.034	1.53 ± 0.16	5.9 ± 1.3
AFTER TEN-DAY INCUBATION							
Plant species; 9 g fr wt L^{-1}							
M.aquaticum	7.0 ± 0.2	20 ± 4	5.3 ± 2.1	0.10 ± 0.05	0.147 ± 0.104	1.41 ± 0.10	5.6 ± 1.3
M.spicatum	7.1 ± 0.2	43 ± 10	3.5 ± 0.2	0.28 ± 0.05	0.868 ± 0.224	1.36 ± 0.07	9.7 ± 3.2
E.densa	8.4 ± 0.5	37 ± 6	8.8 ± 1.2	0.08 ± 0.05	0.103 ± 0.082	1.34 ± 0.14	10.3 ± 3.8
E.canadensis	7.0 ± 0.1	19 ± 6	5.6 ± 1.4	0.34 ± 0.20	0.244 ± 0.120	1.33 ± 0.10	4.2 ± 0.1
V.americana	6.5 ± 0.5	19 ± 15	5.3 ± 3.1	0.13 ± 0.03	0.215 ± 0.022	1.67 ± 0.29	9.4 ± 4.0
P.crispus	6.4 ± 0.1	14 ± 3	6.1 ± 0.7	0.20 ± 0.04	0.297 ± 0.082	1.36 ± 0.16	4.1 ± 0.5
P.pectinatus	6.9 ± 0.1	40 ± 4	1.3 ± 0.4	0.10 ± 0.01	1.226 ± 0.174	6.62 ± 1.23	4.0 ± 0.4
H.dubia	6.1 ± 0.7	16 ± 14	8.3 ± 0.5	3.40 ± 0.63	1.273 ± 0.146	1.59 ± 0.33	3.4 ± 0.2
E.parvula	5.7 ± 0.4	5 ± 3	8.6 ± 0.3	0.49 ± 0.19	0. 860 ± 0.057	3.20 ± 1.55	2.6 ± 2.1
C.vulgaris	7.8 ± 0.0	108 ± 24	6.9 ± 0.3	0.18 ± 0.02	0.071 ± 0.022	2.46 ± 0.61	10.2 ± 2.2
Plant species; 18 g fr wt L^{-1}							
M.aquaticum	6.8 ± 0.2	40 ± 4	0.4 ± 0.4	0.19 ± 0.02	0.131 ± 0.018	1.61 ± 0.33	6.1 ± 2.3
M.spicatum	7.0 ± 0.2	89 ± 18	0.0 ± 0.0	1.94 ± 1.78	2.400 ± 1.542	1.26 ± 0.21	13.2 ± 4.1
E.densa	7.9 ± 0.3	47 ± 2	9.4 ± 0.2	0.07 ± 0.01	0.071 ± 0.025	1.27 ± 0.06	12.0 ± 5.0
canadensis	6.9 ± 0.0	43 ± 6	1.5 ± 1.4	0.44 ± 0.19	0.474 ± 0.114	1.33 ± 0.08	5.0 ± 0.5
americana	7.0 ± 0.2	29 ± 9	5.2 ± 1.8	0.20 ± 0.01	0.242 ± 0.093	2.72 ± 0.74	9.0 ± 2.5
.crispus	6.7 ± 0.2	48 ± 4	0.5 ± 0.4	0.45 ± 0.24	1.273 ± 0.332	2.05 ± 0.84	5.1 ± 1.0
P.pectinatus	7.2 ± 0.1	141 ± 18	1.2 ± 1.0	10.70 ± 3.22	3.336 ± 0.812	4.39 ± 0.34	6.1 ± 1.8
H.dubia	6.7 ± 0.3	28 ± 13	4.2 ± 2.1	6.26 ± 2.67	1.566 ± 0.531	2.15 ± 0.75	0.7 ± 0.3
E.parvula	6.5 ± 0.3	14 ± 4	9.4 ± 7.9	0.27 ± 0.02	2.863 ± 2.182	5.19 ± 0.42	0.3 ± 0.0
Controls							
Groundwater	7.0 ± 0.3	10 ± 4	8.4 ± 0.2	0.04 ± 0.01	0.267 ± 0.026	1.63 ± 0.07	5.0 ± 0.2
Sediment	7.8 ± 0.2	52 ± 2	6.0 ± 0.3	0.07 ± 0.01	0.105 ± 0.003	4.77 ± 3.13	7.8 ± 0.9
Autoclaved sediment	7.5 ± 0.2	67 ± 5	2.4 ± 0.3	0.17 ± 0.08	0.222 ± 0.049	1.29 ± 0.16	11.8 ± 2.9
Fertilized							
Plant species; single density							
M.aquaticum	6.8 ± 0.2	33 ± 10	42.9 ± 1.2	0.10 ± 0.06	0.088 ± 0.013	1.42 ± 0.16	7.8 ± 1.1
M.spicatum	7.3 ± 0.0	60 ± 11	31.7 ± 7.9	0.25 ± 0.04	0.800 ± 0.181	1.36 ± 0.12	13.2 ± 4.7
E.densa	8.4 ± 0.6	42 ± 1	47.2 ± 0.3	0.08 ± 0.01	0.073 ± 0.003	1.40 ± 0.12	12.6 ± 3.4
E.canadensis	6.9 ± 0.1	38 ± 20	30.5 ± 5.6	1.10 ± 0.46	0.188 ± 0.063	1.25 ± 0.04	6.0 ± 0.4
V.americana	6.5 ± 0.3	16 ± 6	45.1 ± 1.5	0.18 ± 0.04	0.275 ± 0.093	1.75 ± 0.19	9.3 ± 1.1
P.crispus	6.7 ± 0.1	19 ± 2	34.7 ± 7.5	0.29 ± 0.00	0.272 ± 0.102	1.35 ± 0.13	5.2 ± 2.0
P.pectinatus	6.9 ± 0.0	34 ± 1	30.0 ± 6.7	0.09 ± 0.00	0.743 ± 0.200	5.75 ± 0.32	5.6 ± 2.6
H.dubia	5.3 ± 0.6	4 ±3	39.7 ± 10.1	2.67 ± 0.52	0.843 ± 0.210	1.44 ± 0.14	4.6 ± 0.8
E.parvula	6.4 ± 0.1	20 ± 11	43.5 ± 7.6	0.19 ± 0.05	0.818 ± 0.165	3.24 ± 1.36	3.7 ± 2.2
C.vulgaris	7.9 ± 0.3	91 ± 15	32.4 ± 7.4	0.15 ± 0.03	0.039 ± 0.018	5.56 ± 2.33	15.7 ± 7.0
Controls							
Groundwater	6.7 ± 0.1	6 ± 0	37.6 ± 12.8	0.07 ± 0.03	0.133 ± 0.118	1.36 ± 0.15	4.1 ± 1.0
Sediment	7.9 ± 0.2	70 ± 10	23.4 ± 8.2	0.10 ± 0.01	0.015 ± 0.019	1.86 ±0.35	6.0 ± 2.8
Autoclaved sediment	7.3 ± 0.1	69 ± 7	27.7 ± 4.6	0.09 ± 0.01	0.027 ± 0.021	1.58 ± 0.23	11.2 ± 2.9

Table 17. Chemical characteristics of wetted Milan soil used in the experiment. Mean values and standard deviations (N=3).

Characteristic		
Parameter	Unit	Concentration
Nitrogen	$g.kg\ DW^{-1}$	1.4659 ± 0.055
Exchangeable NH_4-N	$g.kg\ DW^{-1}$	0.007 ± 0.000
Phosphorus	$g.kg\ DW^{-1}$	0.447 ± 0.014
Available PO_4-P	$g.kg\ DW^{-1}$	0.067 ± 0.002
Bulk density	$g\ DW.ml^{-1}$	1.246 ± 0.009
Moisture	$g\ H_2O.kg\ FW^{-1}$	26.91 ± 0.78
Organic matter	$g.kg\ DW^{-1}$	3.96 ± 0.13

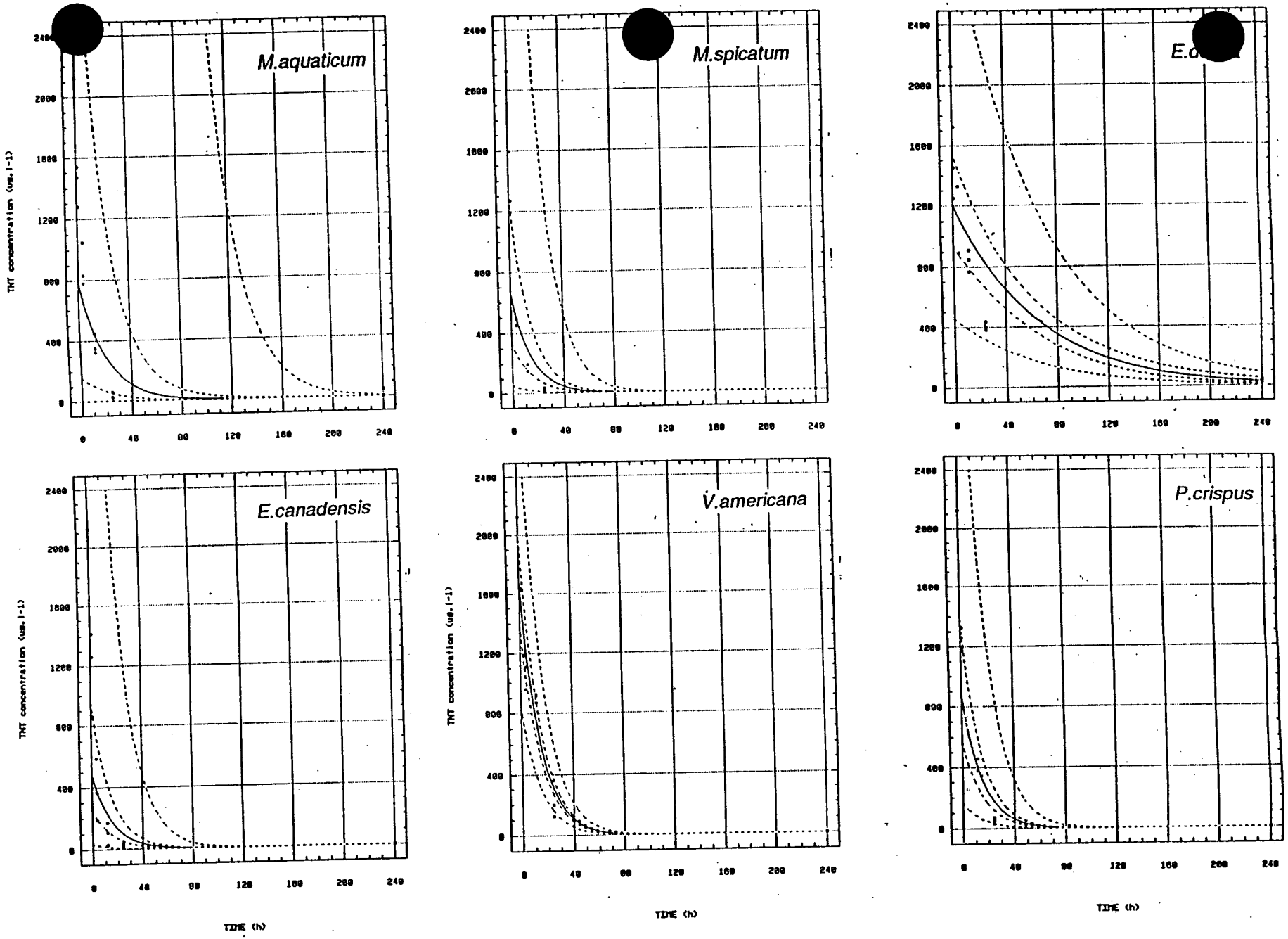


Figure 1. Changes in the TNT concentration with time of groundwater incubated with one of ten different aquatic plant species, groundwater alone or with non/autoclaved sediment. Drawn lines: fitted curves; interrupted lines, respectively, 95 and 90% confidence levels.

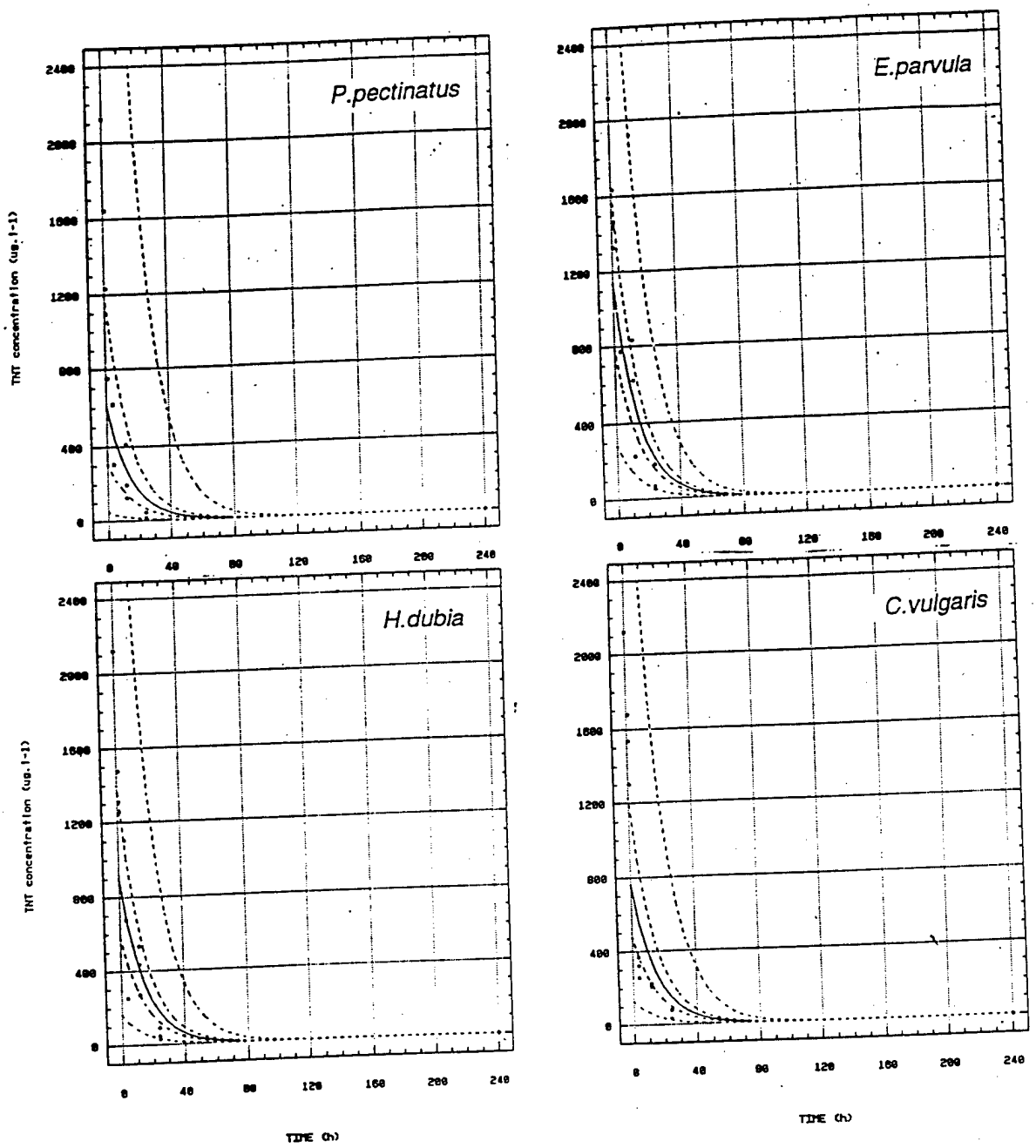
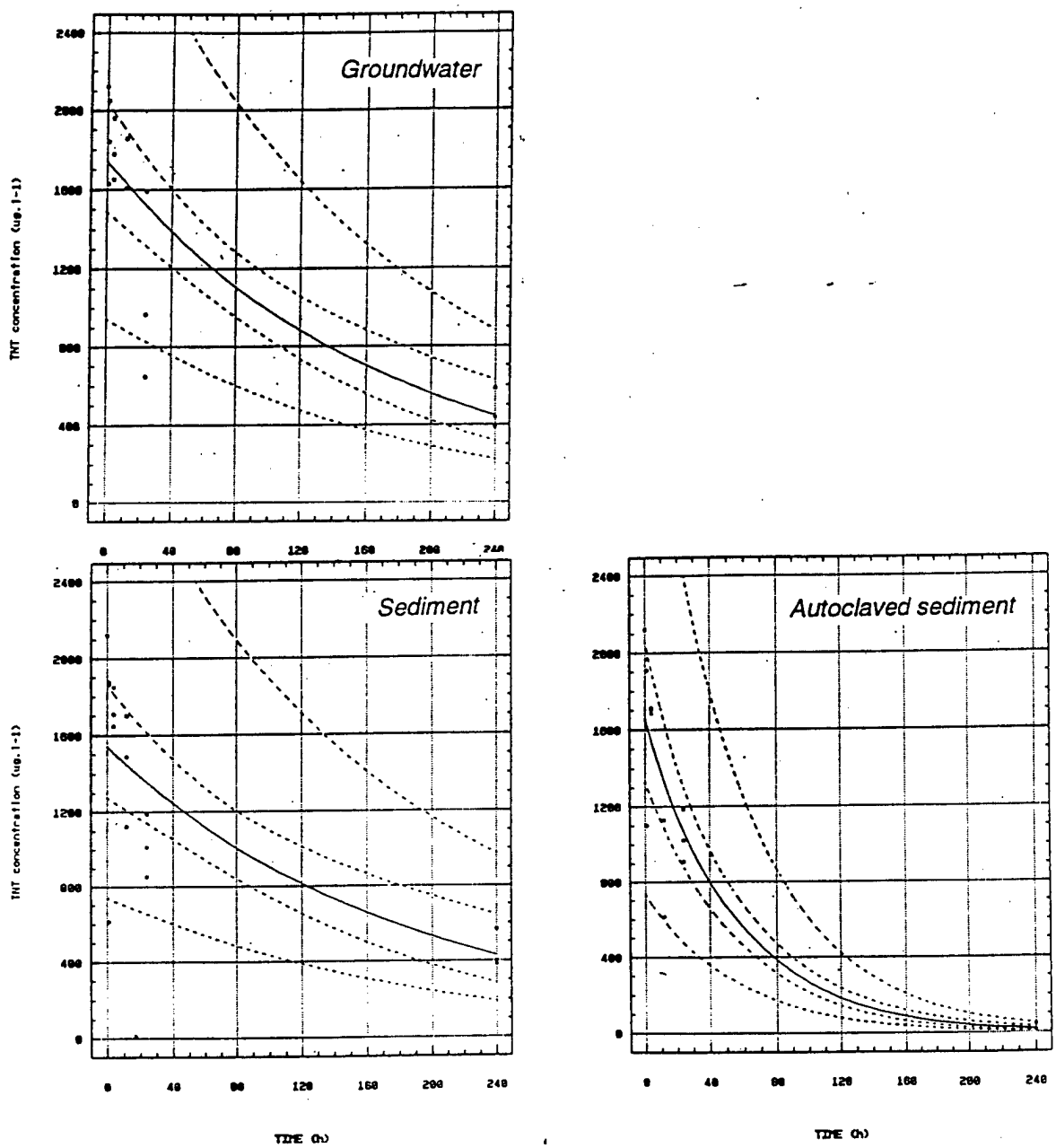


Figure 1. Continued-1.



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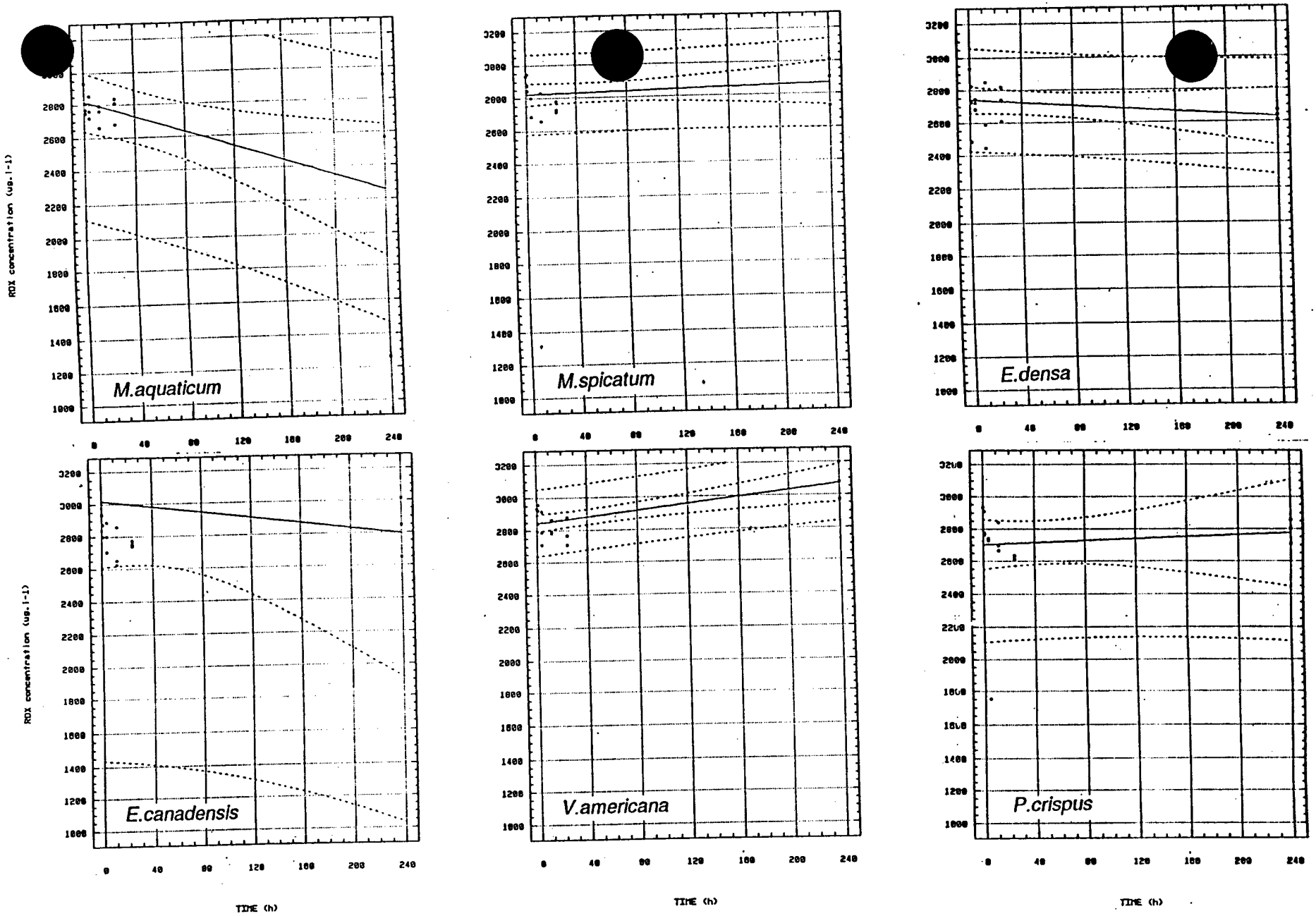

Figure 2. Changes in the RDX concentration with time of groundwater incubated with one of ten different aquatic plant species, groundwater alone or with non/autoclaved sediment. Drawn lines: fitted curves; interrupted lines, respectively, 95 and 90% confidence levels.

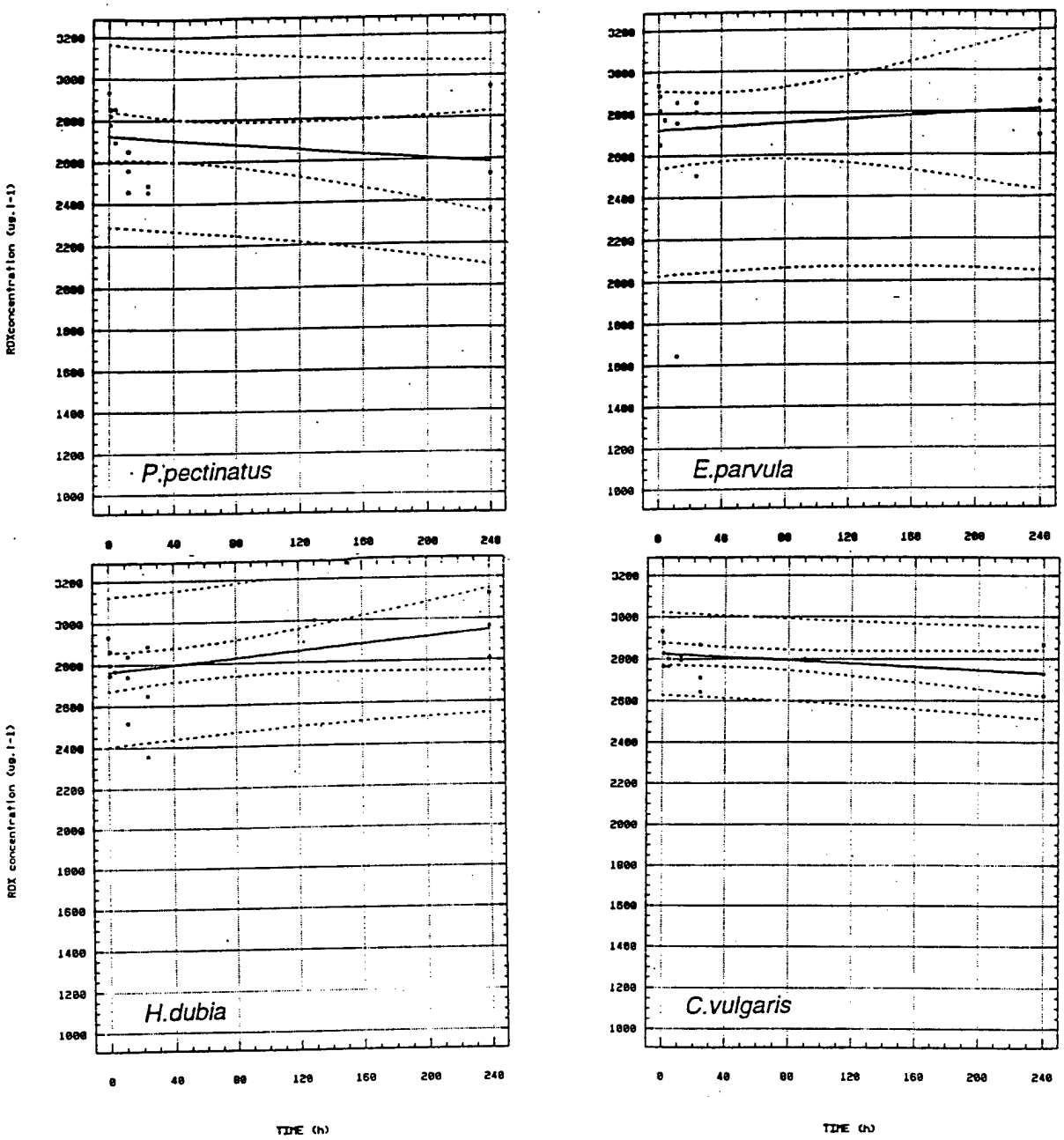

Figure 2. Continued-1.

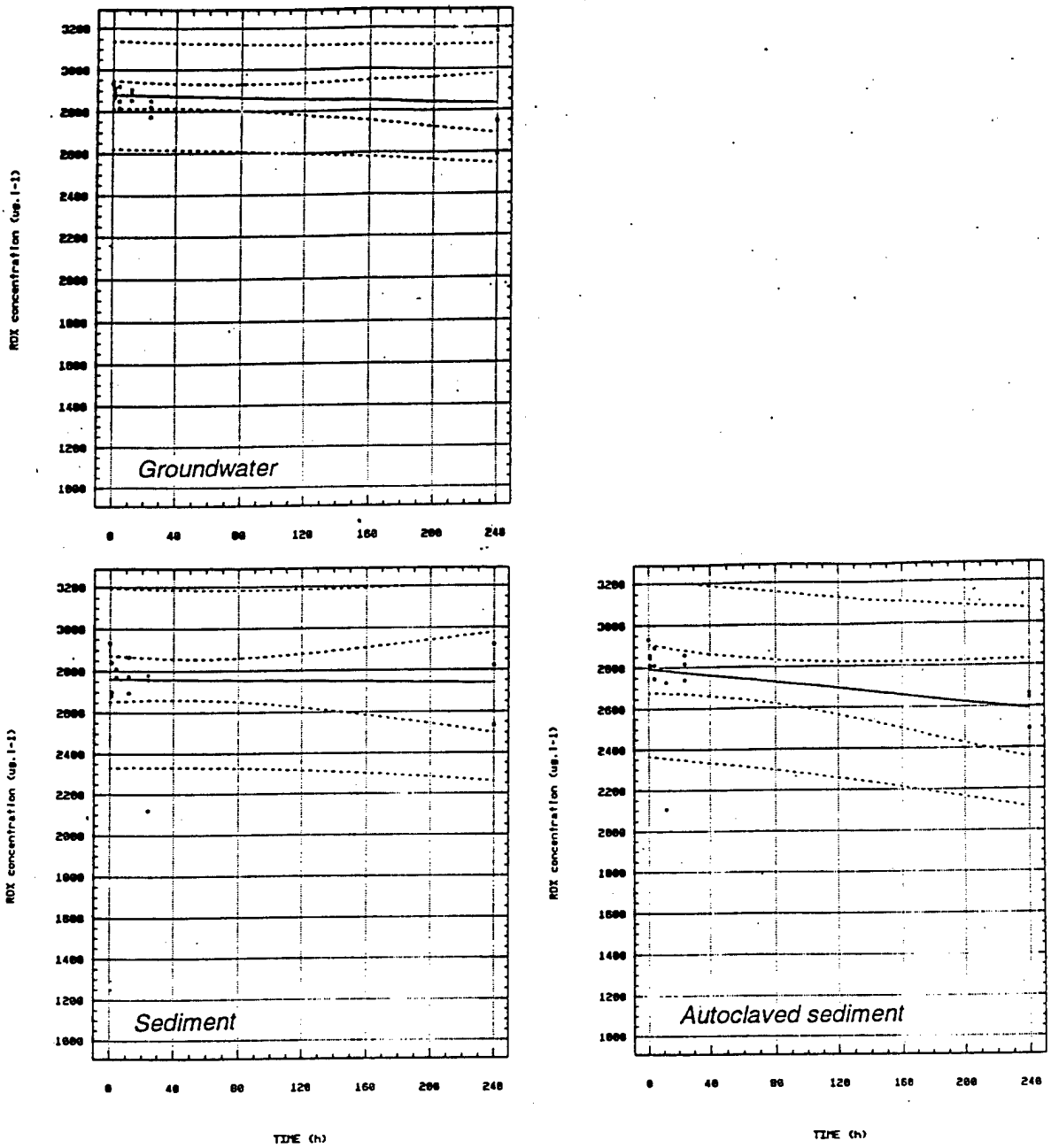

Figure 2. Continued-2.

Plot of First Two Principal Components

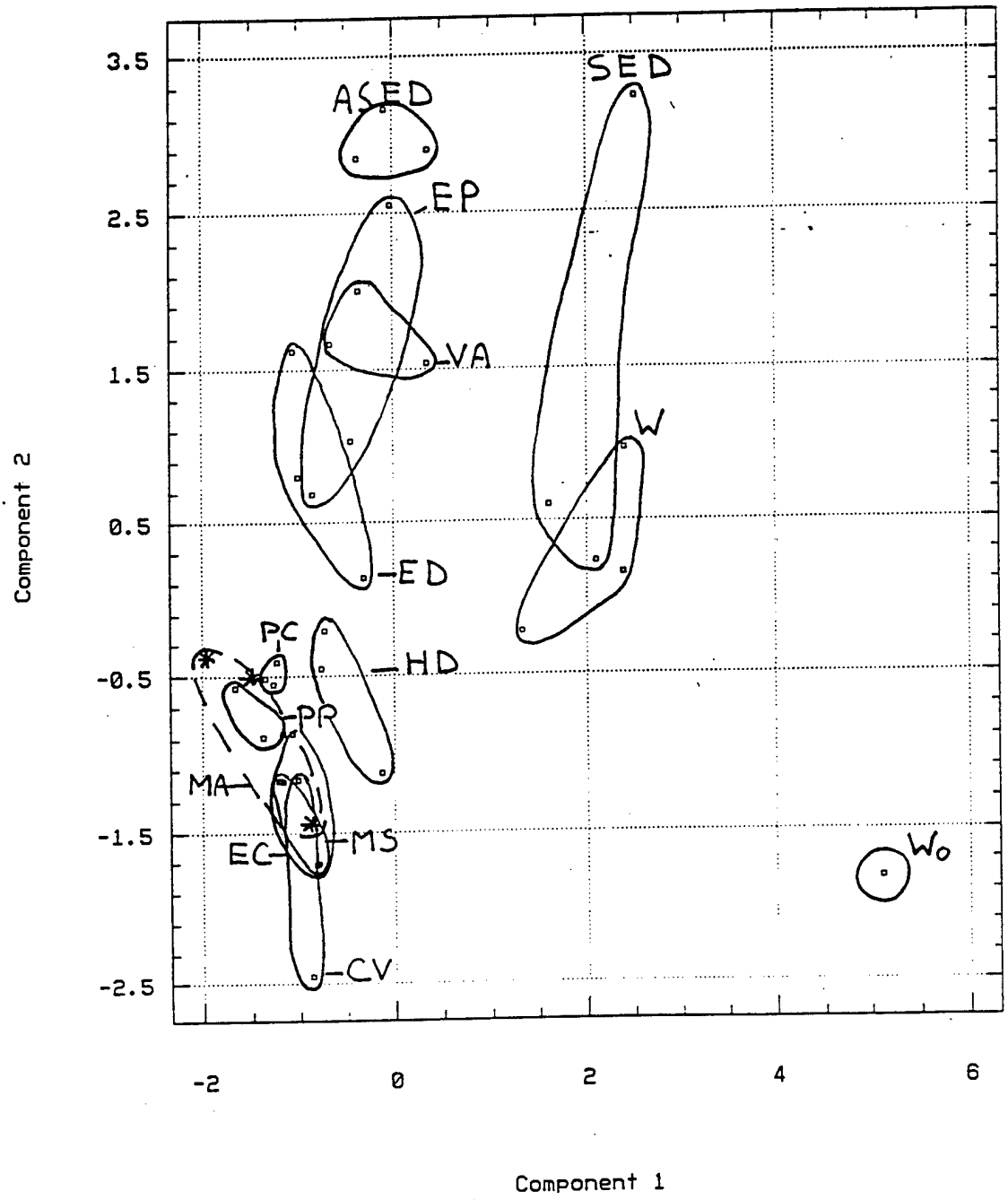

Figure 3. Scatterplot of the first two principal components for the explosives data analysis. Included in the analysis were the explosives data of water samples incubated for ten days with plants, groundwater alone, groundwater+non/autoclaved sediment, and of initial groundwater. Abbreviations: MA, *M.aquaticum*; MS, *M.spicatum*; ED, *E.densa*; EC, *E.canadensis*; VA, *V.americana*; PC, *P.crispus*; PP, *P.pectinatus*; HD, *H.dubia*; EP, *E.parvula*; CV, *C.vulgaris*; W, groundwater; SED, sediment; ASED, autoclaved sediment; W_0, Groundwater at the beginning of the experiment.

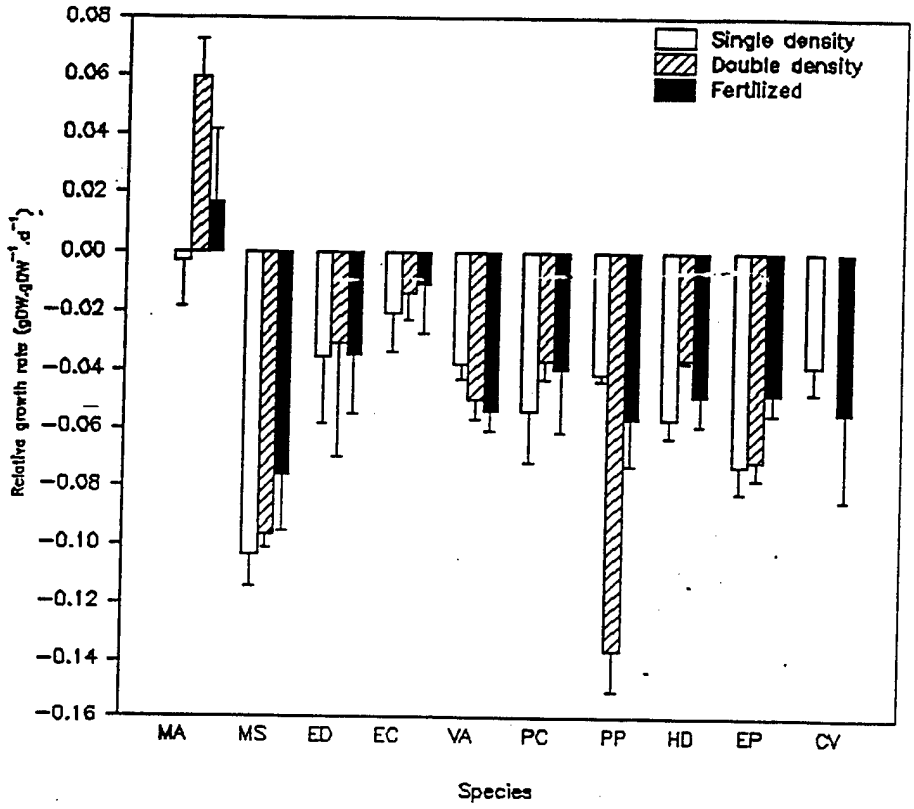

Figure 4. Relative growth rates of aquatic plant species cultivated for ten days in explosives-contaminated groundwater. Mean values and standard deviations (N=3).

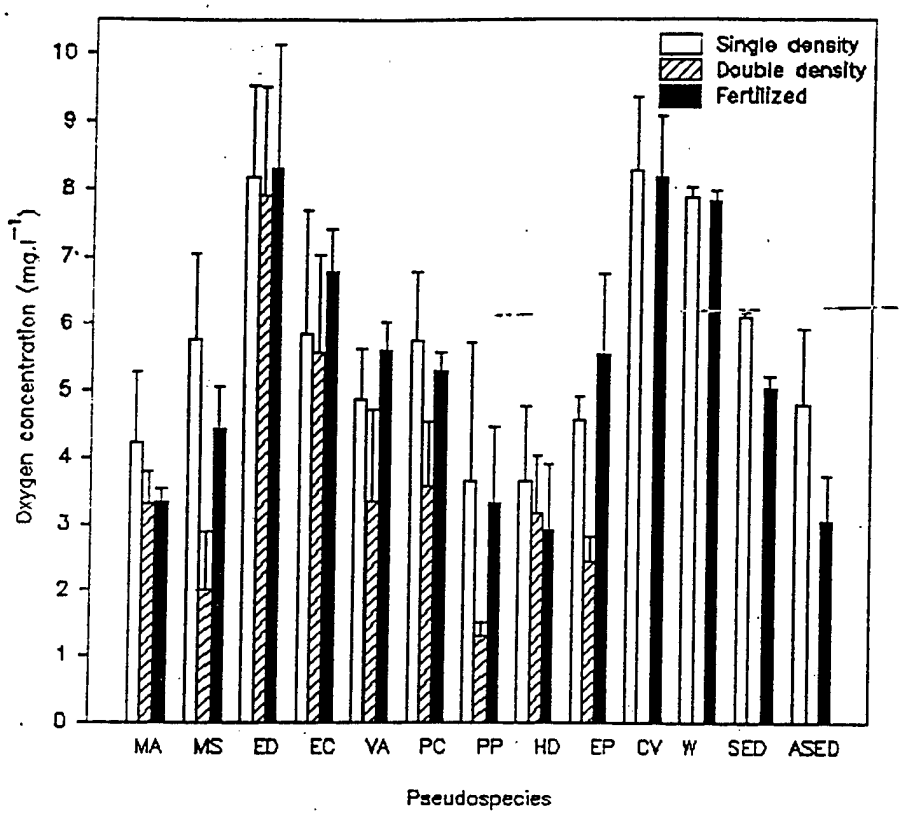

Figure 5. Oxygen concentrations in explosives-contaminated groundwater after a ten-day incubation period with plants, groundwater alone, or groundwater+non/autoclaved sediment. Mean values and standard deviations (N=3).

APPENDIX: ANALYTICAL SPECIFICATIONS, CALIBRATION COMPOUNDS, AND METHOD REFERENCES

1. HPLC Analysis of Explosives in Water

100 ml samples were first concentrated using solid phase extraction (SPE; Waters RDX cartridges, no. 47220; Jenkins et al., 1995). In a few samples, plant debris were removed using Miracloth gauze (Calbiochem). Explosives were eluted from the cartridges using 5 ml acetonitrile. The samples were evaporated to dryness using N_2, redissolved in a 2 ml mixture of acetonitrile:water (50/50 v/v), and analyzed using High Pressure Liquid Chromatography (HPLC).

HPLC separations were performed on a Hewlett-Packard 1090 Series 2/M with ChemStation (Pascal Series) liquid chromatograph equipped with a diode array detector (Series 2), PV5 ternary solvent delivery system, thermostatically controlled column compartment, autosampler, auto-injector and reverse phase analytical C18 column (5μ, 100 mm x 4.6 mm inner diameter) and ODS guard column (5 μ, 20 x 4.0 mm inner diameter). The column compartment was operated at 40 C and the flow rate of the mobile phase was 1.5 ml min^{-1}. The composition of the mobile phase was 68% 20 mM NH_4Cl and 32% a 98:2 mixture of methanol and n-butanol.

Compounds used for calibrations were:

- RDX (NEN Research, Boston, MA).

- 1,3-dinitrobenzene; 2,4-dinitrotoluene; 2,6-dinitrotoluene; 5-nitro-1,3-dimethylbenzene (Aldrich Chemical Company, Milwaukee, WI).

- 1,3,5-trinitrobenzene; 2,4,6-triaminotoluene; 2,4,6-trinitrotoluene; 2-nitrotoluene; 3-nitrotoluene; 4-nitrotoluene; nitrobenzene (Chem Service Chemicals, West Chester, PA).

- 2,4-diamino-6-nitrotoluene; 2,6-diamino-4-nitrotoluene; 2-amino-4,6-dinitrotoluene; 4-amino-2,6-dinitrotoluene; 4-hydroxyamino-2,6-dinitrotoluene, and the azoxy compounds; 4,4',6,6'-tetranitro-2,2'-azoxytoluene and 2,2',6,6'-tetranitro -4,4'-azoxytoluene (Dr. R. Spanggord, SRI International).

A comparison of analytical results from the initial samples of groundwater by WES and TVA show similar mean values for RDX and TNT (Table A-1). RDX values from WES are somewhat lower than the TVA values; some RDX may have remained on the solid phase (SPE) cartridges after elution with acetonitrile, despite the relatively large elution volume of 5 ml.

Table 1. Comparison of explosives analysis in MAAP groundwater performed by WES and by TVA. Groundwater at the beginning of the experiment was analyzed in triplicate by WES and sixfold by TVA. In the WES analysis, 100 ml water samples were concentrated using solid phase extraction, SPE.
REF: Reference data determined by TVA, no concentration step. NA: not analyzed.

Component	Concentration ($\mu g.L^{-1}$)	
	SPE	REF
HMX	NA	178 ± 5
2,6d-4NT	74 ± 3	NA
2,4d-6NT	7± 2	NA
RDX	3002 ± 82	3208 ± 77
TNB	308 ± 17	161 ± 6
1,4DNB	0	NA
1,3DNB	29 ± 14	NA
NB	0	NA
TNT	2197 ± 68	2187 ± 30
2a-DNT	43 ± 1	158 ± 71
4a-2,6DNT	36 ± 1	45 ± 101
2,4-DNT	0	NA
2,6-DNT	0	NA
2-NT	0	NA
4-NT	0	NA
3-NT	0	NA

2. Alkalinity, Macronutrients and Calcium in Water

The pH was calibrated with known buffer solutions (American Public Health Association, APHA, 1992). Alkalinity was determined titrimetrically as CaCO3 (APHA 1992, No2320-B). NH_4-N was measured using a selective ion electrode (Orion 95-12/Orion 940; APHA 1992, No. 4500-NH3-G).

For the remaining analyses, water samples were filtered over a 0.45 μm Gelman GN-6 filter. NO_3-N was measured using HPLC (Waters; APHA 1992, No. 4500-NO3-C). SRP was measured spectrophotometrically using a Shimadzu 1201 UV/VIS Single Beam Spectrophotometer (APHA 1992, No. 4500-P-E). SO_4 was measured turbidimetrically (HACH Ratio turbidimeter; APHA 1992, No. 4500-SO4-E). Total Ca was determined by Atomic Absorption Spectrophotometry after acidification with 1:1 HCl to pH <2 (Varian Model SpectrAA-10; APHA 1992, No. 3500-Ca).

The analytical precision and accuracy of determining macronutrients and Ca in water was checked by comparing the outcomes of determinations of 38 split water samples.

3. Macronutrients, Bulk Density and Organic Matter in Sediment

Total Kjeldahl-nitrogen (N) and phosphorus (P) were determined in soil digests with sulfuric acid, potassium sulfate, and red mercuric oxide. N and P were measured colorimetrically using a Lachat Quikchem AE Automatic Flow Injection Ion Analyzer (QuikChem Methods No. 10-107-06-2-D, 1992, and No. 13-115-01-1-B, 1992). Exchangeable ammonium was extracted from the soil with 1 M NaCl and filtered; the filtrate was analyzed colorimetrically for ammonia via the salicylate method using a Lachat System (QuikChem Method No. 12-107-06-2-A, 1988). Available P was extracted using a dilute HCl acid fluoride modified Bray extraction procedure and was analyzed colorimetrically via the ascorbic acid method using a Lachat System (QuikChem Method No. 12-115-01-1-A, 1988).

Bulk density and moisture content were determined gravimetrically by drying a known quantity of fresh weight to constant dry weight at 105 C (Allen et al., 1974). Concentrations of organic matter were determined by loss on ignition at 550 C.

APPENDIX F

ENVIRONMENTAL BEHAVIOR AND FATE OF EXPLOSIVES IN GROUNDWATER FROM THE MILAN ARMY AMMUNITION PLANT IN AQUATIC AND WETLAND PLANTS - FATE OF TNT AND RDX: USAEC REPORT NO. SFIM-AEC-ET-CR-97060; FEBRUARY 1998

U.S. Army Environmental Center

ENVIRONMENTAL BEHAVIOR AND FATE OF EXPLOSIVES IN GROUNDWATER FROM THE MILAN ARMY AMMUNITION PLANT IN AQUATIC AND WETLAND PLANTS

FATE OF TNT AND RDX

Prepared for

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Environmental Behavior and Fate of Explosives in Groundwater from the Milan Army Ammunition Plant in Aquatic and Wetland Plants

Fate of TNT and RDX

by

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Aberdeen Proving Ground, MD

2 February 1998

Abstract

The present study was performed to elucidate the environmental behavior and fate of TNT and RDX in aquatic and wetland plants collected from a field-scale wetland demonstration deployed at Milan Army Ammunition Plant for removal of explosives from groundwater. The study had three objectives: (1) To establish the physiological capacity of plants to absorb and transport TNT or RDX from explosives-contaminated groundwater in the absence of substrates and their sorptive activities; (2) To quantify partitioning of TNT and RDX between plant portions; and (3) To establish the short-term chemical fate of TNT and RDX in plant tissues of these species. Substrates in which these plants were rooted at the Milan field site (sediment, gravel) were also incubated without plants to investigate sorptive activities, and to evaluate microbial/chemical transformation of TNT and RDX that may affect the explosives availability to plants.

Hydroponic batch incubations of plant or substrate treatments with ^{14}C-TNT or ^{14}C-RDX were used to evaluate explosives fate. The study surveyed seven plant species and two substrates in sequential, independent incubations of 7 and 13 days with TNT and RDX, respectively. Radiolabel distribution in intact plants was followed using autoradiography and radio-analytic imaging. Parent compounds and degradation products were determined through chemical (HPLC) analyses of plant tissue extracts, aqueous phases and substrate extracts. The fate of radiolabel in plants and substrates was followed using thin layer chromatography and radioanalytic imaging.

While growth of most plants except parrot-feather was low in groundwater amended to contain 1.6 to 3.4 mg TNT L^{-1}, TNT disappeared completely from groundwater incubated with plants in 7 days. Highest specific removal rates were found in submersed plants in elodea (0.05 mg TNT g FW^{-1} d^{-1}) and in emergent plants in parrot-feather, sweet-flag, and reed canary grass (0.006 mg TNT g total FW^{-1} d^{-1}). TNT declined less with substrates, and least in unplanted controls. Radiolabel was present in all plants after incubation. In the submersed species radioactivity was concentrated in physiologically active roots and shoots, and in emergent species in roots. Mineralization to CO_2 was very low, and evolution into volatile organic compounds was negligible. TNT residues were extremely low or below chemical detection in plant tissues. Radioactive degradation products accumulated at the sites of uptake and transport was limited. TNT degradation took place via reduction of a single nitro-group. At least five other unknown metabolites were found.

In RDX incubations growth of submersed plants was normal, but growth of emergent plants was reduced in groundwater amended to contain 1.5 mg RDX L^{-1}. RDX disappeared less rapidly than TNT from the incubated groundwater. Highest specific RDX removal rates were

found in submersed plants in elodea (0.004 mg RDX g FW^{-1} d^{-1}), and in emergent plants in reed canary grass (0.001 mg RDX g total FW^{-1} d^{-1}). Radiolabel was present in all plants after incubation. Mineralization to CO_2 was low, but relatively higher than in the TNT incubation. Evolution into volatile organic compounds was negligible. Radioactive degradation products accumulated at physiologically active sites, and transport to leaves was substantial, ranging from 23% of total plant radioactivity in sweet-flag to 81% in parrot-feather. RDX residues were low in most plants, or below detection in the below-ground portions of two emergent species. The RDX residues ranged from 0.3 µg g FW^{-1} in pondweed to 8.6 µg g FW^{-1} in parrot-feather shoots. RDX degradation into at least five unknown compounds was shown to occur.

No detectable residues of either explosive were found in substrates.

The promise of phytoremediation in constructed wetlands as a technology for removal of explosives from groundwater is supported by several results of this study. 1) The rapid decrease in TNT and relatively slower decrease in RDX in the presence of certain aquatic or wetland plants under viable environmental conditions, 2) The relatively rapid metabolism of the parent compounds inside the plants, and 3) Low explosives residues in plant tissues and substrates. However, it must be realized that metabolic pathways of degradation of TNT and RDX in plants are still unknown, and that certain explosives degradation products may exert other biological and toxicological activities. Decreases in TNT and RDX levels in water with plants may also be due partly to chemical binding between explosives transformation products and organic matter. The generation of plant-specific dissolved organic matter and leachates, may also play a role in stimulating microbial activity and result in degradation of explosives.

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Preface

The work reported here was conducted as part of the Army Environmental Center's "Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands" project under a partnering agreement involving the US Army Environmental Center (AEC), Aberdeen Proving Ground, MD, as the lead agency with Ms. Darlene F. Bader as project manager, and the US Army Waterways Experiment Station (WES), Vicksburg, MS, and the Tennessee Valley Authority (TVA), Muscle Shoals, AL, providing technical support. Funding was provided under the DoD's Environmental Security Technology Certification Program (ESTCP).

This study was conducted at WES under the general supervision of Dr. John W. Keeley, Acting Director, Environmental Laboratory (EL), and under the direct supervision of Dr. Richard E. Price, Chief Environmental Processes and Effects Division (EPED).

Technical help was provided by Ms. Linda Nelson and Mr. Robbie B. Godwin, Ecosystem Processes and Effects Branch (EPEB), and Ms. Anne B. Stewart, AScI Corporation, McLean, VA. HPLC analysis of explosives and TNT degradation products in water and plant extracts, and chemical characterization of the groundwater was performed by the Environmental Chemistry Branch, Environmental Engineering Division (EED), EL.

The report was prepared by Dr. Elly P. H. Best of the AScI Corporation, with contributions from Drs. Herb L. Fredrickson and Susan L. Sprecher (EPED), and Dr. Steven Larson (EED). Valuable discussions and comments during the course of the study were provided by Drs. Judith Pennington and Bill Davis, EPED. Technical reviews were provided by Drs. Pennington and John Madsen.

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1- Introduction

Explosives and Phytoremediation

Munitions material such as 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and their combustion and degradation products can enter the environment from production activities and field usage and disposal (Small and Rosenblatt 1974; Spanggord et al. 1980). The presence of these substances is of concern because of their potential toxicity and mutagenicity (Marvin-Sikkema and De Bont 1994).

The utilization of plants for clean-up of the environment has received relatively little attention despite the fact that plants, like microorganisms, play an important role in nature in sustaining and restoring environments. The capabilities of plants to absorb, accumulate and metabolize, directly or indirectly, various organic substances suggests their utilization in the remediation of contaminated environments in a technology named phytoremediation (Salt et al. 1995; Schnoor et al. 1995).

In the aquatic environment, both TNT and RDX can disappear rapidly from water due to photolysis (Spanggord et al. 1980; Gorontzy et al. 1994). Furthermore, adsorption of these explosives to sediment is not significant (Spanggord et al. 1980). Relatively rapid rates of TNT transformation by microorganisms have been reported (Spanggord et al. 1980; Gorontzy et al. 1994), but slower rates of RDX -- the latter predominantly under anaerobic conditions (Binks, Nicklin and Bruce 1995; Sikora et al. 1997). Recently, TNT was found to disappear rapidly from water in the presence of several algae and submersed and emergent plants, while RDX decreased far more slowly (Schnoor et al. 1995; TVA 1995; Best and Sprecher 1996; Best et al. 1997a; Best et al. 1997b; Best et al. 1997c; Best et al. 1997d). The decrease in RDX concentration was largely attributed to plant-stimulated activity of microorganisms inherent to the explosives-contaminated water. Recent flow-through studies of 2-month duration, however, suggested that, in the presence of some aquatic and wetland plants, the initial rapid disappearance of TNT is largely due to adsorption processes, followed by some plant-leachate stimulation of microbial degradation. Disappearance of RDX was gradual and slower, but the actual RDX removal rates were twice as high as for TNT (Best et al. 1997a). Degradation of TNT by freshwater sediments has been shown to be mediated by enzymes of aquatic plant origin (Van Beelen and Burris 1995).

Biotransformation depends on the activity of the organisms involved, and can be decreased by toxic compounds, such as explosives. Both TNT and RDX have been shown to be toxic to aquatic plants at concentrations of $\geq$ 2 mg L^{-1}, depending on the species (Schott and Worthley 1974; Smock, Stoneburner and Clark 1976; Best et al. 1997a). The mechanisms of biotransformation of TNT by plants have been investigated in radiolabel mass balance studies, and shown to be considerable; degradation proceeds via reduction of the nitro-groups. A suite of known (2ADNT, 4ADNT, 24DANT; abbreviations given in Appendix D) and unknown (polar and non-polar) TNT metabolites was identified recently in poplar trees (Thompson and Schnoor 1996). Mineralization to CO_2 and formation of volatile organic compounds has been negligible (Palazzo and Legett 1986; Cataldo et al. 1989; Cataldo, Harvey, and Fellows 1990; Fellows, Harvey, and Cataldo 1995; Mueller et al. 1995; Thompson and Schnoor, 1996; Hughes et al. 1997; Price et al. 1997). Biotransformation of RDX in the presence of plants has been far lower than that of TNT, and accumulation of the parent compound depended upon plant species. Whether RDX degradation was carried out by the plants themselves or by microorganisms associated with plants was not verified; so far one RDX-degradation product has been identified in plant tissue (Cataldo, Harvey, and Fellows 1990; Fellows, Harvey, and Cataldo 1995; Price et al. 1997).

Phytoremediation of Explosives-Contaminated Groundwater from the Milan Army Ammunition Plant

The Milan Army Ammunition Plant (MAAP) located near Milan, TN (longitude 88° 50' W, latitude 35° 45'N) was selected as a demonstration site for phytoremediation of explosives on the basis of high concentrations of TNT and RDX occurring in groundwater (1.4 and 1.9 mg L^{-1}, respectively; TVA 1996, unpublished).

Phase I of this project, encompassing short-term plant screens for ability to remove TNT and RDX from groundwater, generated information on the basis of which suitable plant species were selected for Phase II, a field-scale wetland demonstration at MAAP.

The present study was undertaken to determine the behavior and fate of ^{14}C-labeled TNT and RDX in aquatic and wetland plants collected from the field-scale wetland demonstration with the objective of removing explosives from groundwater. The submersed species evaluated under hydroponic conditions were: *Elodea canadensis* Rich. in Michx. (elodea), *Potamogeton pectinatus* L. (sago pondweed), *Heteranthera dubia* (Jacq) Macm. (water star-grass); and the emergent species: *Myriophyllum aquaticum* (Vell.) Verdc., *Acorus calamus* L. (sweet-flag), *Phalaris aundinacea* L. (reed canary grass), and *Scirpus cyperinus* (L.) Kunth (wool-grass). The

fate of TNT and RDX in the substrates in which the plants were rooted in the Milan Demonstration project was also assessed. Accumulation in plants and substrates over time, and biotransformation in plant portions and substrates, were evaluated.

2- Material and Methods

Hydroponic batch incubations of plant or substrate treatments with [^{14}C]-TNT or [^{14}C]-RDX were used to evaluate explosives transformation. A preliminary study exposing elodea to these respective compounds in separate 8- and 13-day incubations was carried out to evaluate experimental conditions and analytical techniques. The main study surveyed all seven species and two substrates in sequential, independent incubations of 7 and 13 days with TNT and RDX, respectively. Parent compounds and degradation products were determined through both radioisotope and chemical analyses of plant tissues, aqueous phases and substrate extracts.

[^{14}C]-TNT and [^{14}C]-RDX Specific Activity and Radiolabel Purity

The uniformly [^{14}C]-ring-labeled TNT (specific activity of 24.3 x 10^7 Bq mmol^{-1}) and RDX (177.6 x 10^7 Bq mmol^{-1}; NEN Research Products, Boston, MA) had 99% radiochemical purity as reported by the vendor. These purities were verified by diluting small aliquots in methanol, and analyzing by High Performance Liquid Chromatography /radiomatic (HPLC: Waters, see Analyses section; radiomatic: Series A-100 slow-1 beta unit, Packard Instruments, Downers Grove, IL; detection limit 6,500 disintegrations per minute (DPM), 92% efficiency). The purities of TNT and RDX were calculated by comparing DPM to total counts brought onto the HPLC column. The calculated purities matched the specifications provided by the vendor.

Plant Material, Substrates and Groundwater

The three submersed and four emergent plants evaluated for ability to take up and/or degrade explosives in MAAP groundwater were harvested in September 1996, from vegetation cultivated since April 1996 in explosives-contaminated groundwater in a purification lagoon and gravel beds at the Milan field site (Table 1). Whole plants were transported to WES in MAAP groundwater at ambient temperature and incubated the next day.

The submersed plants and the root systems of the emergent plants were freed from sediment by rinsing repeatedly with tap water. Submersed plants and the roots of the emergent plants were then immersed in 500 mL 1% H_2O_2 to reduce the presence of microorganisms. Plants were then rinsed in 500 mL de-ionized water, and adhering water was removed using paper towels.

The plant material used in the incubations represented whole plants as intact shoots or crowns. Each plant incubation consisted of three replicate beakers with each beaker containing 800-mL untreated groundwater and individuals of a species. For elodea and pondweed, approx. 10 g fresh weight (FW) was added to each beaker. For the other plant species, three representative plants were placed in each beaker. All fresh plant weights were determined before and after incubation, for both above-ground and below-ground portions.

Sediment and gravel were also collected from the Milan lagoon and gravel bed cells and incubated with groundwater. Each replicate consisted of 50 mL of substrate, approximately 26 g wet sediment or 20 g wet gravel, incubated with or without previous autoclaving (30 min at 50 psi). The chemical composition of the sediment and gravel were not determined; the composition of the sediment was expected to be similar to that of the wetted soil evaluated for explosives removal in earlier screening tests (Best and Sprecher 1996; Table 2).

Approximately 190 L of groundwater for incubation was collected from well M-146 at the end of August 1996. The water was pumped into a stainless steel drum, transported to WES overnight, and held at 10 °C. The groundwater was filter-sterilized over a 0.2 µm nylon filter to remove most microorganisms, collected in autoclaved 500-mL beakers and held in 1-L autoclaved, opaque Wheaton bottles in the refrigerator to minimize microbial contamination until use. Filtration decreased the concentration of NO_3-N considerably (from 6.0 to 0.1 mg L^{-1}) and the concentrations of TNT and RDX by 27% (Table 3, compare Appendix A). In comparison, explosives levels in the groundwater used in Phase I to screen species for explosives removal ability were 2197 µg L^{-1} and 3002 µg L^{-1} for TNT and RDX (TVA 1995; Best and Sprecher 1996). However, levels in well M-146 may have already been declining by then already.

Incubation Systems

To assess the potential for TNT and RDX transformation in the presence of plant tissue or substrate, and the related release of gaseous products into the atmosphere (volatilization/ aerosolization), the incubations were carried out in air-tight incubators with controlled air flow and individual gas-trapping systems. Two-piece, cylindrical incubators were constructed of 6 mm thick Plexi-glas. Those for submersed species, substrates and water controls were 41 cm high in total

height x 30 cm diameter, and those for emergent species were 79 x 35.5 cm (Figure 1). Each incubator was equipped with an air inlet, and an outlet connected to a vacuum pump. The inlet was fitted with a one-way check valve to prevent air out-flow in the event of a power failure. Each incubator held one treatment, containing three replicate beakers of either groundwater with intact plants, groundwater alone, or groundwater with substrate. The capacities of the incubation beakers were 1.2 L for submersed plants, 2 L for emergent plants, and 1 L for controls (Table 4). The incubators were situated in a walk-in growth chamber. Illumination was at 500 to 600 $\mu E\ m^{-2}\ s^{-1}$ for a 14-h photoperiod at a temperature of 23 °C. The air was pulled into the incubators by vacuum at 15 mm Hg, was passed successively through a 15 mL-XAD resin cartridge (to adsorb volatile organics), a gas-washing bottle containing 1 L 5N KOH (to trap CO_2), a condensation bottle (to trap KOH and moisture), and the pump itself. The pH of the KOH traps was maintained above 7 (unsaturated) by changing the chemical during the course of incubation (once a week).

Incubations

The filtered, explosives-contaminated MAAP groundwater used for incubations contained 988 µg TNT L^{-1} and 1,443 µg RDX L^{-1} (Table 3). Each replicate beaker contained 800 mL of this groundwater and was kept stirred magnetically. Each incubator holding three beakers rested on three stir-plates, one for each beaker. To prevent discontinuous rotation of the stirrer, magnetic bars were covered by stainless-steel gauze domes in the plant treatments, and substrates were suspended in stainless-steel baskets lined with aluminum foil. The groundwater was adjusted from pH 6.6 to 8 to prevent excessive CO_2 evolution into the atmosphere, and was amended with $NaHCO_3$ to an initial concentration of 298 mM to provide a carbon source for photosynthesis by the submersed plants. This was based on preliminary experiments, where elodea initially deteriorated rapidly but resumed growth after pH adjustment and bicarbonate amendment. Bicarbonate amendment was repeated once for the [^{14}C]-TNT incubations (after 3 days), and twice for the [^{14}C]-RDX incubations (after 3 and 7 days).

Radiolabel was initially added to each beaker as 1 mL methanol solution containing 55.5 x 10^4 Bq TNT or RDX as 519 or 69 µg explosive, respectively. Specific activity of TNT was 24.3 x 10^7 Bq $mmol^{-1}$ and of RDX 177.6 x 10^7 Bq $mmol^{-1}$. In the TNT incubations, radiolabel was re-dosed at mid-week, concomitant with addition of 2,175 µg unlabeled TNT per replicate. Total radiolabel used per replicate was 111.0 x 10^4 Bq TNT, or 55.5 x 10^4 Bq RDX (Table 5).

In the [^{14}C]-TNT exposures plants were incubated in solutions initially containing a total of 1,637 µg TNT L^{-1} (1,309 µg per 800mL replicate) with a total of 55.5 x 10^4 Bq radioactivity as 519 µg TNT per replicate (Table 5). The availability of unlabeled and labeled TNT was increased after

3 days of incubation by re-dosing to 3,367 μg L^{-1} (2,694 μg/800 mL replicate), and an additional 55.5 x 10^4 Bq per replicate. Actual total TNT concentration in the incubation water was initially slightly higher than at the Milan field site, i.e.1,637 μg L^{-1} (Table 5) versus 1,359 μg L^{-1} (Appendix A), respectively. Re-dosing with unlabeled analyte TNT was done to restore TNT levels presumed to be below detection after 3-day incubation (Best and Sprecher 1996; Best et al. 1997b) to a level similar to the TNT concentration of 2,200 μg L^{-1} previously measured in unfiltered MAAP groundwater. The TNT level of 3,367 μg L^{-1} in the incubation water after re-dosing was higher than initially.

In the incubations with [^{14}C]-RDX, plants were incubated in solutions initially containing a total of 1,529 μg RDX L^{-1} (1,223 μg/800mL) with a total of 55.5 x 10^4 Bq radioactivity as 69 μg RDX per replicate. This compared to 3,002 μg RDX L^{-1} found in unfiltered MAAP groundwater previously (Best and Sprecher 1996), and to 1,980 μg L^{-1} measured 11 September 1996 (Appendix A). Explosives levels in the groundwater from MAAP decreased between September 1995 and 1996.

The plants and substrates were placed into beakers filled with these groundwater/radiolabel solutions. For the submersed plants and substrates the beakers and their contents were fully exposed to illumination. For the emergent plants, sides and tops of beakers were covered with aluminum foil to prevent illumination of the roots. Two solution blanks without plants were also incubated with radiolabel: one illuminated, and one with side and top covered with aluminum foil to minimize photolysis.

Total radioactivity was determined in groundwater from each beaker using direct liquid scintillation (LS) counting of 3 1-mL samples per beaker at each of 3 sampling times, at the beginning (before addition of plants or substrates), at radiolabel re-dosing, and at the end of the incubation period. Radioactivity was also determined in acidified water samples at the end of the incubation period. Explosives contents of the incubated groundwater was determined at the end of the incubation period by HPLC, on one 100-mL sample per beaker (USEPA 1992).

Following incubation periods of 7 days for TNT and 13 days for RDX, the submersed plants and the root systems of the emergent plants were rinsed by blotting with paper towels, submersion in 500-mL de-ionized water, and additional blotting. Total fresh weight was determined per replicate. The emergent plants were then separated into above-ground and below-ground portions, and fresh weight was determined for these portions separately. One plant per treatment (i.e., per three replicates) was used for direct assessment of radiolabel distribution in the intact plant by radio-analytic imaging.

Plants of each replicate were clipped into small pieces (approx. 1 cm^3) using scissors, thoroughly mixed, wrapped in aluminum foil and stored deep-frozen (- 20 °C) until further tissue analysis. Following freezing, plants were ground with liquid N_2. Portions of 0.1 to 0.2 g FW per ground sample were weighed, and analyzed for radioactivity by combustion and subsequent LS. From submersed plants three sub-samples were analyzed per replicate; from emergents, three sub-samples from each above- and below-ground plant portion were analyzed.

The metal baskets containing the substrate treatments were removed from solution, and the standing water in each was decanted and returned to the incubation water in the beaker. The exterior of the substrates and baskets was freed from adhering incubation solution as described earlier in this section for plants. The substrates were transferred into pre-weighed glass jars, and total wet weight was determined for the substrate contents of each basket. The substrate samples were thoroughly mixed in the glass jars by stirring using a stainless steel scoop, and frozen until analysis. Portions of 0.14 to 0.90 g wet weight per mixed sediment sample, and 0.13 to 1.0 g wet weight (three small stones) per mixed gravel sample, were weighed, and analyzed for radioactivity by LS after combustion, i.e. three sub-samples per replicate.

Any material that may have condensed on the inside of the chamber following evapotranspiration was collected by wiping with paper tissue. The paper was weighed, clipped into small pieces, and mixed, and three sub-samples per incubator were analyzed for radioactivity by oxidation and LS.

Evolved [^{14}C]-CO_2 was quantified by LS of three sub-samples per incubator of the KOH solution in the gas-washing bottles. Resulting values were multiplied by the KOH volumes at the end of the incubation period. To account for changing the KOH solutions once a week, both KOH solutions per treatment were pooled before sub-sampling.

The volatilized [^{14}C]-organics were quantified by eluting the XAD-traps with methanol (4x void volume, 60 mL), LS of the solution, and multiplying the resulting value by the elution volumes (three sub-samples per incubator).

Analyses

Overview

LS determinations of total radioactivity in incubation solutions quantified all aqueous [^{14}C]-labeled compounds: explosives and their degradation products, as well as aqueous bicarbonate and CO_2 that became dissolved in the incubation water. The contribution of $^{14}CO_2$ and $H^{14}CO_3^-$ to the total radioactivity of the water was quantified by taking the difference between direct samples

of the incubation water and those counted following acidification and aeration which removed aqueous CO_2 and bicarbonate.

LS determinations of total radioactivity in plants and substrates quantified all ^{14}C-labeled compounds. These could include explosives, known or unknown explosive degradation products, any photosynthetic metabolites resulting from plant assimilation of aqueous ^{14}C-bicarbonate and $^{14}CO_2$ generated during the incubation period, and ^{14}C produced from any plant- or substrate-associated microbial transformation. Direct LS of the filtered acetonitrile extracts of plants or substrates quantified the free, i.e. extractable, labeled compounds. In the preliminary experiments with elodea, acetonitrile extracts accounted for 27% of the total radiolabel in [^{14}C]-TNT exposed plants, and for 14% in [^{14}C]-RDX exposed plants. Radio-analysis by thin layer chromatography (TLC) of acetonitrile extracts was used to quantify groups of free radiolabeled compounds having similar mobilities. Identification was provided by comparison of Rf values of known, labeled, compounds. Unlabeled compounds, detectable by fluorescence under UV light, provided identification in certain cases. Chemical analysis by HPLC of acetonitrile extracts, following cleanup using Florisil cartridges, was then used to quantify that part of the free explosives and degradation products in plants and substrates that did not adsorb/adhere to the filters. In the elodea preliminary experiments, the radioactivities in these cleaned plant extracts were also determined by LS. Total radioactivities in these extracts accounted for 15% of the total radiolabel in the ^{14}C-TNT exposed plants, and for 5% in the ^{14}C-RDX exposed plants.

Radioactivity in Water and Other Liquids Using Liquid Scintillation Counting

The radioactivity in liquid samples was measured using a Liquid Scintillation Analyzer (Packard Instruments, Downers Grove, IL) with appropriate quench correction. Suitable aliquots of water (1 mL), KOH solution (0.5 mL), methanol (2 mL), or acetonitrile (100 µL), were placed in 20 mL glass vials with 15 mL scintillation cocktail (Ultima Gold, Packard Instruments, Meriden, CT). All samples were counted twice, each time for two min; only the data of the first count were used for the calculations, while those of the second count were used for verification. The detection limit was 50 DPM. Regularly run liquid standards indicated a typical counting efficiency of 98%.

Radioactivity in Plants and Substrates Using Combustion and Liquid Scintillation Counting

Plant, substrate and paper tissue samples were combusted in a Sample Oxidizer (Model 307, Packard Instruments, Meriden, CT) to determine the total amount of radioactivity associated

with each sample. A predetermined weight of ground plant or substrate sample (approx. 0.1 g) was combusted at 900 to 1150 °C for 45 s under a stream of oxygen. The $^{14}CO_2$ evolved was trapped into 10 mL Carbosorb collected in standard counting vials. After adding 10 mL Permafluor (both solutions from Packard Instruments, Downers Grove, IL), the samples were counted using LS. After every other sample, a blank was burned consisting of an empty cellulose combustion cup. After every tenth sample a standard consisting of an empty cellulose combustion cup spiked with several µL explosives stock solution was burned. Counting of the standards indicated recovery efficiencies ranging from 93 to 97%.

Explosives in Water Using High Performance Liquid Chromatography

Analysis by direct injection on HPLC quantified explosives and their degradation products in groundwater (EPA 846 Method 8330; USEPA 1992). Water samples were filtered over 0.5 µm Millex-SR filters (Millipore, Milford, MA) prior to analysis. Concentration of the water samples by solid phase extraction (SPE) and by salting-out with NaCl (Jenkins et al. 1995) was tried prior to the current incubations. However, SPE cartridges were apparently plugged by mucilagenous plant excretion products, and salting-out gave low ^{14}C recoveries in the preliminary experiments with elodea, i.e. 25% with [^{14}C]-TNT and 0.04% with [^{14}C]-RDX, compared to LS determined totals. Therefore, determination by direct injection was used for analysis in the main incubations. While concentration methods such as SPE and salting out can increase accuracy of detection limits when amounts to be analyzed are low, direct injection does not provide the same low limits of detection.

HPLC methods for analysis of water were carried out following standard operating procedures for use of standards and controls, and for Quality Assurance/Quality Control (WES 1996). Determined were: HMX, RDX, TNB, Tetryl, TNT, 4ADNT, 2ADNT, 26DNT, 24DNT, 26DANT, 24DANT and the 4,4'-azoxy-derivative of TNT (2,2', 6,6 tetranitro- 4,4-azoxytoluene). Detection limits for target compounds in groundwater, following direct injection onto HPLC, were 0.020 mg L^{-1} . Exceptions were 2,4 DANT, 2,6-DANT and the 4,4-azoxy-derivative of TNT, with detection limits of 0.200 mg L^{-1}, 0.100 mg L^{-1}, and 0.500 mg L^{-1}, respectively. The HPLC system consisted of a Waters 610 Fluid Unit pump capable of achieving 6,000 psi, a Waters 717 plus Auto-sampler including a 200 µL loop injector, a Waters 486 Tunable UV Absorbance detector monitored at 245 nm and Millenium 2.1 Chromatography software (Waters Chromatography Division, Milford, MA). A Supelco LC-18 reverse phase HPLC column (25 cm x 4.6 mm, 5 µm; Catalog #5-8298) was used as the primary column and a Supelco LC-CN reverse phase HPLC column (25 cm x 4.6 mm, 5 µm; Catalog # 5-8231) as a confirmation column. As pre-column, the

Novapak C-18 (Catalog # WAT015220) or Novapak CN (Catalog # WAT020800; Waters Chromatography Division, Milford, MA) was used. A Cera Column Heater 250 at 30 °C (Catalog # 282-0252; Cera Inc., Baldwin Park, CA) was used to ameliorate retention time shifts due to changes in room temperature.

Explosives in Plants and Substrates Using High Performance Liquid Chromatography

Previously frozen plant samples were quick-frozen in liquid N_2 and ground to a fine powder. Then, 1.37 to 3.87 g FW portions per plant, or 3.12 to 5.83 g wet weight per sediment, or 3.03 to 5.12 g wet weight per gravel replicate were extracted in 10 mL acetonitrile by an 18-hr sonication in a water-cooled sonic bath. Temperature during sonication did not exceed 30 °C. Samples were centrifuged at 2,000 g for 5 min, the extract supernatant was freed from particles by filtering through a 0.50 µm Teflon disposable cartridge, and divided into two portions. One portion was used for direct LS, and for radioactivity and explosives analysis using TLC, and the other portion for explosives analysis using HPLC. The substrates were extracted similarly, without grinding. Polar as well as apolar metabolites of TNT and RDX were expected to appear in incubated plants and substrates; therefore, acetonitrile was used as the extraction solvent as it removes a range of non-polar to polar substances from organic materials, including amino-derivatives of explosives. Freeze-drying of the plant samples was omitted, because ^{14}C recoveries proved low in the preliminary experiments with elodea, i.e. 20% of that in fresh plant material for [^{14}C]TNT and 2% for [^{14}C]RDX, as determined by LS (without Florisil cleanup procedure).

For HPLC, a 0.5-mL extract portion was placed on a cleanup column prepared by layering 0.5 g Florisil and 0.5 g of neutral alumina. The column was washed with 5 mL of acetonitrile, and the resulting extract was diluted 1:1 with de-ionized water and analyzed by HPLC (EPA method 8330). HPLC methods for analysis of substrate extracts were carried out following standard operating procedures for use of standards and controls, and for Quality Assurance/Quality Control (WES 1996). Detection limits for target compounds in plant tissue ranged from 0.041 to 0.324 µg g^{-1} in elodea, and from 0.580 to 13.557 µg g^{-1} in sweet-flag on fresh weight basis, expressed as method detection level (MDL) (Appendix C). Detection limits in soil ranged from 0.5 to 2.0 µg g FW^{-1}.

Radioactivity Distribution in Intact Plants Using Autoradiography

Immediately following incubation, representative whole plant samples of submersed species, and apical and below-ground portions of emergent species, were placed on 20 x 20 cm glass plates, covered with radio-transparent mylar film, and scanned for 40 min. The Ambis Radioanalytic Imaging System (Ambis Inc., San Diego, CA), with a No. 4000 detector (sensitivity 0.07 DPM mm^{-2}) was used to quantify the radioactivity associated with intact plants. Radiographic images and associated radioactivities were stored on computer disk, and conventional camera photographs were taken of the plates.

Radioactivity in Plant and Substrate Extracts Using Thin Layer Chromatography and Autoradiography

For TLC analysis, a 3 to 5 mL acetonitrile extract aliquot was concentrated to approx. 1 mL by evaporation at 30 °C under N_2. Aliquots (20 to 50 µL) of this concentrated extract were analyzed for radiolabeled compounds following migration in various TLC solvent systems, by developing and radio-analytic imaging the plates. TNT and TNT-degradation products were separated from extracts of ^{14}C-TNT exposed plants or substrates on polar and fluorescent Silica Gel 60F plates (EM Science, Gibbstown, NJ), developed by incubation in a toluene:methanol (99:1 v/v) mixture solvent system for 40 min. TNT and known TNT metabolites separated well on these plates, and they could be identified by Rf value and by both color and fluorescence. RDX and RDX degradation products were separated from extracts of ^{14}C-RDX exposed plants or substrates using the same TLC plates as for TNT, and, in addition, on apolar Whatman Reversed Phase LKC18F plates (Octadecylsylane bonded; Whatman, Clifton, NJ), developed by incubation in a water:methanol (50:50 v/v) mixture solvent system for 4 h. Although RDX proved more mobile on the apolar than on the polar plate, most RDX degradation products remained immobile.

The presence of each compound or co-migrating group of compounds in the resulting chromatogram was determined by visual inspection under a fluorescent lamp (254 nm.; mineralight Model UVG-54, San Gabriel, CA). The radioactivity of each labeled compound was quantified by radioanalytic imaging (40 min). Radiographic images and associated radioactivities were stored on computer disk until further data processing. The identity of the compounds was determined by comparison of their Rf values with those of standards of either labeled TNT and RDX, or unlabelled 2ADNT, 4ADNT, 24DANT, 26DANT, 24DNT, and 26DNT, run on the same plate. The standard mix contained all these standards. The distribution of the radioactivity over the separated compounds was calculated relative to the total radioactivity per lane (chromatogram area allotted to each sample). Radiolabel recovery on the TLC plates was at least

20% of the total quantity applied, counted by LS. An example of the information provided by this TLC method is given in Figure 2.

Alkalinity, Macro-nutrients and Ions in Water

The filtered groundwater was analyzed for explosives as described above, and for pH, alkalinity, Kjeldahl-nitrogen (N), nitrate/nitrite-N, total-phosphorus (P), ortho-P, sulfate, calcium and iron at the beginning of the incubation.

The pH meter was calibrated with known buffer solutions bracketing the pH of the samples (American Public Health Association, APHA 1992). Alkalinity was determined colorimetrically as $CaCO_3$ (Method 310.2, USEPA 1979). Sulfate was determined colorimetrically (Method 375.2, USEPA 1979).

Kjeldahl-N and total P were measured colorimetrically in samples digested with sulfuric acid, potassium sulfate, and mercuric sulfate using a Lachat Quikchem AE Automatic Flow Injection Ion Analyzer (QuikChem Methods No. 10-107-06-2-D and No. 13-115-01-1-B, 1992). Ammonia-N was analyzed colorimetrically via the salicilate method using the Lachat System (QuikChem Method No. 12-107-06-2-A) and Nitrate/Nitrite-N was reduced over a cadmium column to Nitrite-N and analyzed colorimetrically via the Lachat system (Quikchem Method No. 10-107-04-1-C). Phosphate-P was analyzed colorimetrically using the Lachat System ascorbic acid method (QuikChem Method No. 12-115-01-1-A).

The concentrations of Ca and Fe were determined after acidification with 1:1 hydrochloric acid to pH<2 using Inductively Coupled Argon Plasma emission spectrometry (ICP; USEPA 1990 and USEPA 1992; SW-846 Method 6010).

3- Results and Discussion

Hydroponic studies addressed three objectives of importance to the field demonstration at MAAP, (1) To establish the physiological capacity of plants to absorb and transport TNT or RDX from explosives-contaminated groundwater in the absence of substrates (sediment, gravel) and their sorbing activities; (2) To quantify the partitioning of TNT and RDX over plant parts; (3) To establish the short-term chemical fate of TNT and RDX in plant tissues of these species. Substrates in which these plants were rooted at the Milan field site were also incubated without plants to investigate adsorption, and to evaluate microbial/chemical transformation of TNT and RDX that may affect explosives availability to plants.

Behavior of TNT in Hydroponic Culture

Plant Growth and Labeling in [^{14}C]-TNT Groundwater

Most plants decreased in weight over the 7-day incubation period in the TNT-amended incubation. Relative growth rates were usually negative (Figure 3; Appendix B - Table 1). Only the emergent parrot-feather thrived. Poor growth was generally attributed to lateness in the growth season (September) and TNT concentration approaching a toxic range for some aquatic plants (above 2.5 mg L^{-1} after re-dosing, cf. Schott and Worthley 1974; Smock, Stoneburner, and Clark 1976; Best et al. 1997a; $\geq$ 5 mg L^{-1} lethal after >2-week exposure for some aquatic and terrestrial plants, cf. Best et al. 1997a; Thompson and Schnoor, 1997). Emergent plants except parrot-feather may also have suffered from nutrient limitation, since they normally have access to interstitial sediment nutrient concentrations higher than those in the groundwater in the current incubations. The evapotranspiration rates in incubations with emergent plants were significantly higher than those with substrates and controls, concentrating the solution further (Figure 4; data in Appendix B - Table 2). However, expressed on above-ground dry weight basis, evapotranspiration rates of emergent plants were highly variable and not significantly different from each other (Figure 4; Appendix B- Table 2).

Radio-analytic imaging (Figure 5; Table 6) showed that in the submersed plants, the physiologically active leaves and roots were highly labeled. These species are known for carbon and nutrient uptake by leaves (elodea), or by leaves and roots (pondweed). In water star-grass, however, a gradient in label intensity was evident ranging from highest in leaves to lowest in roots. This indicates label uptake by leaves in elodea, by roots and leaves in pondweed, and

mostly by leaves in water star-grass. In emergent plants, radiolabel was highest in roots, detectable in lower shoots, below detection in upper shoots, and again detectable in apical tips. This indicates label uptake by roots, limited transport upwards, and concentration in the physiologically active shoot tips.

Fate of [^{14}C]-TNT Radioactivity and Analyte TNT in Groundwater

Radioactivity in the groundwater decreased by a factor of two in most submersed plant incubations, but ten-fold with the three emergent species sweet-flag, reed canary grass and wool-grass (Table 7). Decreases were less in incubations with substrates than with submersed species, and decreases were not observed in groundwater controls. Groundwater TNT concentrations as determined by HPLC were undetectable in plant incubations after 7 days, accounting for a total disappearance of 4.003 mg TNT per beaker. The TNT residues (mg TNT per replicate) in the substrate incubations remained significantly higher in the autoclaved treatments than in the un-autoclaved ones (Table 7; Appendix B -Table 3). The TNT levels in groundwater controls remained relatively high, decreasing by 22% in darkness and by 40% in the light (Table 7), showing a significant effect of photolysis. The average radioactivity removal rates (calculated by dividing the difference between initial and final radioactivities of the incubation water by the number of incubation days), derived from these changes in concentrations were highest for the incubations with the three emergent species mentioned. TNT-equivalent removal rates were calculated as follows: e.g. for elodea, the radioactivity removal rate (3.3 x 10^6 DPM repl.$^{-1}$ d^{-1}) was divided by the total radioactivity per replicate (57.5 x 10^6 DPM replicate^{-1}), and multiplied by the total amount of TNT per replicate (4.003 mg replicate^{-1}) to give 0.23 mg repl.$^{-1}$ d^{-1}. These values were similar in the incubations with submersed plants and sediment, relatively higher with emergent plants, and lowest with gravel.

Specific, mass-based, removal rates were derived from the above-mentioned changes in radioactivity over time (Table 8). Specific TNT-equivalent removal rate was calculated as follows: e.g. elodea, the radioactivity removal rate (4.985 x 10^6 DPM g total DW^{-1} d^{-1}, Table 8) was divided by the total dose of radioactivity per replicate (57.5 x 10^6 DPM replicate^{-1}, Table 7), and multiplied by the total amount of TNT per replicate (4.003 mg replicate^{-1}, Table 7). Specific TNT-equivalent removal rates with plants were highest in the incubations with water star-grass (0.513 mg TNT-equiv. g total DW^{-1} d^{-1}) and lowest with sweet-flag (0.025 mg TNT-equiv. g below-ground DW^{-1} d^{-1}). These removal values correspond with 0.05 mg TNT g FW^{-1} d^{-1} in water star-grass and 0.001 mg TNT g total FW^{-1} d^{-1} in wool-grass. Specific TNT-equivalent removal with substrates was generally lower than with plants, highest with un-autoclaved sediment, and lowest

with autoclaved gravel (Table 8). Adsorption or sorption of TNT to soils has been found to be low (Pennington 1988; Wood and Tiller 1996).

The endpoint explosives composition of the incubated groundwater (Figure 6; Appendix B- Table 3) differed greatly from that of the initial filtered groundwater as determined by HPLC (Table 3). The aqueous phase TNT concentrations were far lower in the plant and un-autoclaved substrate treatments, than in treatments with autoclaved substrates and controls. Little initial 2ADNT (9.3 $\mu g\ L^{-1}$) disappeared from most treatments with plants, increased with water star-grass (HD), but increased up to forty-fold in controls and with substrates. 4ADNT increased in all incubations, but to a lesser extent with most plants and controls, and more with substrates. Of the di-amino TNT-derivatives, 24DANT rose from non-detect to over 1 mg L^{-1} with pondweed (PP) and elodea (EC); 26DANT increased slightly. Traces of the 4,4-azoxy-derivative of TNT were found only in the gravel incubations, at 0.526 mg L^{-1} in one of the non-autoclaved gravel replicates. RDX decreased below detection limits with elodea, pondweed, and reed canary grass and decreased significantly with wool-grass. However, it was seen to increase significantly above dosage concentration with parrot-feather, sweet-flag, and to increase in some of the controls. Residues of 24DNT and TNB were below detection.

[^{14}C]-TNT Radioactivity Distribution over Plants, Substrates and Air

Radiolabel mass balances showed that in incubations with submersed plants and parrot-feather, about half of the [^{14}C]-TNT derived label ended up in the groundwater, and 24 to 79% in the plants (Table 9). With emergent plants most label was recovered in the plants; with substrate most label was recovered in the groundwater. Mineralization to aerial CO_2 was minimal, but $\geq$ 0.09 % in elodea, sweet-flag and most substrate treatments. Label incorporation into aqueous HCO_3 and CO_2 was usually significantly higher than mineralization. Incorporation into volatile organic compounds was negligible (maximally 0.3 x 10^6 DPM, collecting volatiles of three replicates per XAD trap). Most overall recoveries were within 67 to 118 %. High recovery in water star-grass (130%) could be explained by the high variability in radiolabel distribution over the plant (Figure 5; Table 6; and individual combustion values- not shown). Low recovery in reed canary grass, 60%, may be due to the evolution of methane, which was not recovered in the XAD and KOH traps. Reed canary grass is known for its ability to decrease oxygen levels rapidly in its rhizosphere (TVA, personal communication 1995), favoring chemical and/or microbial transformation of CO_2 to methane.

Label distribution varied over different plant species and organs (Table 10; Figure 5). The tissues of submersed plants incorporated more label than the above-ground portions of the emergent plants (radioactivity per g FW). In emergent plants, label was concentrated in the

below-ground plant portions. Substrates incorporated less label than submersed plants; sediments incorporated approximately 1.5 x more than gravel, with a small decreasing effect of heat-inactivation.

Only a small part of the [^{14}C]-TNT-derived radiolabel associated with plant tissues, ranging from 9 to 33 %, proved 'free', i.e. un-conjugated into plant compounds and extractable in acetonitrile (Table 10). A similar ratio was extractable from the un-autoclaved substrates; however, more label was extracted from the autoclaved substrates (176% in autoclaved gravel and 464% in autoclaved sediment; calculated as average of three replicates) than found by combustion. The latter phenomenon may be explained by adsorption of TNT to the substrates and, consequently, high counts after combustion. This was verified as follows. An aliquot of [^{14}C]-TNT labeled solution with known strength was mixed with a known amount of autoclaved sediment, and a sub-sample was combusted and counted by LS (triplicate). The total radioactivity recovered by combustion of sediment sub-samples exceeded the radioactivity administered by 45%. Another explanation may be non-homogeneity of the substrates from which sub-samples were taken for combustion and extraction, and this is borne out by variability seen in autoclaved sediment (464 $\pm$ 428 %). Also, relatively more adsorption or transformation may have occurred at the substrates surface exposed to the labeled groundwater than at the unexposed substrate portions.

Fate of [^{14}C]-TNT Radioactivity and Analyte TNT in Plants and Substrates

The results of TLC analyses showed that most of the [^{14}C]-TNT-derived radiolabel in the plant and substrate extracts was polar, and did not move with the toluene:methanol solvent on the polar Silica Gel plates (Figure 7; Table 11). Labeled TNT was absent from all plants, except for the below-ground portion of reed canary grass, and from sediment. Labeled TNT was recovered in the autoclaved sediment, and in both un-autoclaved and autoclaved gravel, where it amounted to 9 to 17% of the radioactivity (Table 11). Radiolabel incorporation into ADNTs was found (2ADNT in submersed plants and substrates, and 4ADNT in emergent plants), but not into other known TNT degradation products. A total of five unknown labeled metabolites or groups of metabolites was found; three found only in plants (U3, U4, U5) and one found only in substrates (U2). The first unknown metabolite, U1, could be a mixture of more than one compound; its location at the origin suggests that it consists of polar compounds that did not migrate. These metabolites were not chemically identified. However, their behavior during separation by TLC could be characterized by mobility relative to standards and Rf. Relative mobilities were: DANTs<U2<ADNTs, and U3, U4 and U5>TNT on Silica Gel plates.

Explosives residues as determined by HPLC in plant tissue were limited to 4ADNT (and RDX; Figure 8; Appendix B- Table 4). 4ADNT concentrations were relatively high in the submersed plants (0.8 to 2.6 μg g FW^{-1}) and in the below-ground portions of parrot-feather (2.5 μg g^{-1}), reed canary grass (0.8 μg g^{-1}) and wool-grass (1.0 μg g^{-1}). RDX was only detected in water star-grass (1.0 μg g^{-1}) and in the below-ground portions of parrot-feather (2.0 μg g^{-1}). The 4,4-azoxy-derivative of TNT occurred in water star-grass (0.2 μg g^{-1}), and the below-ground portions of sweet-flag (1.9 μg g^{-1}) and reed canary grass (0.2 μg g^{-1}; Appendix B- Table 4). These residues of TNT metabolites are extremely low, even if 85% of total radioactivity was lost during clean-up of plant extracts, as is suggested by loss found in preliminary tests of elodea (see Materials and Methods, Analyses - Overview). They are far lower than earlier data on plant tissue residues in terrestrial plants, which were derived from radioactivity data and given as TNT equivalents, and which overestimated TNT since TNT (but not radiolabel) rapidly degrades (Cataldo et al. 1989). The present TNT metabolite residue levels in the plants are somewhat higher than found in more recent studies on terrestrial plants, indicating 4ADNT residues below detection (Fellows, Harvey, and Cataldo 1995). Comparison of the TNT and TNT metabolite residue levels of the present study with those found by Hughes et al. 1997 is not meaningful, since in the latter case plants were incubated in darkness and at high (30 to 95 mg L^{-1}), lethal TNT levels, conditions which did not allow normal plant metabolism-derived biotransformation of explosives. Low 4ADNT and RDX levels were found in the substrates, 0.25 and 0.50 μg g^{-1}, respectively. Autoclaving appeared to increase the 4ADNT and RDX residues in sediment by a factor 1.5 to 2, but only those of 4ADNT in gravel. Although autoclaving minimizes microbial activity in the substrates, it also changes the substrate structure, presumably increasing the adsorption sites. However, the latter adsorbed explosives remained extractable. No azoxy-compounds were recovered from the substrates.

Behavior of RDX in Hydroponic Culture

Plant Growth and Labeling in [^{14}C]-RDX Groundwater

Submersed species increased in weight over the 13-day incubation period in the RDX amended incubation. Relative growth rates were positive (Figure 9; Appendix B - Table 5), and those for elodea and pondweed were within normal ranges for field conditions at the end of the growth season (Best and Dassen 1987; Van Wijk 1989). Emergent plants decreased in weight, except for reed canary grass, which showed a growth rate considered normal for grasses at the end of the growth season. Emergent plants may have suffered from nutrient limitation, as in the

TNT amended incubations. However, wool-grass probably also suffered from root desiccation, since only 60 mL solution was left at the end of the incubation period. The evapotranspiration rates in the incubations with submersed plants and in the darkened control were significantly lower than in the remaining incubations (Figure 10; Appendix B- Table 6). In emergents, the trend in relative growth rate was reflected by the evapotranspiration rates, which were highest for reed canary grass.

Partitioning of radiocarbon in intact plants was assessed from the radio-analytic images (Figure 11; Table 12). The submersed plants were in general uniformly labeled. However, newly formed shoots of elodea and pondweed obviously served as either uptake site or sink for RDX, since they were very highly labeled. In the emergent plants, radiolabel was detectable in roots and in lower shoots, usually below detection in upper shoots, and extremely high in apical shoots. This indicates label uptake by roots, transport upwards, and concentration in the physiologically active shoot tips.

Fate of [^{14}C]-RDX Radioactivity and Analyte RDX in Groundwater

The radioactivity in the groundwater decreased by approx. 30 % in most plant incubations, by 79% with pondweed and by 91% with wool-grass (Table 13). Decreases in substrate incubations were 27 to 31% with sediment, and 4 to 6% with gravel. No decrease occurred in groundwater controls. The RDX concentrations as determined by HPLC (Table 13; Figure 12) decreased from the initial 1.5 mg L^{-1} by 40 to 50% of these levels in most plant and sediment incubations. Exceptions were RDX concentrations with pondweed (decreased by 98%) and wool-grass (by 100%), and with gravel (remained unchanged; Table 13). The radioactivity removal rates derived from these changes in concentrations were similar for the incubations with most plants and sediments, significantly higher with pondweed, and extremely low with gravel and in controls (Table 13). The analyte RDX removal rates generally reflected the radioactivity removal rates, but were usually somewhat higher than the RDX-equivalent removal rates.

Specific mass-based removal rates were derived from the above-mentioned changes in radioactivity over time (Table 14), and from the changes in analyte-RDX. RDX-equivalent removal rates were calculated as for TNT-equivalents. The RDX-equivalent removal rates indicated activity only in the plant treatments. The incubation with elodea had the highest activity (0.042 mg RDX-equiv. g DW^{-1} d^{-1}) and those with sweet-flag and wool-grass the lowest (0.007 mg RDX-equiv. g below-ground DW^{-1} d^{-1}). These removal values correspond with 0.004 mg RDX g FW^{-1} d^{-1} for elodea and 0.0002 mg RDX g total FW^{-1} d^{-1} for wool-grass. Specific analyte RDX removal rates were usually somewhat higher than the RDX-equivalent removal rates.

The explosives composition of the groundwater following incubation differed greatly from the initial filtered groundwater (Figure 12; Appendix B- Table 7; Table 3). TNT was only recovered in the controls, and had decreased significantly more in the light than in darkness. 2ADNT was below detection in all plant treatments, but had increased in the substrate treatments and controls; it was higher in the treatments with gravel and both autoclaved substrates than with plants. 4ADNT was below detection in all plant treatments except parrot-feather, and had increased in all non-plant treatments; it was significantly higher in gravel and both autoclaved substrates. Only very low levels of 24DNT were recovered in controls. TNB was below detection in treatments with plants or sediments, had decreased less with gravel, and least in controls. RDX had decreased in all plant treatments, except parrot-feather, and in sediment, but increased in the remaining substrate treatments, with parrot-feather and in the illuminated control. RDX increases in parrot-feather and control treatments may be due to the high evapotranspiration rates, which were particularly variable for parrot-feather (Figure 10; Appendix B - Table 6).

[^{14}C]-RDX Radioactivity Distribution over Plants, Substrates and Air

Radiolabel mass balances showed that in elodea, water star-grass, emergent plants (except for wool-grass), and in substrate treatments, most [^{14}C]-RDX-derived label ended up in the incubated groundwater, but that in pondweed and wool-grass treatments most label was recovered in the plants (Table 15). Mineralization to aerial CO_2 was generally low (< 1%), but higher in the sediment (2.08 %), pondweed (2.76 %), sweet-flag (4.06 %), reed canary grass (5.05 %) and particularly wool-grass (10.17 %). The pondweed and sediment incubations showed not only considerable $^{14}CO_2$ evolution, but also high (4 to 8%) incorporation of radiolabel in the aqueous HCO_3/CO_2. Incorporation into volatile organic compounds was negligible. Overall recoveries ranged from 56 to 112 %. Low recovery in wool-grass may be associated with some RDX crystal formation on the outside of the roots due to high solution loss. The latter radiolabel was not recovered, because it was probably rinsed from the plants after incubation (no radio-assay of the rinsing water was done).

Label distribution varied over plant species and organs (Table 16). The tissues of submersed plants and emergent plants were generally labeled to a similar extent. Exceptions were pondweed and the above-ground portions of reed canary grass, which incorporated relatively high amounts of label. Considerable root-to-shoot transport of label occurred in the emergent plants, as could be concluded by dividing the radioactivities in the shoots by those in the whole plants. It ranged from 23% of total plant radioactivity in sweet-flag to 81% in parrot-feather. Substrates were labeled far less than plants, with sediments approximately twice as much as gravel with no heat-inactivation effect.

A sometimes considerable part of the radiolabel found in the plant tissues, up to 61 %, proved 'free', i.e. extractable in acetonitrile (Table 16). The extractable fraction in the sediment was higher when autoclaved than un-autoclaved, 59% versus 25%, and was around 60% in gravel, where autoclaving did not appear to have an influence.

Fate of [^{14}C]-RDX Radioactivity and Analyte RDX in Plants and Substrates

Most of the [^{14}C]-RDX radiolabel in the tissue extracts of elodea, pondweed, and parrot-feather was incorporated into polar compounds that did not move with the toluene:methanol solvent on the polar Silica Gel plates (Table 17; Figure 13). Labeled RDX was detected in the acetonitrile extracts of all plants and substrates using separation of compounds by TLC on Silica Gel plates. RDX accounted for $\leq$2 to 63% of the radioactivity in the plant extracts, and $\leq$20 to 80% of the radioactivity in the substrate extracts. Substantial amounts of labeled compounds comigrated with RDX in this TLC system (polar Silica Gel) in extracts of water star-grass and most of the emergents. However, since these activities were often higher than those separated using a TLC system with higher resolution for RDX (apolar Whatman plates; Table 18; Figure 14), it was concluded that in the Silica Gel separation the RDX was accompanied by (an) unknown metabolite(s). A total of five spots attributable to labeled RDX metabolites was found, of which one (polar, U4) was found only in two plant species. The spots U1 and U3 could represent mixtures of more than one compound. These metabolites were not chemically identified. Their mobilities relative to known compounds were: DANTs<U2<ADNTs and equal to RDX on Silica Gel plates; U4 and U5>RDX on Whatman plates.

Explosives residues as determined by HPLC in plant tissue were limited to RDX (and 4ADNT; Figure 15; Appendix B- Table 8). RDX was detected in all plants; however, levels were below detection in the below-ground portions of sweet-flag and wool-grass. RDX residues ranged from 0.32 μg g FW^{-1} in pondweed to 8.57 μg g FW^{-1} in parrot-feather shoots. These RDX concentrations are extremely low, even if 95% was lost during cleanup of the plant extract, as suggested by recoveries in preliminary elodea incubations (see Materials and Methods, Analyses - Overview). They are far lower than plant tissue residues derived from radioactivity data elsewhere and given as RDX equivalents; the latter may have overestimated RDX somewhat since RDX (but not radiolabel) degrades rather slowly (Cataldo, Harvey, and Fellows 1990). The present RDX residue levels in the plants are somewhat lower than more recent data on terrestrial plants (>18 μg g^{-1} in corn and >180 μg g^{-1} in alfalfa; Fellows, Harvey and Cataldo 1995), and similar to those recovered in recent similar mass balance studies of terrestrial plants (maximum of 16 μg g^{-1} in foliage; Price et al. 1997). No explosives nor known metabolites were recovered in

the substrates. 4ADNT concentration was considerable in the below-ground portions of parrot-feather. Azoxy-compounds were absent from all plants and substrates evaluated.

General Discussion

Behavior of TNT and RDX in Groundwater

Although plants did not grow well, TNT was removed, and RDX was greatly decreased in treatments with plants. TNT disappearance from groundwater incubated with plants over 7 days was associated with the subsequent presence of explosive-derived radioactivity in plant tissues. Highest specific TNT removal rates were found in submersed plants in star-grass (0.05 mg TNT g FW^{-1} d^{-1}), and in emergent plants in parrot-feather, sweet-flag, and reed canary grass (0.006 mg TNT g total FW^{-1} d^{-1}). RDX disappeared less rapidly than TNT from the incubated groundwater, and was associated with the subsequent presence of explosives-derived radioactivity in plant tissues. Highest specific RDX removal rates were found in submersed plants in elodea (0.004 mg RDX g FW^{-1} d^{-1}), and in emergent plants in reed canary grass (0.001 mg RDX g total FW^{-1} d^{-1}).

The more rapid decrease in TNT levels in illuminated controls than in dark controls without plants supports photolysis of TNT (Spanggord et al. 1980; Gorontzy et al. 1994), similar to that reported for dissolved organic matter (DOM) originating from both live and decomposing plants in water bodies (Wetzel, Hatcher, and Bianchi 1995). The more rapid decrease in TNT and RDX levels with plants may also be due to the generation of plant-specific DOM and leachates, both providing small fatty acids or assimilates readily available to microbes (Wetzel, Hatcher, and Bianchi 1995; Mann and Wetzel 1996), and enhancing microbial degradation of explosives. These products may have given the problems in SPE columns.

Behavior of TNT and RDX in Plants

The behavior of TNT and RDX seen in this study does, in fact, generally follow that of a herbicide in contact with plants. Plant detoxification of herbicides (Kreuz, Tommasini, and Martinoia 1996; Trapp and Matthies 1995) is generally enzyme-mediated, in which a primary step often includes oxidation or hydrolysis, which may provide a functional group suitable for subsequent covalent binding to an endogenous moiety. This first step often results in the formation of glycosides. Another important conjugation reaction in plant herbicide metabolism is that with the major cellular thiol, GSH (γ-glutamyl cysteinyl glycine); this conjugation was shown to occur in plants tolerant to atrazine under aerobic conditions . It is noted that atrazine is a

herbicide similar in structure to RDX. The resulting conjugates are 1) generally inactive toward the initial target site, 2) more hydrophilic and less mobile in the plant than the parent compound, and 3) susceptible to further processing which may include secondary conjugation, degradation, and compartmentalization. Metabolism of herbicides to glycosides or to GSH conjugates is usually considered a detoxification process, but the products are not always themselves benign and may possess toxicological activities. Recent unpublished work indicates incorporation of amino transformation products of TNT in coniferyl alcohol, a precursor of lignin, in tree species (K.Thorn, USGS, unpublished 1997). This finding supports the hypothesis that explosives-tolerant and -degrading plants may possess detoxification mechanisms similar to those identified in herbicide-resistant agricultural crops, and that the degradation products are utilized as secondary plant substances.

In the present study, specific, mass-based, TNT-equivalent removal rates, derived from the changes in [^{14}C]-TNT derived radioactivity over time, were far higher in the incubations with submersed plants than in those with emergents. Plant tissue labeling strength was consequently higher also. A relatively small part of the tissue radiolabel was 'free', i.e. non-conjugated, and extractable. Radiolabel mass balances indicated considerable [^{14}C]-TNT derived label incorporation into plants, low mineralization to CO_2/HCO_3, and negligible evolution into volatile organic compounds. The [^{14}C]-TNT-derived radiolabel was taken up by physiologically active roots and leaves in submersed plants, and appeared to remain at the sites of uptake. The label was taken up by the roots of emergent plants, and it was transported to a limited extent in an apical direction. TNT may have been transformed (conjugated) prior to transport (Cataldo et al. 1989; Michels and Gottschalk 1994; Fellows, Harvey, and Cataldo 1995; T.F.Jenkins 1996, unpublished; Thompson and Schnoor 1996), since virtually no labeled TNT residues were recovered in the plant extracts after 7 days. [^{14}C]-TNT was reduced to 2ADNT in submersed plants and to 4ADNT in emergent plants. Five other unknown metabolites or groups of metabolites were separated by TLC, but not identified. These metabolites can be attributed to plant activity. No evidence for degradation via nitro-group removal, i.e. no DNTs, was found. Only a small quantity of labeled TNT remained in the below-ground portions of one species, reed canary grass. HPLC analysis confirmed the presence of 4ADNT, but not of TNT, in most plant tissues. The absence of labeled 4ADNT in some plants after 7 days does not preclude its presence and subsequent transformation into other unknown metabolites. Toxic azoxy-compounds had accumulated only in water star-grass and in the below-ground portions of sweet-flag and reed canary grass. HPLC analysis proved RDX to be absent from most plants, except in water star-grass and the below-ground portions of parrot-feather.

Specific, mass-based, RDX-equivalent removal rates, derived from the changes in [^{14}C]-RDX derived radioactivity over time, were highest in the incubations with elodea. The tissues of

submersed plants incorporated as much as those of most emergent plants. A relatively larger part of the tissue radiolabel than in the case of TNT was 'free'. Radiolabel mass balances indicated considerable label incorporation in plants, some mineralization to CO_2/HCO_3, and negligible evolution into volatile organic compounds. The [^{14}C]-RDX derived radiolabel was probably taken up over the whole plant surface of the submersed plants, but it tended to accumulate in newly-formed shoots. The label was taken up by the roots of emergent plants, and considerable transport in an apical direction took place. Part of the RDX was probably transformed (conjugated) prior to transport (this part was relatively higher than in the case of TNT) (Cataldo, Harvey, and Fellows 1990; Fellows, Harvey, and Cataldo 1995; T.F.Jenkins 1996, unpublished), since only part of the labeled RDX residues was recovered in the plant extracts after 13 days. The five unknown metabolites separated by TLC were not identified. One unknown, U4, was unique to plants, but the other four were also found in substrates. The lowest [^{14}C]-RDX level occurred in pondweed and was close to the detection limit for radio-analytic imaging. Analyte RDX concentrations determined by HPLC were usually higher in emergent than in submersed plants, and accumulation occurred in the above-ground plant portions. Azoxy compounds were not found.

To address the question of which plant species would be most effective in a constructed wetland with the objective of removing explosives from groundwater, several plant characteristics have to be taken into account. These are the high, specific, plant mass based explosives removal rates, which were 4 to 8 x higher for TNT, and 2 to 10 x higher for RDX in submersed than in emergent plants, and the high metabolization of the parent explosives, almost complete for TNT in all plants, highest for RDX in elodea and pondweed. From these results, submersed plants, particularly elodea and pondweed, would be most suitable. However, emergent plants can be as effective as submersed species per wetland unit area, presuming that removal rate and metabolic activity are proportional to standing crop (g plant mass produced per m^2), which is typically 2 to 5 x higher in emergent species.

Behavior of TNT and RDX in Substrates

Specific mass-based TNT-equivalent removal rates in the incubations with substrates were generally lower than with plants. Substrate labeling strength ranked between that of submersed plants and that of below-ground portions of emergent plants. Although [^{14}C]-TNT incorporation/adsorption in sediment and gravel was considerable, no analyte TNT was recovered in the substrate extracts by HPLC analysis. Labeled TNT was recovered in the extracts of three of the four substrates using TLC. Part of the TNT had been transformed, with metabolite(or group of metabolites) identical to that extracted from plants (ADNTs), and one

unique for what was presumably the microbial component of the substrates (U2). HPLC analysis confirmed the presence of 4ADNT (and of RDX) in all substrates. No evidence for TNT degradation via nitro-group removal was found.

Specific mass-based RDX-equivalent removal rates in the substrate incubations were negligible. Specific analyte RDX removal rates were extremely low. [^{14}C]-RDX incorporation/ adsorption, as determined by combustion was very low in sediment and gravel. Labeled RDX was recovered in extracts of all substrates using TLC. RDX residues proved also detectable by HPLC analysis. A relatively small part of the [^{14}C]-RDX had been transformed, with four metabolites identical to those extracted from plants. However, HPLC analysis did not identify known RDX degradation products (MNX, TNX).

Summary

This mass balance shows that:

1. TNT was rapidly (0.001 mg TNT g total FW^{-1} d^{-1} in wool-grass to 0.05 mg TNT g FW^{-1} d^{-1} in water star-grass) transformed by explosives-adapted emergent and submersed plants. Neither periphyton nor substrates (sediment and gravel) significantly contributed to this transformation.
2. Mono-aminodinitrotoluene and di-amino-dinitrotoluene levels did not accumulate in the incubation water. Azoxy compounds were only recovered from water from incubations with gravel.
3. 2ADNT levels decreased in groundwater incubated with plants, but 2ADNT was not recovered from plant tissues; 4ADNT levels remained unchanged in groundwater with submersed plants but increased with emergent plants. 4ADNT appeared in the tissues of submersed plants and in the below-ground parts of emergent plants.
4. 24DANT and 26DANT levels increased in groundwater incubated with submersed plants, but neither compound was recovered from plant tissue.
5. Five TNT transformation products were separated from plant and substrate extracts using TLC, but not identified. Three products occurred only in plants. Azoxy compounds had accumulated only in one submersed and in the below-ground portions of two emergent plants.
6. Only a small amount of TNT-derived ^{14}C was extractable with solvent from plant tissue which became radiolabeled.
7. Mineralization of TNT, i.e. $^{14}CO_2$ evolution, was extremely low.
8. Photolysis of TNT was demonstrated in the illuminated water control.

9. RDX was rapidly (0.0002 mg RDX g total FW^{-1} d^{-1} in wool-grass to 0.004 mg RDX g FW^{-1} d^{-1} in elodea) transformed by explosives-adapted emergent and submersed plants. Transformation was slower than that of TNT. Neither periphyton nor substrates (sediment and gravel) significantly contributed to this transformation.
10. RDX levels remained detectable in the incubation water of all plant treatments, except wool-grass. No known RDX transformation products were found in the water.
11. RDX and five RDX transformation products were separated from plant and substrate extracts using TLC, but not identified. One product occurred only in plants.
12. Only a small amount of RDX-derived ^{14}C was extractable with solvent from plant tissue which became radiolabeled.
13. Mineralization of RDX, i.e. $^{14}CO_2$ evolution, was extremely low.

The promise of phytoremediation in constructed wetlands as a technology for removal of explosives from groundwater is supported by several results of this study. 1) The rapid decrease in TNT and relatively slower decrease in RDX in the presence of certain aquatic or wetland plants under viable environmental conditions, 2) The relatively rapid metabolism of the parent compounds inside the plants, and 3) Low explosives residues in plant tissues and substrates. However, it must be realized that metabolic pathways of degradation of TNT and RDX in plants are still unknown, that certain explosives degradation products may exert other biological and toxicological activities, and that decreases in TNT and RDX levels in water with plants may also partly be due to chemical binding between explosives transformation products and organic matter, or to the generation of plant-specific dissolved organic matter and leachates, both stimulating microbial activity and resulting in degradation of explosives.

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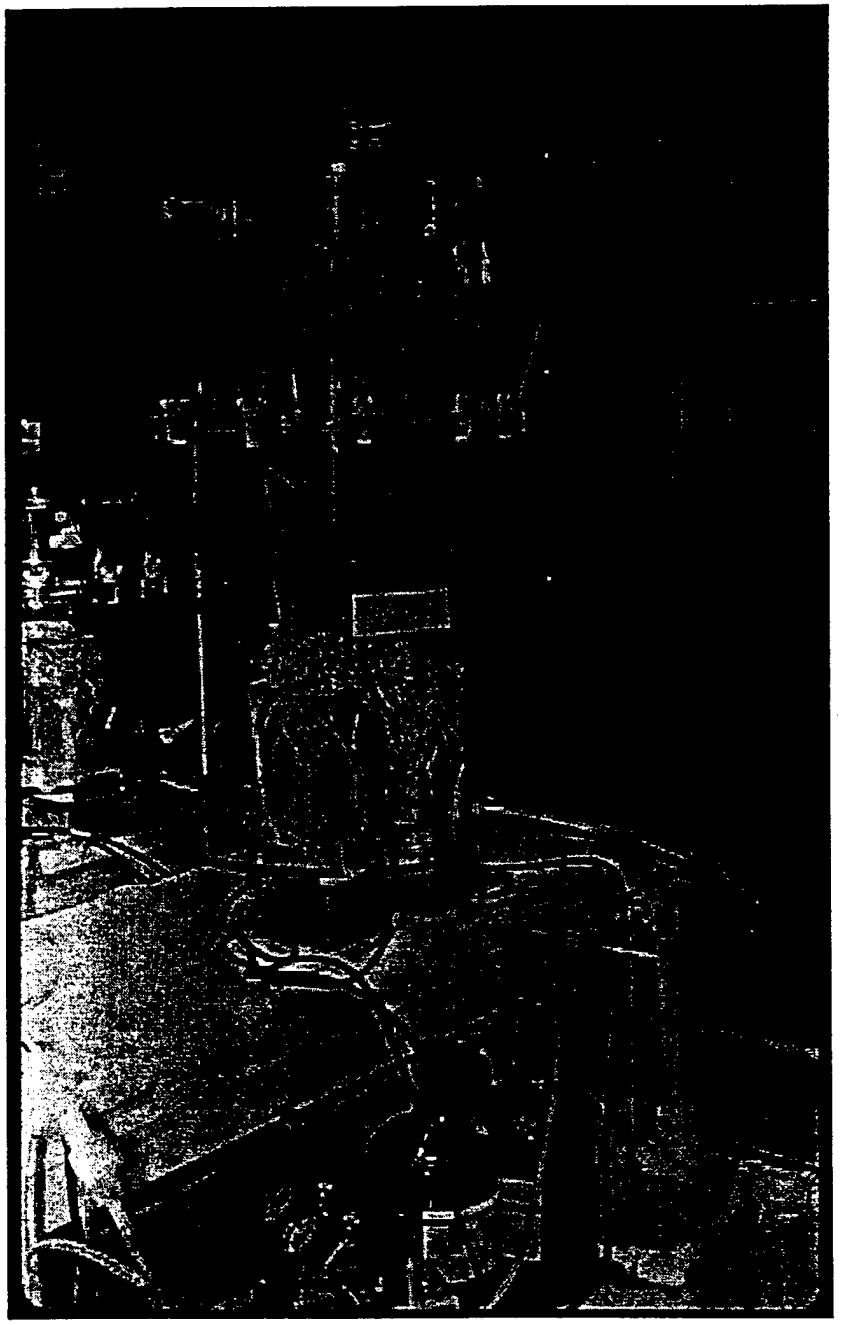


Figure 1. Incubation systems used for mass balance studies.

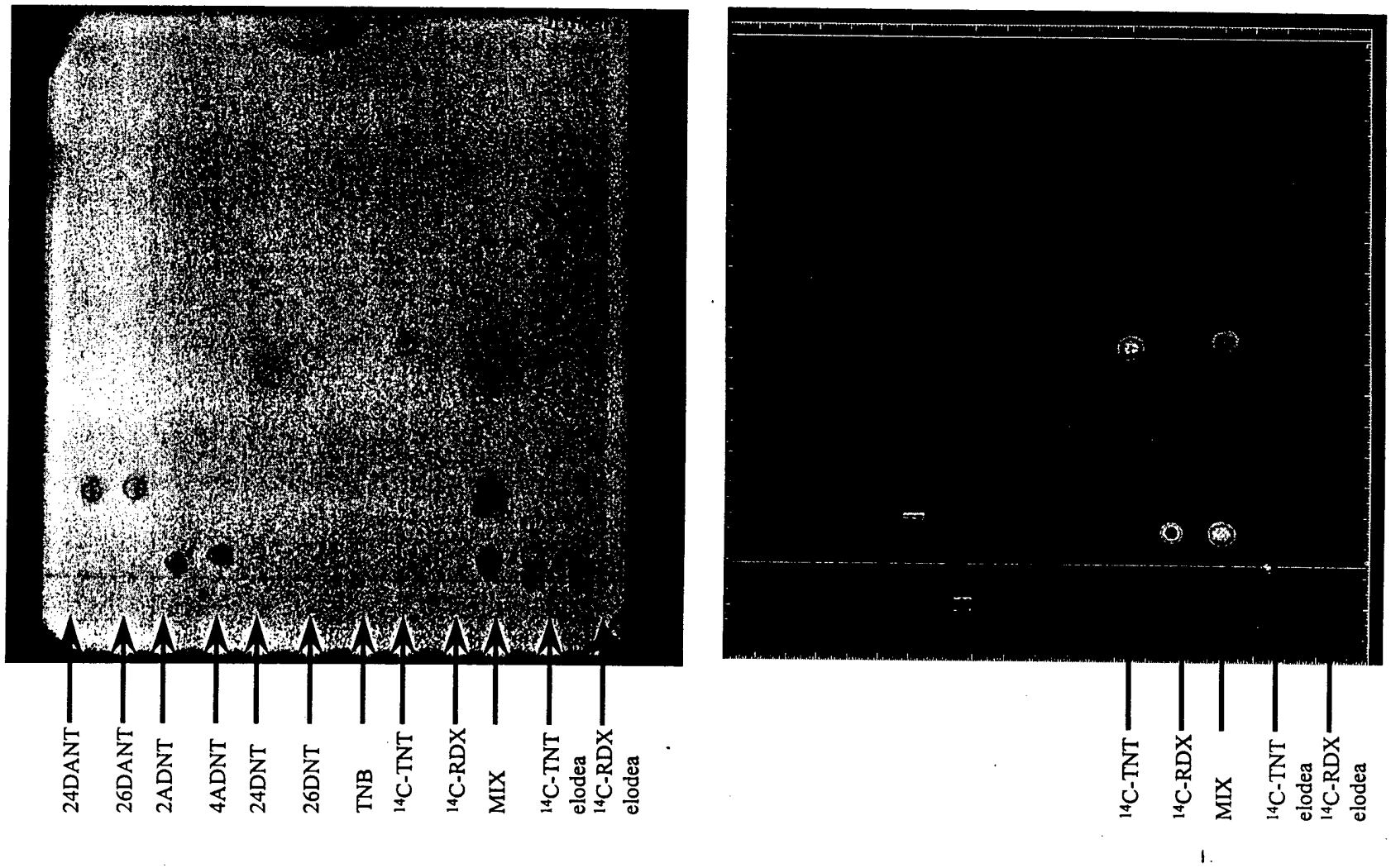


Figure 2. Conventional camera photograph with fluorescent illumination (left side) and radio-analytic image of a thin layer chromatogram (right side) used to separate radiolabeled and unlabeled explosives and degradation products.

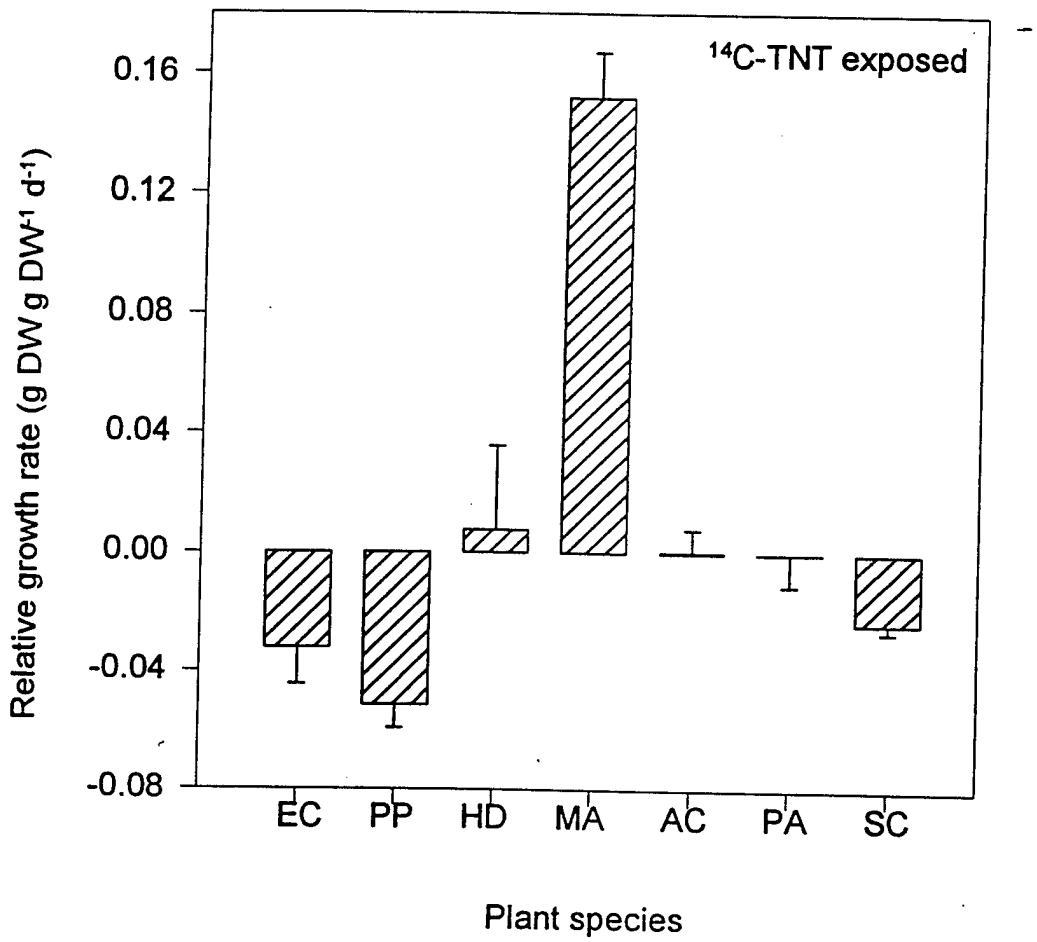


Figure 3. Relative growth rates of plants over 7-day incubation in [^{14}C]-TNT amended groundwater containing 1.64 to 3.37 mg TNT L^{-1}. Mean values and standard deviations(N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass.

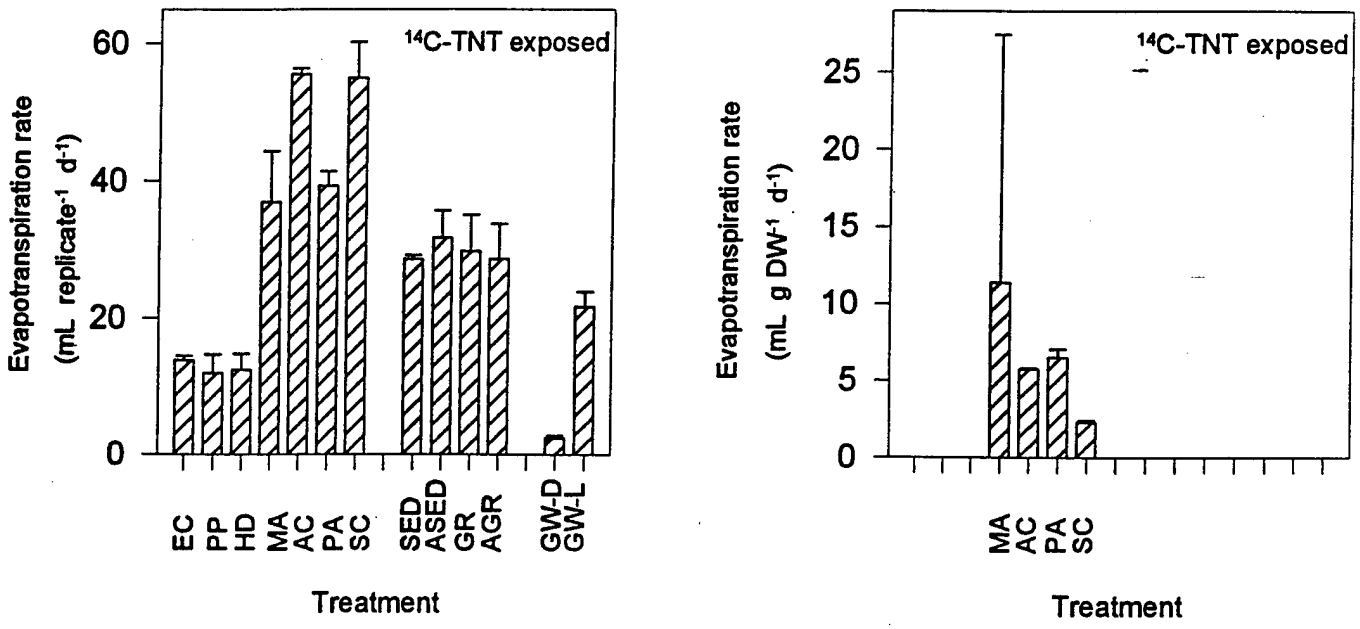


Figure 4. Evapotranspiration rates in [^{14}C]-TNT groundwater over 7-day incubation with plants, substrates, or controls. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel; GW-D, groundwater darkened; GW-L, groundwater illuminated.

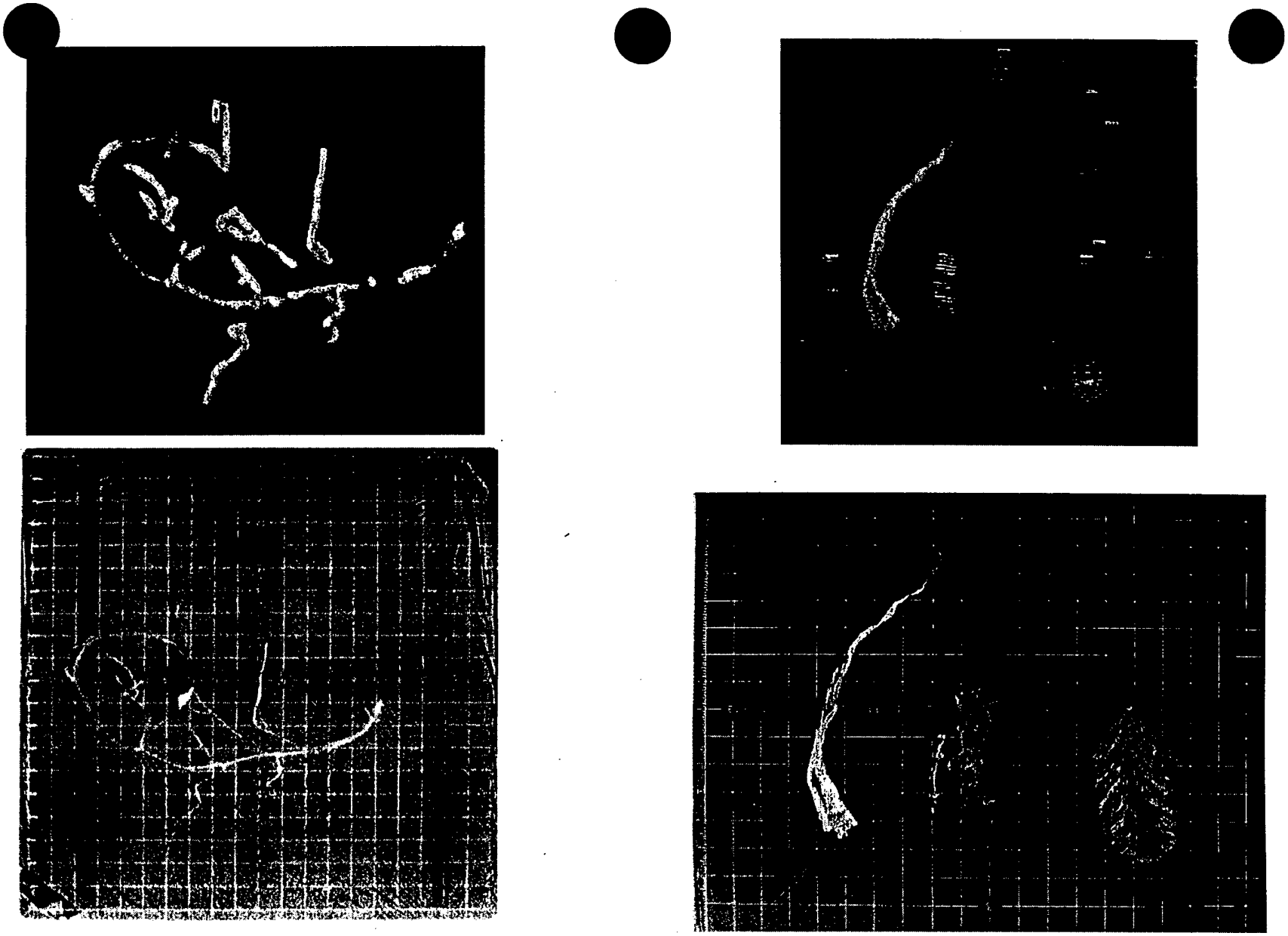


Figure 5. Typical distribution of radio carbon over plant organs or portions after 7-day incubation in [^{14}C]-TNT explosives-contaminated groundwater, as indicated by radio-analytic imaging (upper). Conventional camera-photographs provided visual information on plant morphology and orientation on the plant-supporting glass plate (lower).

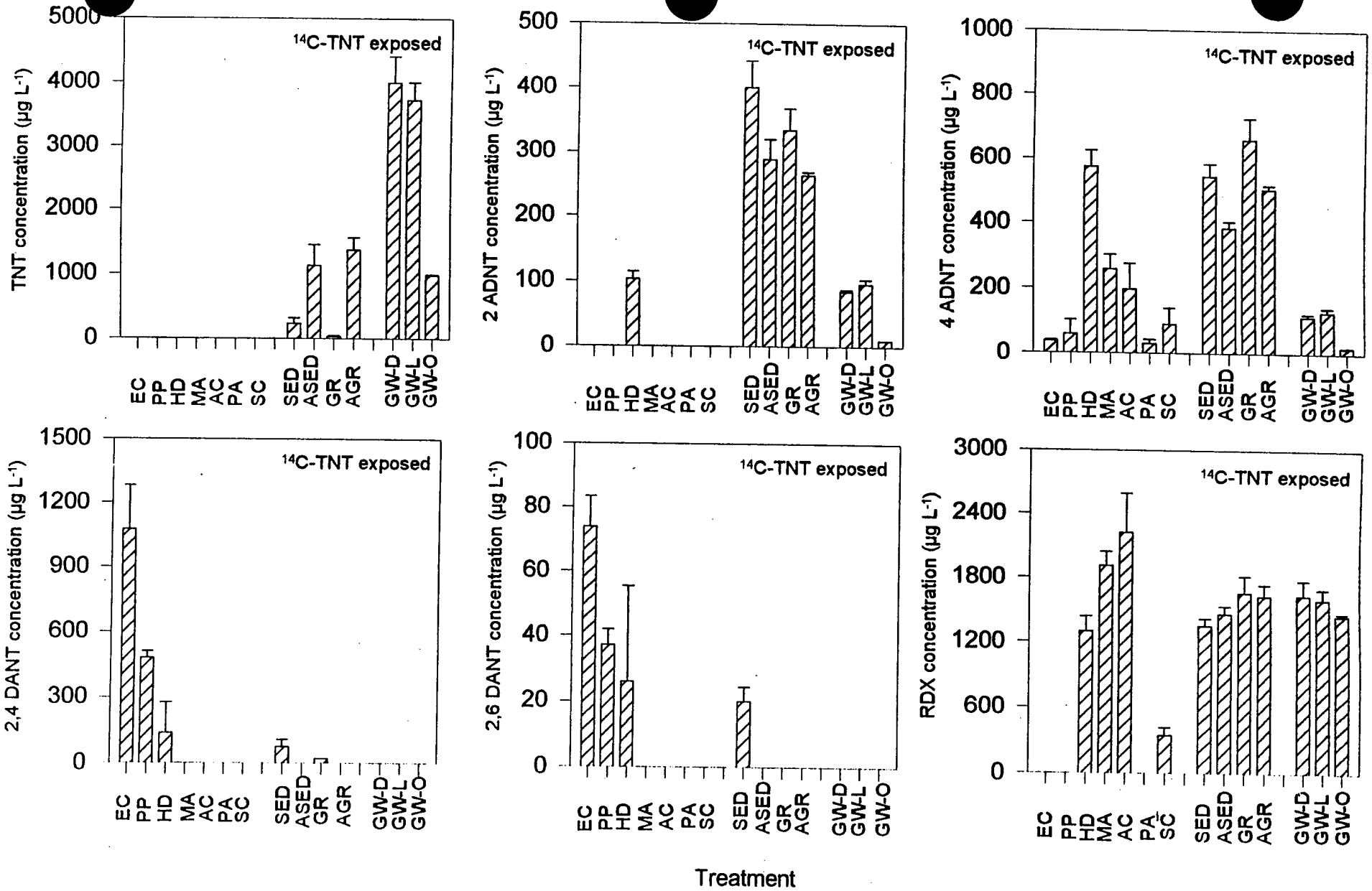


Figure 6. Explosives concentrations in [^{14}C]-TNT groundwater after 7-day incubation with plants, substrates, controls, and initially, as determined by HPLC analysis. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel; GW-D, groundwater darkened; GW-L, groundwater illuminated; GW-O, initial groundwater.

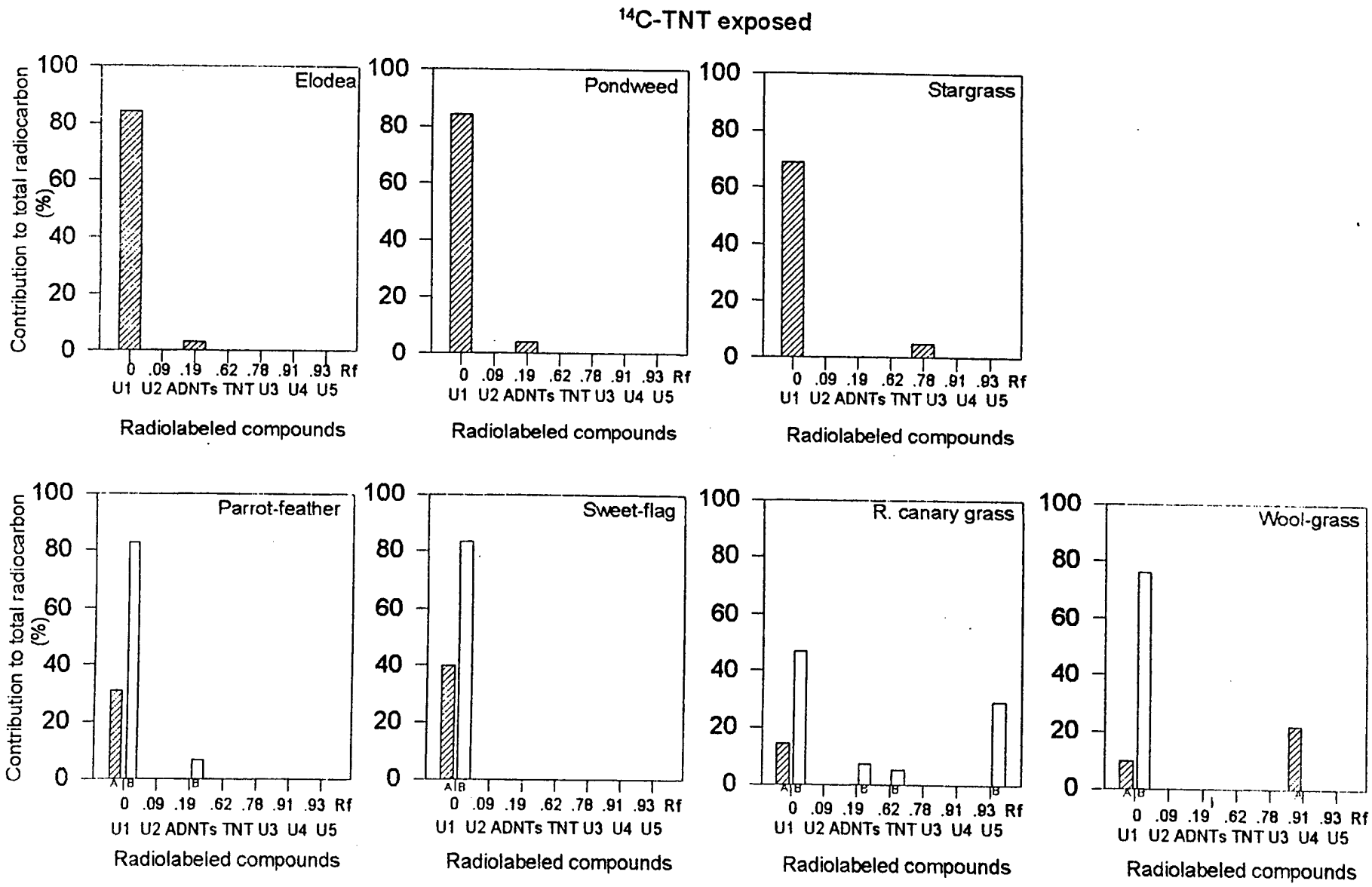
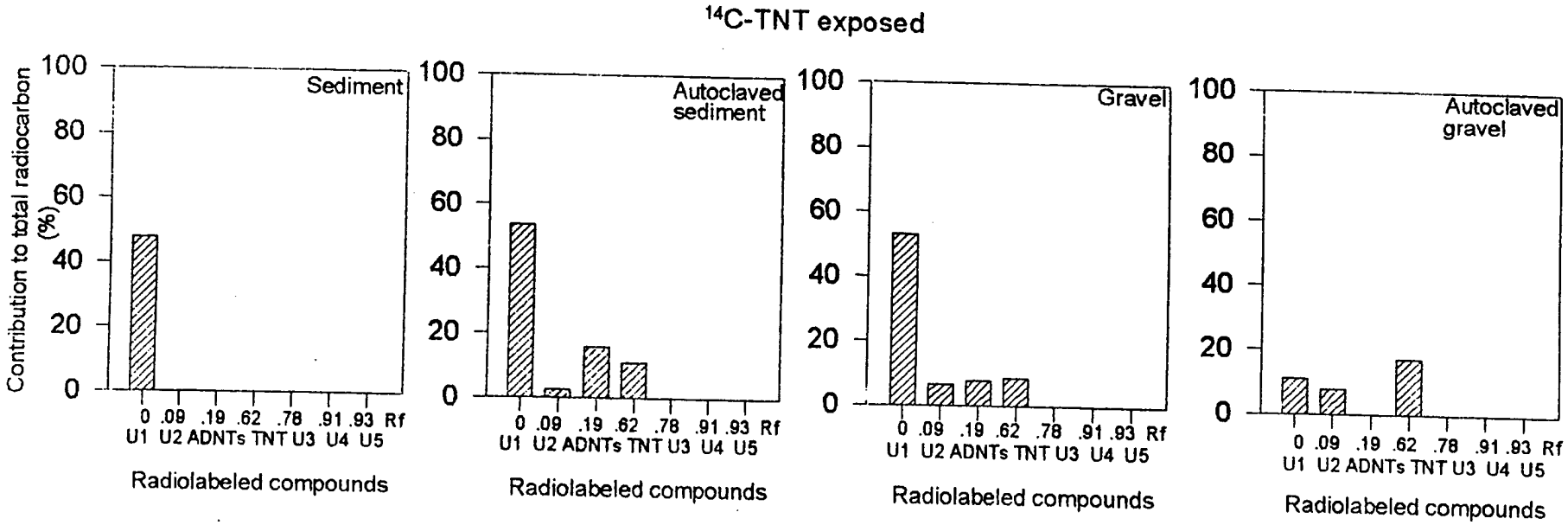


Figure 7. Distribution of TNT-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by thin layer chromatography of extracts and references on Silica Gel 60F plates in a toluene:methanol mixture (98:2), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Abbreviations: A, aboveground; B, below-ground; U, unknown.



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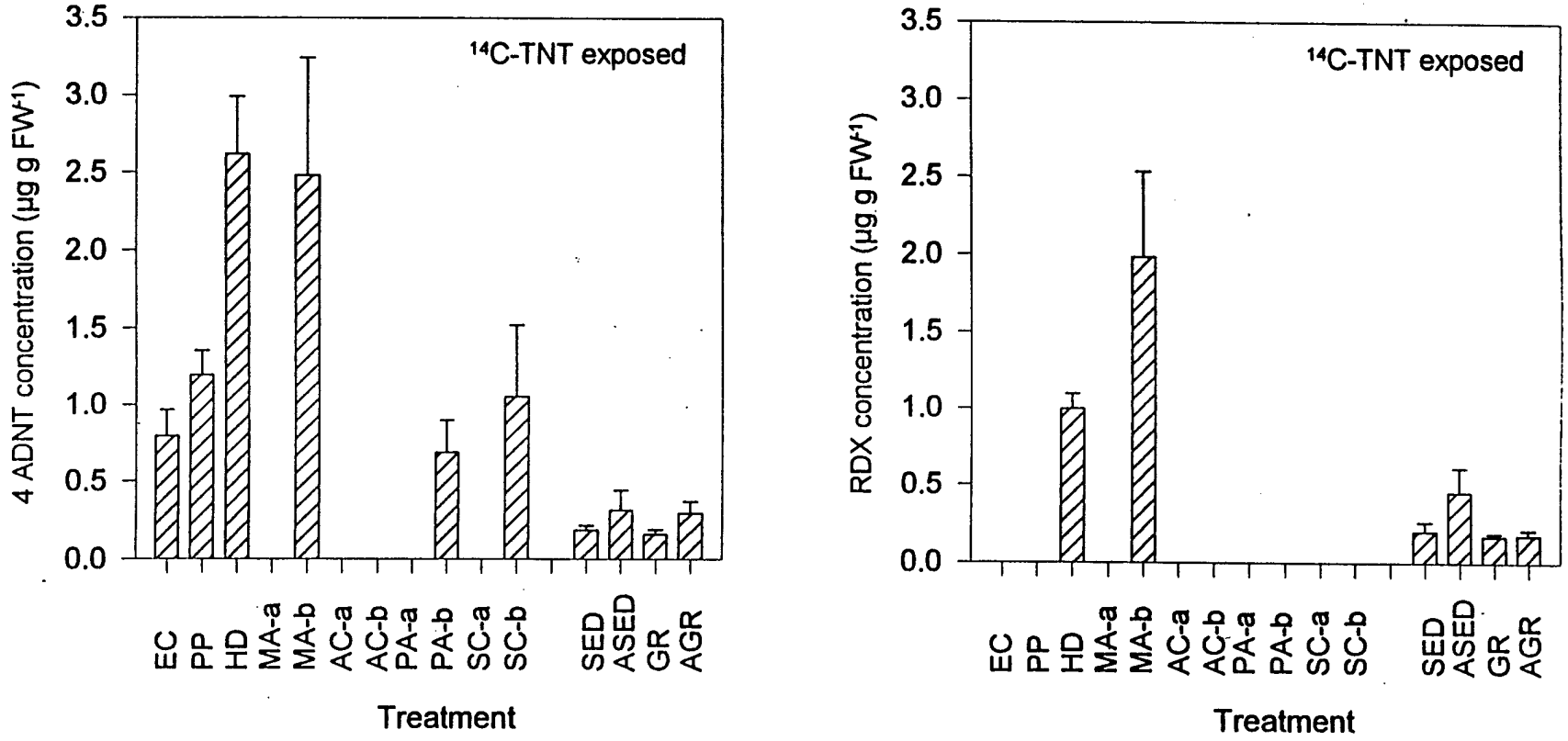


Figure 8. Explosives residues in plants and substrates after 7-day incubation in [^{14}C]-TNT groundwater, as determined by HPLC analysis. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather, -a above-ground and -b below-ground; AC, sweet-flag, -a above-ground and -b below-ground; PA, reed canary grass, -a above-ground and -b below-ground; SC, wool-grass, -a above-ground and -b below-ground; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel.

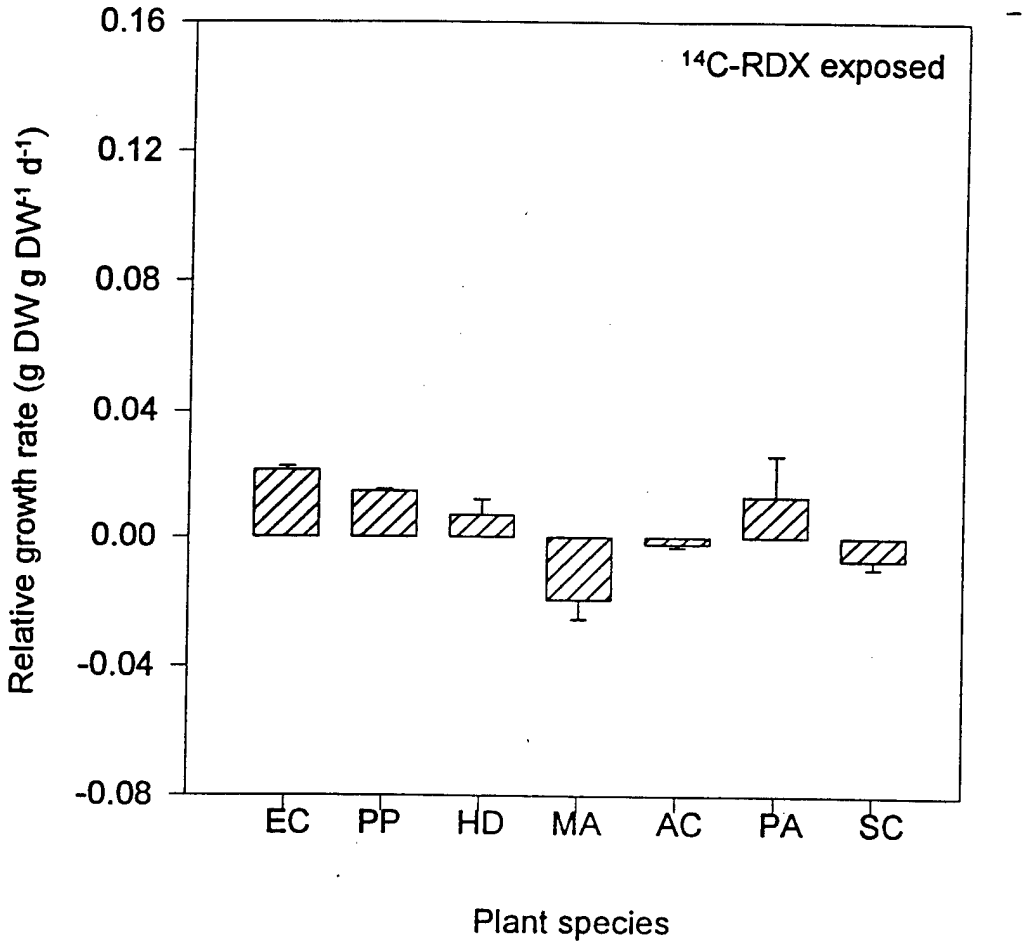
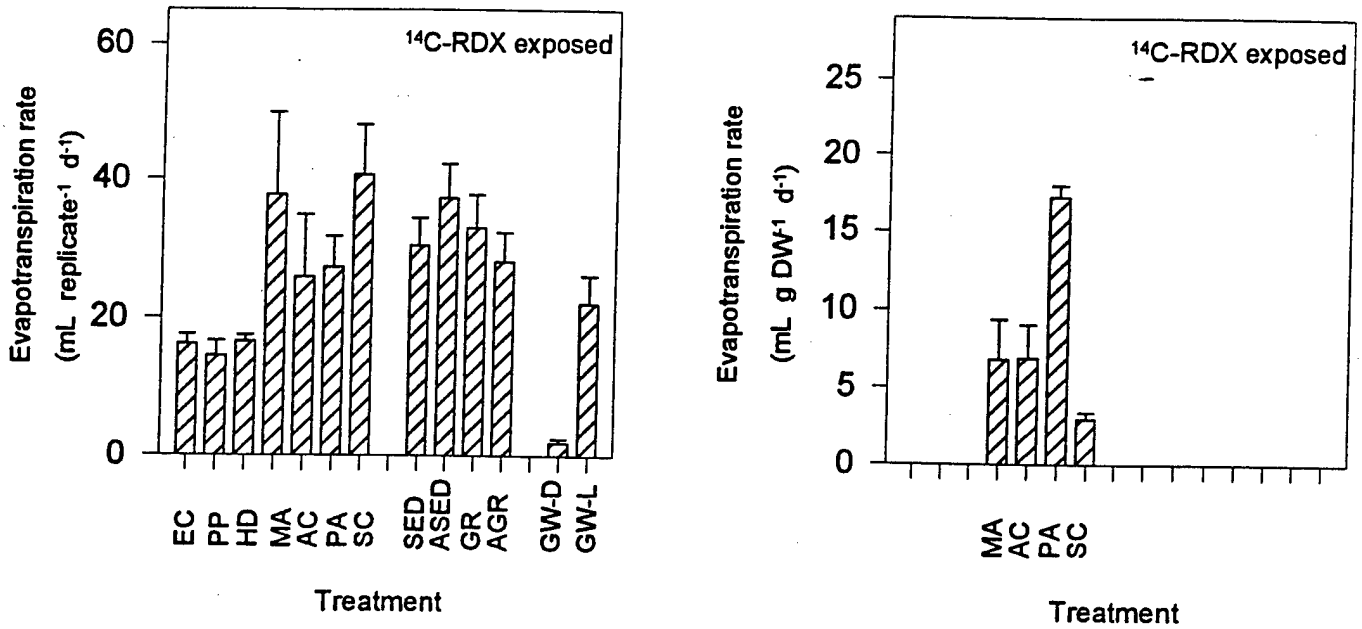


Figure 9. Relative growth rates of plants over 13-day incubation in [^{14}C]-RDX amended groundwater containing up to 1.53 mg RDX L^{-1}. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass.



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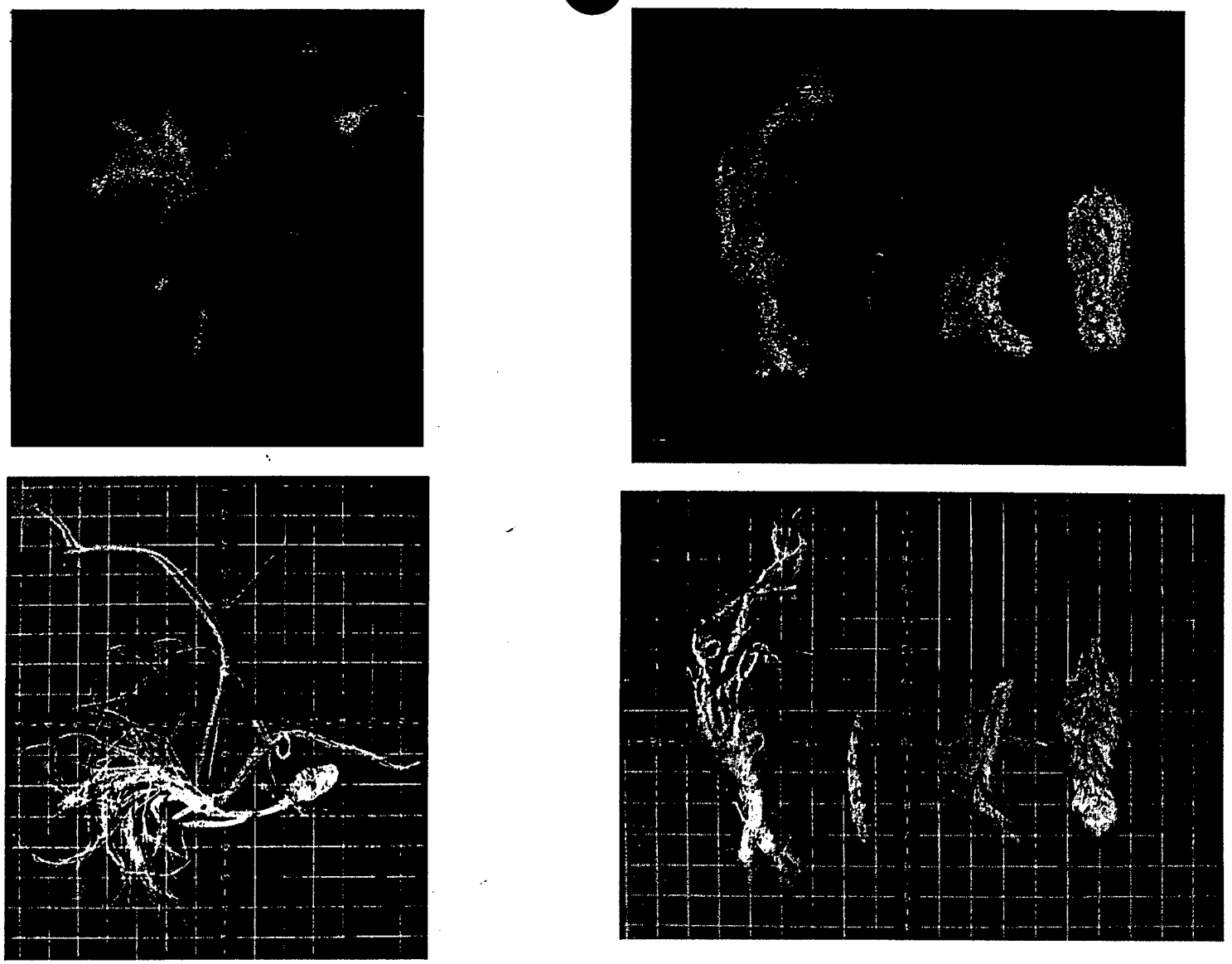

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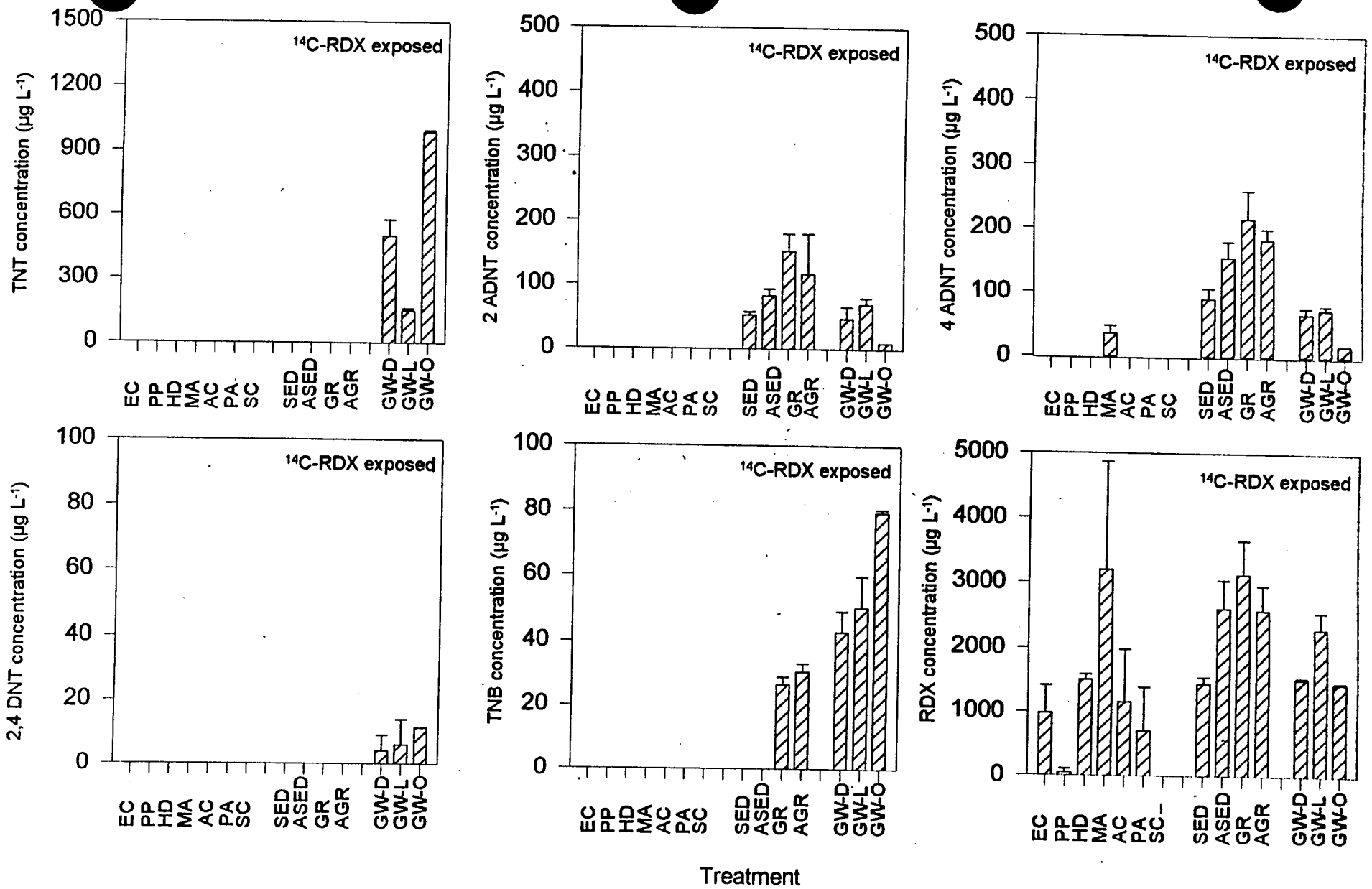


Figure 12. Explosives concentrations in [^{14}C]-RDX groundwater after 13-day incubation with plants, substrates, controls, and initially, as determined by HPLC analysis. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather; AC, sweet-flag; PA, reed canary grass; SC, wool-grass; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel; GW-D, groundwater darkened; GW-L, groundwater illuminated; GW-O, initial groundwater.

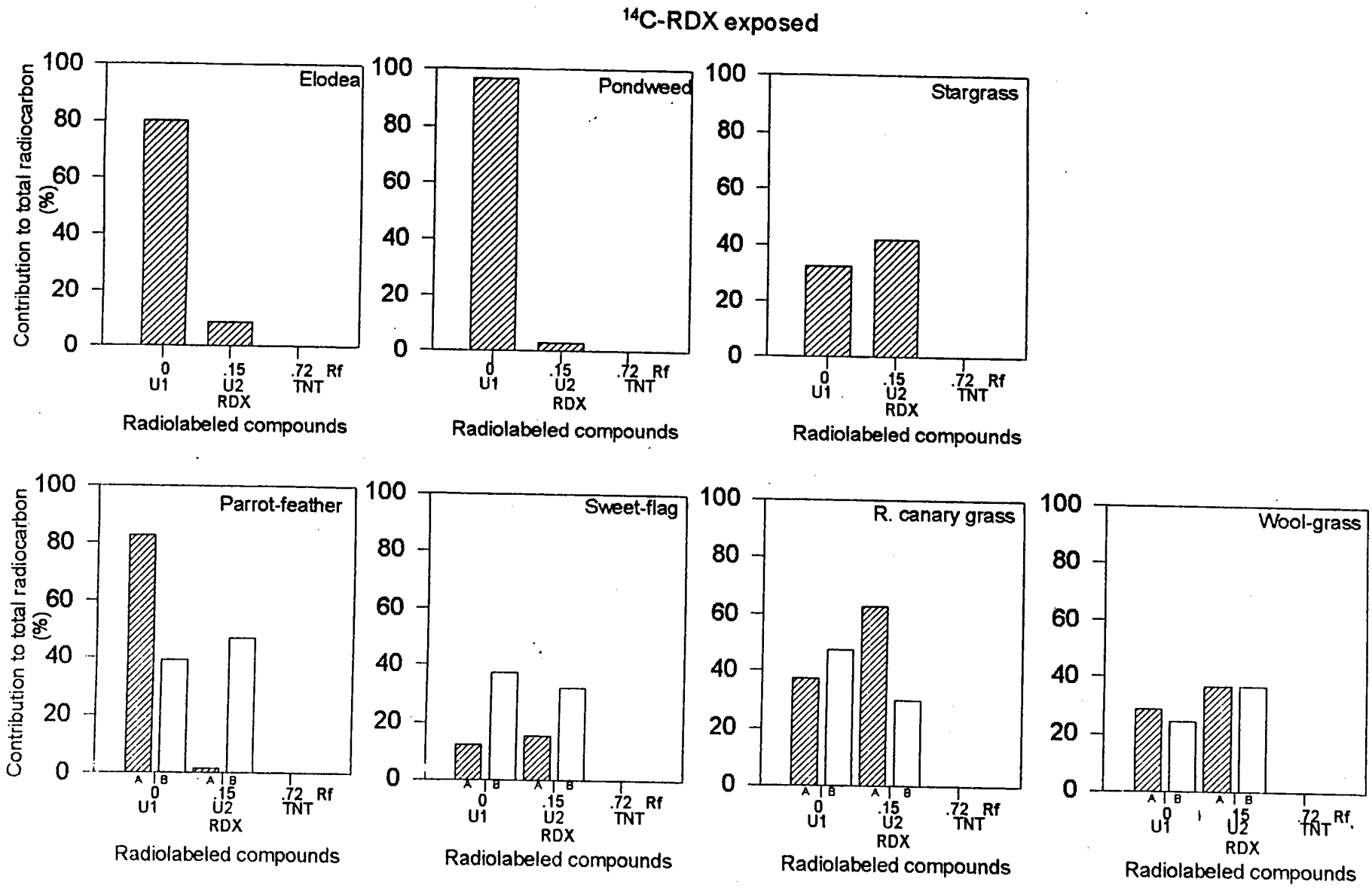
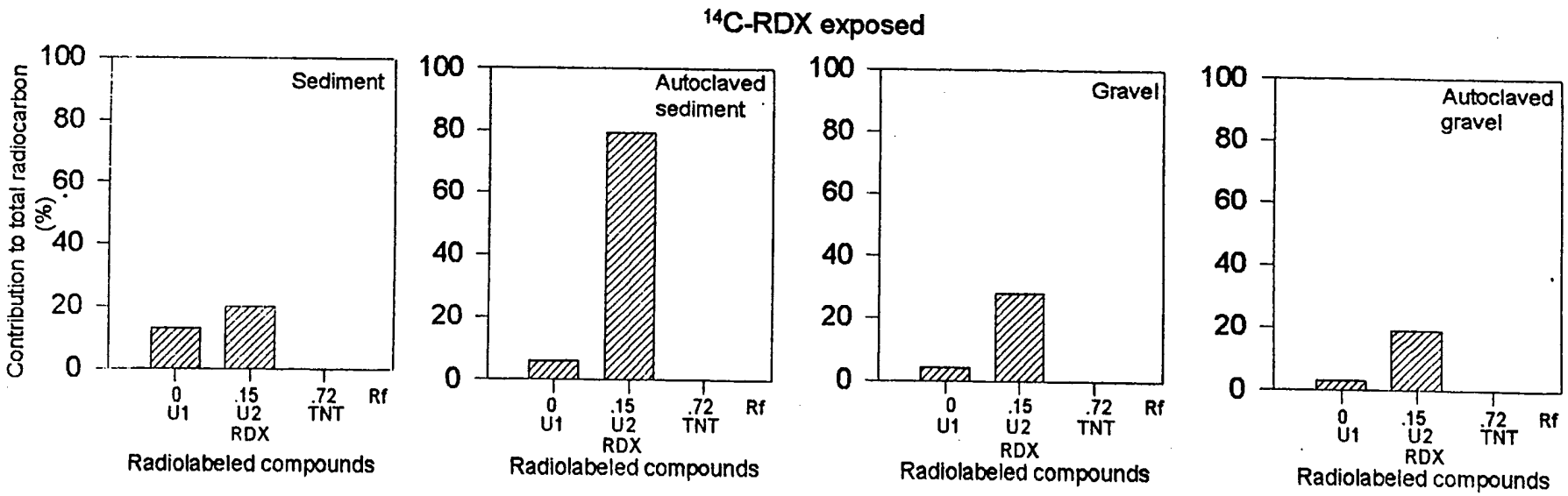


Figure 13. Distribution of RDX-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by thin layer chromatography of extracts and references on Silica Gel 60F plates in a toluene:methanol mixture (98:2), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Abbreviations: A, above-ground; B, below-ground; U, unknown.



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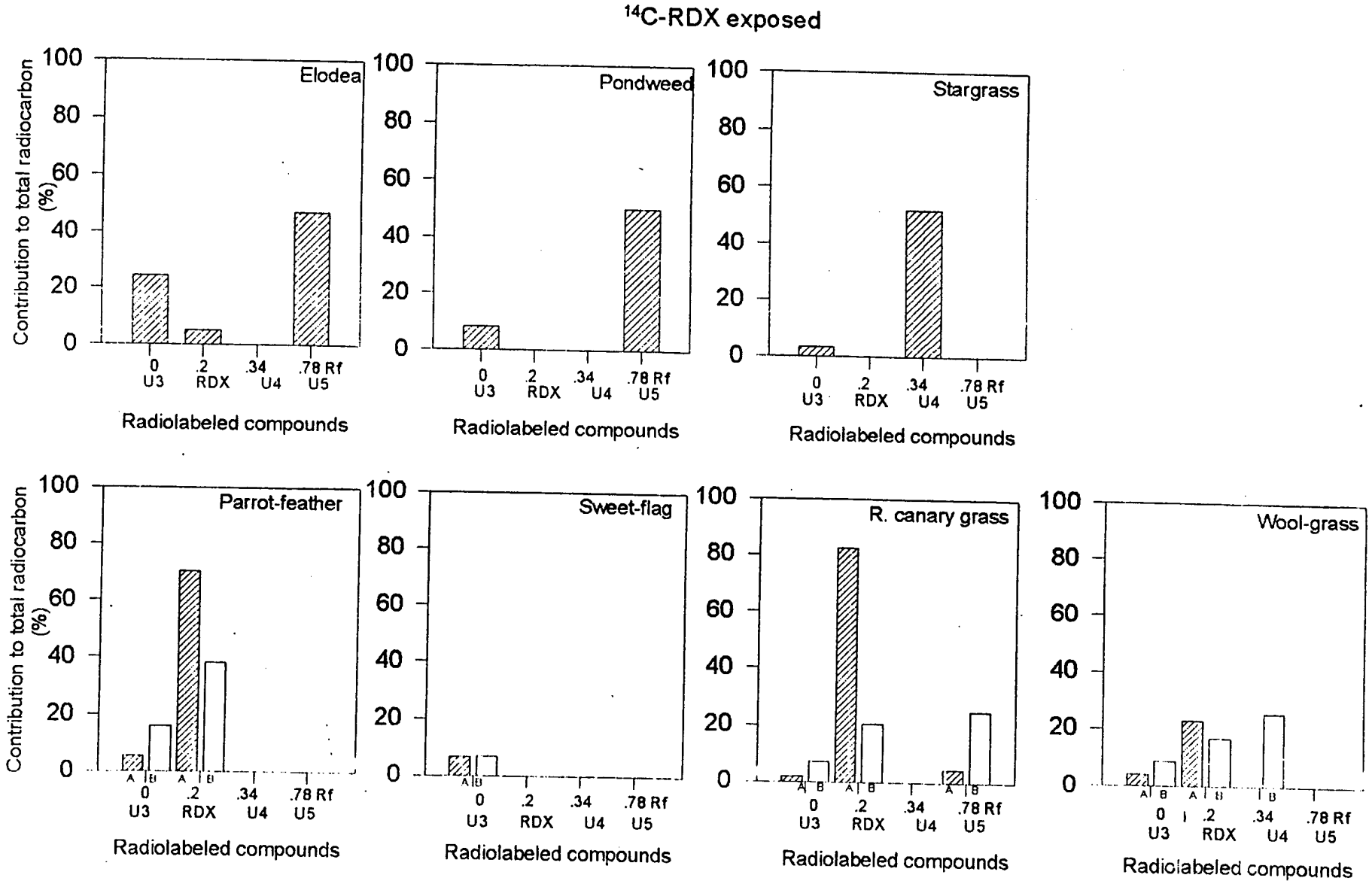


Figure 14. Distribution of RDX-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by thin layer chromatography of extracts and references on Whatman Reversed Phase LKC18F plates in a water:methanol mixture (50:50), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Abbreviations: A, above-ground; B, below-ground; U, unknown.

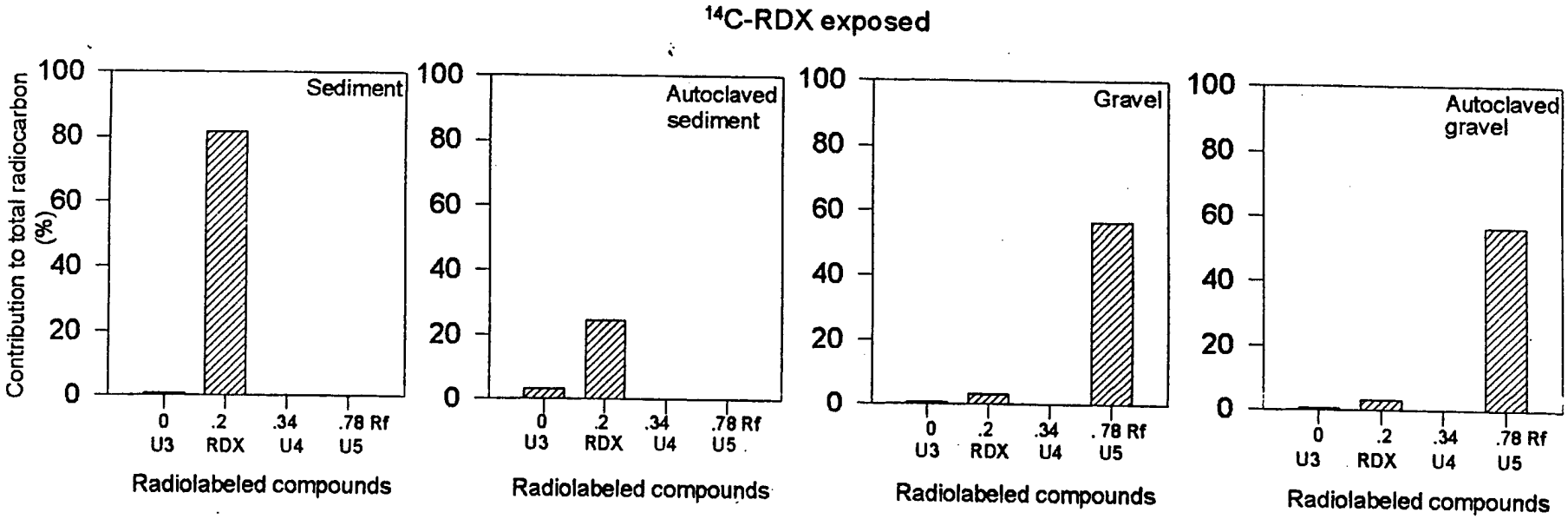
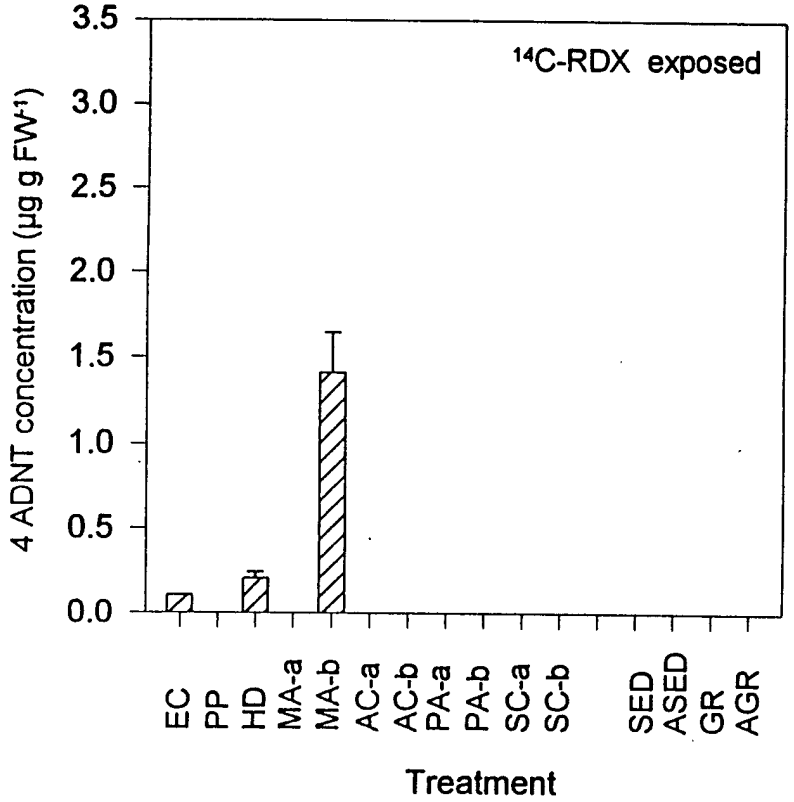


Figure 14- continued.



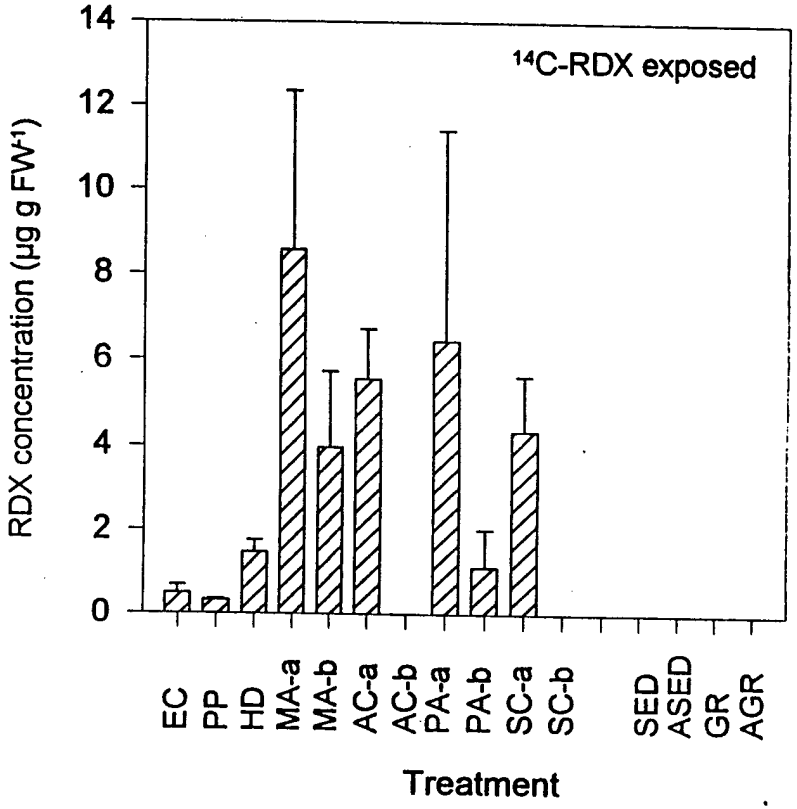


Figure 15. Explosives residues in plants and substrates after 13-day incubation in [^{14}C]-RDX groundwater, as determined by HPLC analysis. Mean values and standard deviations (N=3). Abbreviations: EC, elodea; PP, pondweed; HD, water-stargrass; MA, parrot-feather, -a above-ground and -b below-ground; AC, sweet-flag, -a above-ground and -b below-ground; PA, reed canary grass, -a above-ground and -b below-ground; SC, wool-grass, -a above-ground and -b below-ground; SED, sediment; ASED, autoclaved sediment; GR, gravel; AGR, autoclaved gravel.

Table 1. Aquatic and wetland plant species used to evaluate the behavior and fate of TNT and RDX in incubations with [^{14}C]-TNT or [^{14}C]-RDX amended groundwater from the Milan Army Ammunition Plant.

Group	Family	Plant species	
		Scientific name	Common name
Submersed			
Monocotyledons	Hydrocharitaceae	*Elodea canadensis* Rich. in Michx.	Elodea
	Potamogetonaceae	*Potamogeton pectinatus* L.	Sago pondweed
	Pontederiaceae	*Heteranthera dubia* (Jacq.) MacM.	Water star-grass
Emergent			
Dicotyledons	Haloragaceae	*Myriophyllum aquaticum* (Vell.) Verdc.	Parrot-feather
Monocotyledons	Araceae	*Acorus calamus* L.	Sweet-flag
	Gramineae	*Phalaris arundinacea* L.	Reed canary grass
	Cyperaceae	*Scirpus cyperinus* (L.) Kunth	Wool-grass

Note: U.S.Army Engineer Waterways Experiment Station, September-October 1996. Common names used in the text.

Table 2. Chemical characteristics of wetted Milan soil used in earlier screens for explosives removal (Best and Sprecher 1996). Soil of similar composition served as substrate in the Milan lagoons, from which the presently used sediment was sampled. Mean values ± s.d. (N=3).

Parameter	Concentration	Unit
Nitrogen	1.4659 ± 0.055	g kg DW^{-1}
Exchangeable NH_4-N	0.007 ± 0.000	g kg DW^{-1}
Phosphorus	0.447 ± 0.014	g kg DW^{-1}
Available PO_4-P	0.067 ± 0.002	g kg DW^{-1}
Bulk density	1.246 ± 0.009	g DW mL^{-1}
Moisture	269.1 ± 0.78	g H_2O kg FW^{-1}
Organic matter	39.6 ± 0.13	g kg DW^{-1}

Abbreviations: DW = dry weight; FW = fresh weight

Table 3. Chemical characteristics of the filtered groundwater from the Milan Army Ammunition Plant. pH and bicarbonate concentrations of this water were modified subsequent to these data before incubation. Mean values ± s.d. (N=3).

Characteristic	Value
pH	6.6 ± 0.1
Macro-, micro-nutrients (mg L^{-1})	
Alkalinity	20 ± 1
Kjeldahl-N	0.114 ± 0.161
NO_3-N	0.092 ± 0
NH_3-N	0.323 ± 0.009
Total-P	-
PO_4-P	0.0002 ± 0
SO_4	0.76 ± 0.03
Ca	4.7 ± 0.1
Fe	-
Explosives (µg L^{-1})	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	92.7 ± 1.2
2,6-Diamino-,4-nitro-toluene (2,6DANT)	-
2,4-Diamino-,6-nitrotoluene (2,4DANT)	-
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	1443.3 ± 17.0
1,3,5-Trinitro-benzene (TNB)	79.4 ± 1.0
1,3-Dinitro-benzene (1,3DNB)	-
2, 4, 6-Trinitrotoluene (TNT)	988.0 ± 9.1
2-Amino-dinitrotoluene (2ADNT)	9.3 ± 0
4-Amino-, 2, 6-dinitrotoluene (4ADNT)	18.2 ± 0.2
2,4-Dinitrotoluene (2,4DNT)	11.4 ± 0
2,6-Dinitrotoluene (2,6DNT)	-
2, 2', 6, 6-Tetranitro- 4, 4-azoxytoluene	-

Note: - Below detection.

Table 4. Experimental design and initial fresh weights (mean values ± s.d.; N=3) per incubation type. Incubations were explosives-contaminated MAAP groundwater: 1) amended with [^{14}C]-TNT for 7 days and re-dosed with [^{14}C]-TNT plus unlabeled TNT halfway; and 2) amended with [^{14}C]-RDX for 13 days. Incubated groundwater volume was 0.8 L.

Treatment	Incubator	Beakers and dimensions			Illumination	Initial fresh weight (g FW)	
		Number	Height (cm)	Diameter (cm)		[^{14}C]-TNT incubation	[^{14}C]-RDX incubation
Plant species							
Submersed							
Elodea	1	3	25	9	+	10.44 ± 0.23	13.47 ± 0.21
Sago pondweed	1	3	25	9	+	11.51 ± 0.66	23.30 ± 0.62
Water star-grass	1	3	25	9	+	11.39 ± 0.92	21.67 ± 1.49
Emergent							
Parrot-feather	1	3	19	12	±	23.50 ± 0.98	41.33 ± 3.13
Sweet-flag	1	3	19	12	±	131.60 ± 0.33	50.27 ± 2.81
Reed canary grass	1	3	19	12	±	67.90 ± 9.06	17.43 ± 2.21
Woolgrass	1	3	19	12	±	164.00 ± 11.11	92.03 ± 6.50
Substrates							
Sediment	1	3	19	8	+	26.45 ± 0	26.45 ± 0
Autoclaved sediment	1	3	19	8	+	26.45 ± 0	26.45 ± 0
Gravel	1	3	19	8	+	20.05 ± 0	20.05 ± 0
Autoclaved gravel	1	3	19	8	+	20.05 ± 0	20.05 ± 0
Controls							
Groundwater	1	3	19	8	-		
Groundwater	1	3	19	8	+		

Table 5. Explosives concentrations and quantities initially present in MAAP groundwater, and added as analyte or as radiolabel for incubation.

Timing	TNT Concentration Incubation Water ($\mu g\ L^{-1}$)	Volume Added (mL)	TNT Incubation Water (μg replicate^{-1})	Radiolabel Incubation Water (Bq replicate $^{-1}$)
[^{14}C]-TNT incubation				
1. Initial				
Groundwater	988	800	790	0
Added anal.-TNT	-	-	-	-
Added [^{14}C]-TNT		1	519	55.5×10^4
Total	1637		1309	55.5×10^4
2. At redosing				
Groundwater	NM*	NM	NM*	
Added anal.-TNT		1	2175	0
Added [^{14}C]-TNT		1	519	55.5×10^4
Total			2694	55.5×10^4
Overall exposure	5004		4003	111.0×10^4

Timing	RDX Concentration Incubation Water ($\mu g\ L^{-1}$)	Volume Added (mL)	RDX Incubation Water (μg replicate^{-1})	Radiolabel Incubation Water (Bq replicate $^{-1}$)
[^{14}C]-RDX incubation				
1. Initial				
Groundwater	1443	800	1154	0
Added [^{14}C]-RDX		1	69	55.5×10^4
Total exposure	1529		1223	55.5×10^4

Abbreviation: NM, not measured.
* Presumed to be below detection.

Table 6. Distribution of radioactivity over plant organs or portions after 7-day incubation in [^{14}C]-TNT groundwater, as indicated by radioanalytic imaging. Relative intensity of labeling expressed as +.

Plant species	Organ or portion			
	Root	Stem	Leaf	New shoot
Submersed				
Elodea			+	
Pondweed	++	+	++	
Water star-grass	+	++	+++	
	Root	Lower shoot	Upper shoot	Apical shoot
Emergent				
Parrot-feather	++	+		+
Sweet-flag	+++	+		+
R.canary grass	++	+		
Wool-grass	+++			

Tab[illegible] Initial+ redosed radioactivity and analyte TNT in [^{14}C]-TNT groundwater, and after 7-day incubation with plants, sub[illegible]es, or controls. Mean radioactivity, analyte TNT, and TNT-equivalent removal rates were calculated from these values. Radioactivity and analyte TNT: mean values and s.d. between parentheses (relative % of mean; N=3). TNT-equivalents: mean values.

Treatment	Initial + redosed		After 7-day incubation			Mean removal rate		
	DPM x 10^6 per repl.	mg TNT per repl.	DPM x 10^6 per repl.	^{14}C recovery (% total added)	mg TNT per repl.	DPM x 10^6 repl.$^{-1}$ d^{-1}	mg TNT repl.$^{-1}$ d^{-1}	TNT-equiv. mg repl.$^{-1}$ d^{-1}
Plant species								
Submersed								
Elodea	57.5 (14%)	4.003*	34.3 (14%)	52.0 (7%)	-	3.3 (14%)	---	0.230
Pondweed	70.5 (10%)	4.003*	35.0 (4%)	53.0 (2%)	-	5.1 (18%)	---	0.290
Water star-grass	68.5 (0%)	4.003*	33.5 (9%)	50.8 (4%)	-	4.7 (4%)	---	0.275
Emergent								
Parrot-feather	70.3 (2%)	4.003*	29.5 (10%)	44.7 (4%)	-	5.8 (6%)	---	0.330
Sweet-flag	71.3 (1%)	4.003*	5.8 (19%)	8.8 (2%)	-	9.4 (2%)	---	0.528
R.canary grass	67.3 (2%)	4.003*	5.6 (26%)	8.5 (2%)	-	8.8 (1%)	---	0.523
Wool-grass	64.4 (2%)	4.003*	4.6 (39%)	7.0 (2%)	-	9.3 (5%)	---	0.578
Substrates								
Sediment	70.7 (0%)	4.003*	39.8 (6%)	60.3 (3%)	0.140 (38%)	4.4 (7%)	0.552 (1%)	0.249
Au[illegible] sediment	71.3 (2%)	4.003*	42.3 (5%)	64.1 (3%)	0.645 (23%)	4.1 (2%)	0.480 (3%)	0.230
G[illegible]	72.7 (3%)	4.003*	47.4 (3%)	71.8 (2%)	0.021 (29%)	3.6 (11%)	0.569 (0%)	0.198
Au[illegible].gravel	70.5 (2%)	4.003*	49.7 (3%)	75.3 (2%)	0.813 (8%)	3.0 (13%)	0.456 (1%)	0.170
Controls								
Groundwater/D	65.0 (1%)	4.003*	68.0 (1%)	103.0 (1%)	3.127 (10%)	NA	0.125 (16%)	NA
Groundwater/L	68.7 (3%)	4.003*	66.8 (6%)	101.2 (6%)	2.408 (6%)	NA	0.228 (6%)	NA

Abbreviations: repl., replicate; D dark; L light; NA, not applicable.
*, Initial concentration 1.637 mg TNT L^{-1}; redosed with 3.367 mg TNT L^{-1} after 3 days; initial incubation volume 0.8 L.
-, below detection.
---, Removal was 100% at the end of incubation; therefore per day rate not calculated.

Table 8. Removal rates of radioactivity and TNT-equivalents from [^{14}C]-TNT groundwater over 7-day incubation with plants, substrates, or controls. Removal rates on basis of initial mass. Radioactivity: mean values and s.d. between parentheses (relative % of mean; N=3).TNT-equivalents: mean values.

Treatment	Removal rate			
	DPM x 10^6 g total DW^{-1} d^{-1}	TNT-equiv. mg g total DW^{-1} d^{-1}	DPM x 10^6 g below-gr.DW^{-1} d^{-1}	TNT-equiv. mg g below-gr.DW^{-1} d^{-1}
Plant species				
Submersed				
Elodea	4.985 (14%)	0.347		
Pondweed	5.549 (21%)	0.315		
Water star-grass	8.785 (11%)	0.513		
Emergent				
Parrot-feather	1.150 (2%)	0.065	3.154 (2%)	0.180
Sweet-flag	0.309 (2%)	0.056	0.454 (2%)	0.025
Reed canary grass	0.617 (14%)	0.059	1.065 (14%)	0.063
Wool-grass	0.199 (4%)	0.012	0.415 (4%)	0.026
Substrates				
Sediment	0.266 (7%)	0.015		
Autoclaved sediment	0.250 (2%)	0.014		
Gravel	0.191 (11%)	0.011		
Autoclaved gravel	0.156 (13%)	0.009		
Controls				
Groundwater/Dark	NA	NA		
Groundwater/Light	NA	NA		

Abbreviations: below-gr., below-ground; NA, not applicable

Table 9. Mass balances for [^{14}C]-TNT-derived radioactivity in 7-day incubations of groundwater with plants, substrates, or controls. Radioactivity in plants and substrates determined by combustion. Compartment contributions in percent of total radioactivity added (mean values and s.d. between parentheses; N=3).

Treatment	Groundwater		Aerial CO_2-C*	Volatile organic C*	Carbon in plants or substrates	Recovery
	Total C	[(HCO_3^- + CO_2)-C]				
Plant species						
Submersed						
Elodea	51.97 (7)	[5.27 (4)]	0.09	0.01	23.91 (4)	75.98
Pondweed	52.96 (2)	[0]	0.07	0.01	58.39 (5)	111.43
Water star-grass	50.77 (4)	[2.09 (1)]	0.07	0	79.46 (19)	130.30
Emergent						
Parrot-feather	44.68 (4)	[0]	0.04	0.01	73.13 (19)	117.86
Sweet-flag	8.80 (2)	[0.45 (0)]	0.15	0.01	83.39 (14)	92.35
R.canary grass	8.43 (2)	[0.87 (0)]	0.07	0	51.23 (41)	59.73
Wool-grass	7.00 (3)	[0.05 (0)]	0.06	0	87.36 (5)	94.42
Substrates						
Sediment	60.30 (3)	[5.15 (2)]	0.14	0.09	35.42 (14)	96.95
Autocl.sediment	63.94 (3)	[4.16 (6)]	0.11	0.13	39.80 (34)	103.98
Gravel	71.89 (2)	[10.06 (5)]	0.13	0.06	28.18 (3)	100.26
Autocl.gravel	75.35 (2)	[5.81 (3)]	0.08	0.13	21.98 (3)	97.54
Controls						
Groundwater/D	103.07 (1)	[0]	0.04	0		103.11
Groundwater/L	101.26 (6)	[4.70 (5)]	0.05	0.16		101.47

Abbreviations: D dark; L light; conc., concentration.
*, Single value. **, S.d. between parentheses (relative % of mean; N=3). Condensation was always <0.01%.
[ ], not included in balance.

Table 10. Distribution of radioactivity in plants or substrates after 7-day incubation in [^{14}C]-TNT groundwater. Total radioactivity was determined by combustion, extractable radioactivity by extraction with acetonitrile and LS. Radioactivity per g and total radioactivity in mass: mean values in DPM x 10^6 and s.d. between parentheses (relative % of mean; N=3). Final mass: mean values ± s.d. (N=3). Extractable radioactivity, in 1) mean values in DPM x 10^6 and s.d. between parentheses (relative % of mean; N=3), and 2) relative as % of total radioactivity in mass determined by combustion and LS (mean values ± s.d.; N=3).

Species or substrates	Radioactivity per g (DPM x 10^6 g FW^{-1})	Final plant or substrate mass (g FW)	Total radioactivity in mass (DPM x 10^6)	Extractable radioactivity from plants or substrates	
				(DPM x 10^6 g FW^{-1})	(% total radio-activity mass)
Plant species					
Submersed					
Elodea	1.83 (24%)	8.36 ± 0.77	15.78 (18%)	0.309 (28%)	18.04 ± 6.15
Pondweed	2.88 (9%)	8.02 ± 0.02	38.54 (9%)	0.473 (13%)	16.68 ± 3.19
Water star-grass	2.78 (9%)	11.22 ± 1.62	52.45 (24%)	0.685 (7%)	24.91 ± 2.18
Emergent					
Parrot-feather/a	0.03 (25%)	36.97 ± 6.18	0.80 (30%)	0.006 (20%)	21.67 ± 1.69
---------- /b	1.61 (34%)	19.37 ± 3.71	47.47 (26%)	0.136 (26%)	8.79 ± 1.93
Sweet-flag/a	0.02 (78%)	48.90 ± 6.21	0.82 (88%)	0.003 (59%)	22.92 ± 4.09
---------- /b	0.72 (30%)	82.83 ± 11.11	54.22 (16%)	0.114 (53%)	15.04 ± 3.40
R.canary grass/a	0.01 (57%)	11.63 ± 8.35	0.18 (67%)	0.001 (19%)	17.73 ± 10.47
----------/b	0.89 (96%)	31.33 ± 23.27	33.62 (81%)	0.143 (36%)	33.24 ± 20.00
Wool-grass/a	0.01 (8%)	49.03 ± 2.46	0.29 (12%)	0.001 (28%)	12.82 ± 3.18
----------/b	0.75 (29%)	90.60 ± 14.20	95.04 (14%)	0.065 (41%)	8.37 ± 1.76
Substrates					
Sediment	0.42 (40%)	60.26 ± 5.63	23.38 (42%)	0.042 (34%)	9.92 ± 2.14
Autocl. sediment	0.36 (83%)	79.50 ± 6.94	26.27 (88%)	0.588 (44%)	464 ± 428
Gravel	0.26 (%)	70.76 ± 1.31	18.60 (11%)	0.057 (10%)	22.64 ± 4.68
Autocl. gravel	0.22 (%)	65.00 ± 0.75	14.51 (12%)	0.378 (19%)	176 ± 31

Abbreviations: /a, above-ground; /b, below-ground.

Table 11. Distribution of [^{14}C]-TNT-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by TLC of extracts and references on Silica Gel 60F plates in a toluene:methanol mixture (98:2), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Recovery: radioactivity counted in spots relative to that counted per lane, as percent.

Species or substrates	Rf-value							Reco -very (%)	Radio-label lane^{-1} (counts)	Front (cm)
	0 U1	0.09 U2	0.19 ADNTs	0.62 TNT	0.78 U3	0.91 U4	0.93 U5			
Plant species										
Submersed										
Elodea	84.3		3.4					87.7	18536	16.2
Pondweed	84.2		4.2					88.4	28482	16.2
Water star-grass	68.7				4.9			73.6	13066	16.2
Emergent										
Parrot-feather/a	30.8							30.8	1414	16.2
----------/b	82.6		6.6					89.2	12231	16.2
Sweet-flag/a	39.7							39.7	1515	16.2
----------/b	83.1							83.1	8590	16.2
R.canary grass/a	14.1							14.1	1755	16.2
----------/b	46.7		7.1	4.9			29.0	87.7	19829	16.2
Wool-grass/a	9.6					21.9		31.5	1478	16.2
----------/b	76.0							76.0	6472	16.2
Substrates										
Sediment	48.0							48.0	2977	16.2
Autocl.sediment	53.7	2.7	15.7	11.0				83.1	12755	16.2
Gravel	53.1	6.8	7.8	8.7				76.4	8583	16.2
Autocl.gravel	11.0	7.7		17.2				35.9	8380	16.2

Abbreviations: /a, above-ground; /b, below-ground; U, unknown.

Table 12. Distribution of radioactivity over plant organs or portions after 13-day incubation in [^{14}C]-RDX-labeled groundwater, as indicated by radioanalytic imaging. Relative intensity of labeling expressed as +.

Plant species	Organ or portion			
	Root	Stem	Leaf	New shoot
Submersed				
Elodea				+++
Pondweed	++	+	+	+++
Water star-grass	+	+	+	
	Root	Lower shoot	Upper shoot	Apical shoot
Emergent				
Parrot-feather	+	+	++	+++
Sweet-flag	+			++
R.canary grass	++	+		+++
Wool-grass	+	+		++

Table 3. Initial radioactivity and analyte RDX in [^{14}C]-RDX groundwater, and after 13-day incubation with plans, substrates, or controls. Mean radioactivity, analyte RDX, and RDX-equivalent removal rates were calculated from these values. Radioactivity and analyte-RDX: mean values and s.d. between parentheses (relative % of mean; N=3). RDX-equivalents: mean values.

Treatment	Initial		After 13-day incubation			Mean removal rate		
	DPM x 10^6 per repl.	mg RDX per repl.	DPM x 10^6 per repl.	^{14}C recovery (% total added)	mg RDX per repl.	DPM x 10^6 repl.$^{-1}$ d^{-1}	mg RDX repl.$^{-1}$ d^{-1}	RDX-equiv. mg repl.$^{-1}$ d^{-1}
Plant species								
Submersed								
Elodea	33.9 (0%)	1.223*	20.8 (12%)	63.0 (7%)	0.568 (42%)	1.0 (19%)	0.050 (37%)	0.036
Pondweed	33.8 (1%)	1.223*	7.2 (43%)	21.8 (9%)	0.024 (141%)	2.0 (14%)	0.092 (3%)	0.072
Water star-grass	33.5 (2%)	1.223*	24.7 (7%)	74.8 (5%)	0.875 (6%)	0.7 (12%)	0.027 (15%)	0.026
Emergent								
Parrot-feather	34.8 (6%)	1.223*	20.9 (25%)	63.3 (6%)	0.725 (25%)	1.1 (45%)	0.038 (37%)	0.039
Sweet-flag	35.8 (1%)	1.223*	16.7 (41%)	50.6 (21%)	0.455 (73%)	1.5 (37%)	0.059 (44%)	0.051
R.canary grass	35.9 (1%)	1.223*	18.7 (19%)	56.7 (1%)	0.319 (93%)	1.3 (22%)	0.070 (33%)	0.044
Wool-grass	39.2 (11%)	1.223*	3.7 (42%)	11.2 (4%)	-	2.7 (11%)	0.094 (0%)	0.084
Substrates								
Sediment	34.4 (1%)	1.223*	22.5 (8%)	68.2 (5%)	0.583 (8%)	0.9 (17%)	0.049 (8%)	0.032
Autocl.sediment	34.2 (0%)	1.223*	24.0 (6%)	72.7 (5%)	0.798 (5%)	0.8 (16%)	0.032 (10%)	0.029
Gravel	34.3 (0%)	1.223*	31.5 (0%)	95.5 (0%)	1.131 (1%)	0.2 (5%)	0.007 (18%)	0.007
Autocl.gravel	34.2 (0%)	1.223*	31.1 (0%)	94.2 (0%)	1.104 (1%)	0.2 (3%)	0.008 (8%)	0.007
Controls								
Groundwater/D	33.1 (5%)	1.223*	33.8 (5%)	102.4 (5%)	1.172 (0%)	NA	0.004 (10%)	NA
Groundwater/L	33.6 (1%)	1.223*	34.5 (0%)	104.5 (0%)	1.165 (1%)	NA	0.004 (16%)	NA

Abbreviations: repl., replicate; D dark; L light; NA, not applicable.
*, Initial concentration 1.529 mg RDX L^{-1}; initial incubation volume 0.8 L. -, below detection;

e 14. Removal rates of radioactivity, analyte RDX, and RDX-equivalents from [^{14}C]-RDX groundwater over 13-day bation with plants, substrates, or controls. Removal rates on basis of initial mass. Radioactivity and analyte-RDX: mean values and s.d. between parentheses (relative % of mean; N=3). RDX-equivalents: mean values.

Treatment	Removal rate					
	DPM x 10^6 g tot.DW^{-1} d^{-1}	mg RDX g tot.DW^{-1} d^{-1}	RDX-equiv. mg g tot.DW^{-1}d^{-1}	DPM x 10^6. g below-gr. DW^{-1} d^{-1}	mg RDX g below-gr. DW^{-1} d^{-1}	RDX-equiv. mg g below-gr. DW^{-1}d^{-1}
Plant species						
Submersed						
Elodea	1.175 (19%)	0.058 (36%)	0.042			
Pondweed	0.794 (12%)	0.035 (2%)	0.029			
Water star-grass	0.491 (7%)	0.019 (9%)	0.018			
Emergent						
Parrot-feather	0.123 (50%)	0.004 (43%)	0.004	0.338 (50%)	0.012 (43%)	0.012
Sweet-flag	0.130 (43%)	0.005 (50%)	0.004	0.192 (43%)	0.008 (50%)	0.007
R.canary grass	0.359 (23%)	0.019 (36%)	0.012	0.619 (23%)	0.032 (36%)	0.021
Wool-grass	0.104 (5%)	0.004 (7%)	0.002	0.217 (5%)	0.007 (7%)	0.007
Substrates						
Sediment	0.055 (17%)	0.003 (8%)	0			
l. sediment	0.047 (16%)	0.002 (10%)	0			
el	0.011 (5%)	0.0003 (17%)	0			
Autocl. gravel	0.013 (3%)	0.00004 (84%)	0			
Controls						
Groundwater/D	NA	NA				
Groundwater/L	NA	NA				

Abbreviations: below-gr., below-ground; tot., total; D dark; L light; NA, not applicable

Table 15. Mass balances for [^{14}C]-RDX-derived radioactivity in 13-day incubations of groundwater with plants, substrates, or controls. Radioactivity in plants and substrates determined by combustion. Compartment contributions in percent of total radioactivity added (mean values and s.d. between parentheses; N=3.

Treatment	Groundwater		Aerial CO_2-C*	Volatile organic C*	Carbon in plants or substrates	Recovery
	Total C	[(HCO_3^- + CO_2)-C]				
Plant species						
Submersed						
Elodea	63.04 (7)	[1.27 (0)]	0.70	0.01	47.83 (8)	111.58
Pondweed	21.86 (9)	[4.19 (1)]	2.76	0.01	57.98 (13)	82.61
Water star-grass	74.76 (5)	[1.01 (1)]	0.97	0	18.31 (3)	94.04
Emergent						
Parrot-feather	63.25 (16)	[0.32 (0)]	1.02	0.01	20.52 (10)	84.80
Sweet-flag	50.47 (21)	[0.87 (1)]	4.06	0.01	20.60 (4)	75.14
R.canary grass	56.64 (11)	[1.30 (1)]	5.05	0.03	21.36 (7)	83.08
Wool-grass	11.22 (4)	[0.37 (0)]	10.17	0.03	35.01 (11)	56.43
Substrates						
Sediment	68.19 (5)	[7.94 (1)]	2.68	0.08	3.40 (2)	74.35
Autocl.sediment	72.66 (5)	[0.99 (0)]	1.03	0.11	3.45 (2)	77.25
Gravel	95.48 (0)	[1.61 (2)]	0.55	0.03	1.63 (0)	97.69
Autocl.gravel	94.24 (0)	[0]	0.91	0.05	1.60 (0)	96.80
Controls						
Groundwater/D	103.65 (1)	[0.59 (1)]	0.30	0		103.95
Groundwater/L	104.65 (0)	[0]	0.80	0.02		105.47

Abbreviations: D dark; L light; conc., concentration.
* Single value. ** S.d. between parentheses (relative % of mean; N=3). Condensation was always <0.01%.
[ ], not included in balance.

Table 16. Distribution of radioactivity in plants or substrates after 13-day incubation in [^{14}C]-RDX groundwater. Total radioactivity was determined by combustion, extractable radioactivity by extraction with acetonitrile and LS. Radioactivity per g and total radioactivity in mass: mean values in DPM x 10^6 and s.d. between parentheses (relative % of mean; N=3). Final mass: mean values ± s.d. (N=3). Extractable radioactivity, in 1) mean values in DPM x 10^6 and s.d. between parentheses (relative % of mean; N=3), and 2) relative as % of total radioactivity in mass determined by combustion (mean values ± s.d.; N=3).

Species or substrates	Radioactivity per g (DPM x 10^6 g FW^{-1})	Final plant or substrate mass (g FW)	Total radioactivity in mass (DPM x 10^6)	Extractable radioactivity from plants or substrates	
				(DPM x 10^6 g FW^{-1})	(% total radio-activity mass)
Plant species					
Submersed					
Elodea	0.419 (24%)	17.75 ± 0.53	8.123 (21%)	0.100 (16%)	24.47 ± 3.26
Pondweed	0.628 (21%)	28.09 ± 0.82	19.135 (23%)	0.083 (3%)	13.96 ± 3.19
Water star-grass	0.246 (12%)	23.71 ± 2.12	6.041 (18%)	0.072 (10%)	29.58 ± 2.01
Emergent					
Parrot-feather/a	0.383 (49%)	21.16 ± 1.55	5.506 (54%)	0.239 (56%)	59.94 ± 5.19
---------- /b	0.199 (37%)	8.78 ± 0.88	1.268 (31%)	0.122 (38%)	60.93 ± 4.88
Sweet-flag/a	0.211 (40%)	11.83 ± 2.36	1.562 (48%)	0.025 (60%)	10.55 ± 3.88
---------- /b	0.138 (12%)	35.33 ± 0.42	5.237 (13%)	0.050 (32%)	35.23 ± 7.67
R.canary grass/a	0.721 (12%)	5.50 ± 2.24	3.204 (41%)	0.205 (19%)	28.15 ± 1.95
----------/b	0.231 (13%)	15.74 ± 2.92	3.845 (27%)	0.059 (25%)	26.90 ± 10.62
Wool-grass/a	0.341 (52%)	30.8 ± 3.83	8.797 (54%)	0.051 (34%)	16.82 ± 6.14
----------/b	0.106 (19%)	52.07 ± 2.58	7.275 (16%)	0.022 (29%)	20.70 ± 2.70
Substrates					
Sediment	0.017 (53%)	76.97 ± 5.12	1.124 (48%)	0.003 (47%)	24.79 ± 11.55
Autoclaved sediment	0.017 (51%)	72.93 ± 7.07	1.139 (61%)	0.011 (72%)	59.30 ± 13.62
Gravel	0.008 (4%)	63.60 ± 2.38	0.538 (10%)	0.005 (10%)	61.76 ± 10.26
Autoclaved gravel	0.008 (15%)	65.93 ± 1.20	0.527 (16%)	0.004 (6%)	57.69 ± 8.39

Abbreviations: /a, above-ground; /b., below-ground

Table 17. Distribution of [^{14}C]-RDX-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by TLC of extracts and references on Silica Gel 60F plates in a toluene:methanol mixture (98:2), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Recovery: radioactivity counted in spots relative to that counted per lane, as percent.

Species or substrates	Rf-value			Recovery (%)	Radio-label lane^{-1} (counts)	Front (cm)
	0 U1	0.15 U2/RDX	0.72 TNT			
Plant species						
Submersed						
Elodea	80.3	8.7		89.0	10095	17.0
Pondweed	96.7	3.0		99.7	10359	17.0
Water star-grass	32.6	41.9		74.5	6354	17.0
Emergent						
Parrot-feather/a	82.6	1.5		84.1	6166	17.0
----------/b	38.9	46.8		85.7	8431	17.0
Sweet-flag/a	12.3	15.3		27.6	1425	17.0
----------/b	37.1	32.1		69.2	5486	17.0
R.canary grass/a	37.2	62.8		100.0	40903	17.0
----------/b	47.1	29.8		76.9	5381	17.0
Wool-grass/a	28.6	36.7		65.3	3704	17.0
----------/b	24.5	36.7		61.2	3422	17.0
Substrates						
Sediment	12.8	19.9		32.7	1429	17.0
Autocl.sediment	5.9	79.5		85.4	8304	17.0
Gravel	4.2	28.0		32.2	1389	17.0
Autocl.gravel	3.0	19.1		22.1	1784	17.0

Abbreviations: /a, above-ground; /b, below-ground; Unknown.

Table 18. Distribution of [^{14}C]-RDX-derived radioactivity over acetonitrile extracted compounds from plants and substrates, expressed as percent of total counts per TLC lane. Separation by TLC of extracts and references on Whatman Reversed Phase LKC18F plates in a water:methanol mixture (50:50), using the Ambis Radioanalytic Imaging System for measuring radioactivity. Recovery: radioactivity counted in spots relative to that counted per lane, as percent.

Species or substrates	Rf-value				Recovery (%)	Radio-label lane^{-1} (counts)	Front (cm)
	0 U3	0.20 RDX	0.34 U4	0.78 U5			
Plant species							
Submersed							
Elodea	24.3	5.2		47.1	76.6	8403	10.2
Pondweed	8.5			50.2	58.7	5005	10.2
Water star-grass	3.5		52.1		55.6	4920	10.2
Emergent							
Parrot-feather/a	5.3	70.4			75.7	11930	10.2
----------/b	15.8	38.0			53.8	7175	10.2
Sweet-flag/a	6.4				6.4	1413	10.2
----------/b	6.7				6.7	2727	10.2
R.canary grass/a	1.9	82.4		4.4	88.7	30184	10.2
----------/b	7.0	20.4		25.0	52.4	5544	10.2
Wool-grass/a	3.9	22.8			26.7	3316	10.2
----------/b	8.4	16.9	25.7		51.0	3267	10.2
Substrates							
Sediment	20.5				20.5	1390	10.2
Autocl.sediment	0.6	81.6			82.2	7711	10.2
Gravel	3.2	24.6			27.8	1103	10.2
Autocl.gravel	0.7	3.2		56.6	60.4	6582	10.2

Abbreviations: /a, above-ground; /b, below-ground; U, unknown.

Appendix A
Chemical Characteristics Milan Army Ammunition Plant Groundwater Before Filtration

Table. Chemical characteristics of unfiltered and untreated groundwater from the Milan Army Ammunition Plant (Well M-146) measured on 11 September 1996 by the Tennessee Valley Authority.

Characteristic	Value
pH	5.8
Macro-, micronutrients (mg L^{-1})	
Kjeldahl-N	0.37
NO_3-N	6.0
NH_3-N	0.32
PO_4-P	0.01
Ca	4.5
Fe	0.03
Explosives (µg L^{-1})	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	106
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	1980
1,3,5-Trinitro-benzene (TNB)	109
2, 4, 6-Trinitrotoluene (TNT)	1359
2-Amino-dinitrotoluene (2ADNT)	-
4-Amino-, 2, 6-dinitrotoluene (4ADNT)	21.3
2,4-Dinitrotoluene (2,4DNT)	23

-, Below detection

Appendix B
Tables Supporting Figures

Appendix B- Table 1. Relative growth rates of plants over 7-day incubation in [^{14}C]-TNT groundwater. Mean values ± s.d. (N=3).

Plant species	Relative growth rate (g DW g DW^{-1} d^{-1})
Submersed	
Elodea	-0.0323 ± 0.0123
Pondweed	-0.0514 ± 0.0079
Water star-grass	0.0074 ± 0.0280
Emergent	
Parrot-feather	0.1516 ± 0.0152
Sweet-flag	0.0003 ± 0.0077
Reed canary grass	-0.0001 ± 0.0108
Wool-grass	-0.0237 ± 0.0027

Appendix B - Table 2. Evapotranspiration rates in [^{14}C]-TNT groundwater over 7-day incubation with plants, substrates, or controls. Mean values ± s.d. (N=3). Initial incubation volume 0.8 L.

Treatment	Evapotranspiration rate	
	mL replicate^{-1} d^{-1}	mL g above-ground DW^{-1} d^{-1}
Plant species		
Submersed		
Elodea	13.81 ± 0.67	
Pondweed	11.90 ± 2.69	
Water star-grass	12.38 ± 2.36	
Emergent		
Parrot-feather	36.90 ± 7.38	11.38 ± 16.08
Sweet-flag	55.48 ± 0.89	5.72 ± 0.08
Reed canary grass	39.29 ± 2.10	6.48 ± 0.57
Wool-grass	55.00 ± 5.25	2.27 ± 0.11
Substrates		
Sediment	28.57 ± 0.58	
Autoclaved sediment	31.67 ± 3.97	
Gravel	29.76 ± 5.29	
Autoclaved gravel	28.57 ± 5.18	
Controls		
Groundwater/Dark	2.38 ± 0.34	
Groundwater/Light	21.62 ± 2.15	

Appendix B - Table 3. Explosives concentrations in [^{14}C]-TNT groundwater, initially and after 7-day incubation with plants, substrates, or controls. 24DNT (11 μg L^{-1}) and TNB (79 μg L^{-1}) were only present in the initial groundwater. Mean values ± s.d. (N=3).

Treatment	Explosives concentration (μg L^{-1})					
	TNT	2ADNT	4ADNT	24DANT	26DANT	RDX
Initial						
Groundwater	988* ± 9	9 ± 0	18 ± 0	-	-	1443 ± 17
Plant species						
Submersed						
Elodea	-	-	37 ± 2	1074 ± 206	74 ± 10	-
Pondweed	-	-	58 ± 45	478 ± 31	37 ± 5	-
Water star-grass	-	103 ± 12	572 ± 51	134 ± 142	26 ± 29	1296 ± 143
Emergent						
Parrot-feather	-	-	257 ± 44	-	-	1906 ± 132
Sweet-flag	-	-	196 ± 78	-	-	2220 ± 370
R.canary grass	-	-	30 ± 13	-	-	-
Wool-grass	-	-	89 ± 50	-	-	339 ± 74
Substrates						
Sediment	234 ± 88	400 ± 42	542 ± 40	72 ± 33	20 ± 4	1346 ± 69
Autocl.sediment	1130 ± 317	289 ± 31	382 ± 20	-	-	1456 ± 76
Gravel	36 ± 13	334 ± 34	657 ± 68	20 ± 0	-	1653 ± 162
Autocl.gravel	1366 ± 191	264 ± 5	503 ± 11	-	-	1623 ± 111
Controls						
Groundwater/D	3993 ± 417	85 ± 3	113 ± 9	-	-	1630 ± 142
Groundwater/L	3716 ± 282	96 ± 7	128 ± 13	-	-	1593 ± 97

Abbreviations: D dark; L light.
-, below detection. * Total (initial + redosed) TNT concentration was 5004 μg L^{-1}.

Appendix B - Table 4. Explosives residues in plants and substrates after 7-day incubation in [^{14}C]-TNT groundwater. Mean values ± s.d. (N=3).

Treatment	Explosives concentration (µg g FW^{-1})		
	4ADNT	RDX	4,4-Azoxytoluene
Plant species			
Submersed			
Elodea	0.794 ± 0.169	-	-
Pondweed	1.190 ± 0.161	-	-
Water star-grass	2.613 ± 0.375	0.995 ± 0.097	0.248 ± 0.201
Emergent			
Parrot-feather/a	-	-	-
----------/b	2.480 ± 0.759	1.980 ± 0.552	-
Sweet-flag/a	-	-	-
----------/b	-	-	1.927 ± 0.438
R.canary grass/a	-	-	-
----------/b	0.688 ± 0.209	-	0.243 ± 0.344
Wool-grass/a	-	-	-
----------/b	1.052 ± 0.467	-	-
Substrates			
Sediment	0.180 ± 0.031	0.197 ± 0.061	-
Autocl.sediment	0.311 ± 0.130	0.451 ± 0.159	-
Gravel	0.156 ± 0.030	0.167 ± 0.021	-
Autocl.gravel	0.292 ± 0.078	0.173 ± 0.036	-

Abbreviations: /a, above-ground; /b, below-ground
-, below detection.

Appendix B - Table 5. Relative growth rates of plants over 13-day incubation in [^{14}C]-RDX groundwater. Mean values ± s.d. (N=3).

Plant species	Relative growth rate (g DW g DW^{-1} d^{-1})
Submersed	
Elodea	0.0212 ± 0.0013
Pondweed	0.0144 ± 0.0007
Water star-grass	0.0068 ± 0.0050
Emergent	
Parrot-feather	-0.0197 ± 0.0058
Sweet-flag	-0.0022 ± 0.0011
Reed canary grass	0.0128 ± 0.0132
Wool-grass	-0.0072 ± 0.0026

Appendix B - Table 6. Evapotranspiration rates in [^{14}C]-RDX groundwater over 13-day incubation with plants, substrates, or controls. Mean values ± s.d. (N=3). Initial incubation volume 0.8 L.

Treatment	Evapotranspiration rate	
	mL replicate^{-1} d^{-1}	mL g above-ground DW^{-1} d^{-1}
Plant species		
Submersed		
Elodea	16.15 ± 1.44	
Pondweed	14.49 ± 2.09	
Water star-grass	16.54 ± 0.94	
Emergent		
Parrot-feather	37.69 ± 12.26	6.78 ± 2.57
Sweet-flag	25.77 ± 8.99	6.87 ± 2.16
Reed canary grass	27.18 ± 4.41	17.21 ± 0.75
Wool-grass	40.64 ± 7.37	2.97 ± 0.39
Substrates		
Sediment	30.26 ± 3.98	
Autoclaved sediment	37.18 ± 5.03	
Gravel	32.82 ± 4.80	
Autoclaved gravel	28.08 ± 4.08	
Controls		
Groundwater/Dark	1.92 ± 0.54	
Groundwater/Light	22.05 ± 4.04	

Appendix B - Table 7. Explosives concentrations in [^{14}C]-RDX groundwater, initially and after 13-day incubation with plants, substrates, or controls. Mean values ± s.d. (N=3).

Treatment	Explosives concentration (μg L^{-1})					
	TNT	2ADNT	4ADNT	24DNT	TNB	RDX
Initial						
Groundwater	988 ± 9	9 ± 0	18 ± 0	11 ± 0	79 ± 1	1443* ± 17
Plant species						
Submersed						
Elodea	-	-	-	-	-	976 ± 431
Pondweed	-	-	-	-	-	42 ± 59
Water star-grass	-	-	-	-	-	1496 ± 83
Emergent						
Parrot-feather	-	-	35 ± 12	-	-	3196 ± 1665
Sweet-flag	-	-	-	-	-	1156 ± 822
R.canary grass	-	-	-	-	-	704 ± 681
Wool-grass	-	-	-	-	-	-
Substrates						
Sediment	-	52 ± 6	89 ± 16	-	-	1443 ± 95
Autocl.sediment	-	82 ± 11	154 ± 25	-	-	2606 ± 427
Gravel	-	152 ± 27	215 ± 44	-	26 ± 2	3120 ± 530
Autocl.gravel	-	116 ± 63	182 ± 17	-	30 ± 3	2583 ± 365
Controls						
Groundwater/D	500 ± 75	48 ± 18	66 ± 9	4 ± 5	43 ± 6	1513 ± 19
Groundwater/L	148 ± 12	69 ± 10	73 ± 6	6 ± 8	50 ± 9	2296 ± 258

Abbreviations: D dark; L light.
-, below detection.* Total initial RDX concentration was 1529 μg L^{-1}.

Appendix B - Table 8. Explosives residues in plants or substrates after 13-day incubation in [^{14}C]-RDX groundwater. Mean values ± s.d. (N=3).

Treatment	Explosives concentration (µg g FW^{-1})		
	4ADNT	RDX	4,4-Azoxytoluene
Plant species			
Submersed			
Elodea	0.108 ± 0	0.481 ± 0.198	-
Pondweed	-	0.315 ± 0.036	-
Water star-grass	0.204 ± 0.039	1.470 ± 0.289	-
Emergent			
Parrot-feather/a	-	8.567 ± 3.777	-
----------/b	1.407 ± 0.236	3.960 ± 1.757	-
Sweet-flag/a	-	5.560 ± 1.150	-
----------/b	-	-	-
R.canary grass/a	-	6.437 ± 4.985	-
----------/b	-	1.117 ± 0.894	-
Wool-grass/a	-	4.350 ± 1.276	-
----------/b	-	-	-
Substrates			
Sediment	-	-	-
Autocl.sediment	-	0.299 ± 0.244	-
Gravel	-	0.165 ± 0.021	-
Autocl.gravel	-	0.134 ± 0.029	-

Abbreviations: /a, above-ground; /b, below-ground.
-, below detection.

Appendix C
Detection Levels for Explosives in Plants

Detection limits of explosives in plant material, calculated cf. EPA method 8330 (USEPA 1992).
Method Detection Level (MDL): 2.998 x SD, calculated for seven replicates.
Laboratory Reporting Limit (LRL): 10 x SD, calculated for seven replicates

Compound	Spiked conc. (mg L^{-1})	Recovery (%)	Explosives concentration (µg g FW^{-1})		
			Mean ± SD	MDL	LRL
Elodea					
TNT	0.750	71.39	0.803 ± 0.018	0.054	0.182
2ADNT*	0.750	61.03	0.687 ± 0.032	0.096	0.323
4ADNT*	0.750	21.01	0.236 ± 0.025	0.074	0.246
24DNT	0.750	80.99	0.911 ± 0.045	0.135	0.451
26DNT	0.750	85.28	0.959 ± 0.015	0.044	0.147
NB	0.750	64.90	0.730 ± 0.023	0.068	0.226
DNB	0.750	85.41	0.960 ± 0.040	0.121	0.405
TNB	0.750	61.01	0.686 ± 0.014	0.041	0.137
2NT	0.750	87.81	0.988 ± 0.044	0.132	0.440
3NT	0.750	91.79	1.033 ± 0.022	0.066	0.220
4NT	0.750	90.42	1.017 ± 0.012	0.036	0.119
RDX	0.750	133.41	1.501 ± 0.108	0.324	1.080
HMX	0.750	121.89	1.371 ± 0.054	0.161	0.538
Tetryl	0.750	18.46	0.208 ± 0.021	0.062	0.208
Sweet-flag/above-ground					
TNT	2.000	104.57	3.137 ± 0.270	0.810	2.703
2ADNT*	2.000	88.50	2.655 ± 0.101		
4ADNT*	2.000				
24DNT	2.000	158.14	4.744 ± 0.390	1.170	3.902
26DNT	2.000	85.93	2.578 ± 0.513	1.539	5.135
NB	2.000	83.357	2.501 ± 0.761	2.281	7.609
DNB	2.000	177.36	5.321 ± 0.596	1.786	5.958
TNB	2.000	246.71	7.401 ± 4.522	13.557	45.221
2NT	2.000	159.79	4.794 ± 1.656	4.965	16.560
3NT	2.000	88.64	2.659 ± 0.384	1.151	3.838
4NT	2.000	73.79	2.214 ± 0.257	0.771	2.573
RDX	2.000	403.21	12.096 ± 2.740	8.213	27.396
HMX	2.000	58.79	1.764 ± 0.586	1.757	5.860
Tetryl	2.000	77.29	2.320 ± 0.131	0.392	1.307

Appendix C - Table continued

Compound	Spiked conc. (mg L^{-1})	Recovery (%)	Explosives concentration (µg g FW^{-1})		
			Mean ± SD	MDL	LRL
Sweet-flag/below-ground					
TNT	2.000	105.29	3.159 ± 0.193	0.580	1.934
2ADNT*	2.000	98.50	2.955 ± 0.149		
4ADNT*	2.000				
24DNT	2.000	169.07	5.072 ± 0.493	1.478	4.931
26DNT	2.000	95.86	2.876 ± 0.390	1.169	3.899
NB	2.000	97.71	2.931 ± 0.901	2.700	9.007
DNB	2.000	174.71	5.241 ± 0.554	1.662	5.544
TNB	2.000	78.37	2.351 ± 0.298	0.892	2.977
2NT	2.000	163.93	4.918 ± 1.434	4.298	14.335
3NT	2.000	49.71	1.491 ± 1.123	3.366	11.228
4NT	2.000	65.143	1.954 ± 0.892	2.673	8.915
RDX	2.000	431.57	12.947 ± 2.239	6.711	22.385
HMX	2.000	23.34	0.700 ± 0.377	1.129	3.767
Tetryl	2.000	105.71	3.171 ± 0.786	2.357	7.860

Two gram fresh plant material, ground in liquid N_2, was spiked with an acetonitrile solution containing known concentrations of explosives, extracted with acetonitrile, and cleaned up using Florisil and neutral alumina as described in the 'Materials and Methods' section. Recovery and concentrations as determined using a C18 column; CN column determinations served to confirm compound identity.
*4ADNT and 2ADNT co-elute on C18 column; for elodea CN column-values, and for sweet-flag 2ADNT+4ADNT concentrations are given.

Concentration in mg explosive per L plant extract was converted to µg g FW^{-1} by multiplication with 0.005 x ½ x 1000; i.e., 5 mL extract volume, 2 g FW extracted.

Appendix D
Abbreviations

AC	sweet-flag
2ADNT	2-amino-4,6-dinitrotoluene
4ADNT	4-amino-2,6-dinitrotoluene
ADNTs	total monoamino-dinitrotoluenes (= 2ADNT, 4ADNT)
AGR	autoclaved gravel
ASED	autoclaved sediment
4,4-azoxytoluene	2,2', 6,6-tetranitro-4,4-azoxytolene
24DANT	2,4-diamino-6-nitrotoluene
26DANT	2,6-diamino-4-nitrotoluene
DNB	dinitrobenzene
1,3DNB	1,3-dinitrobenzene
1,4DNB	1,4-dinitrobenzene
DNT	dinitrotoluene
24DNT	2,4-dinitrotoluene
26DNT	2,6-dinitrotoluene
DOM	dissolved organic matter
DPM	desintegrations per minute
DW	dry weight
EC	elodea
FW	fresh weight
GR	gravel
GSH	γ-glutamyl-cysteinylglycine
GW-D	groundwater incubated in darkness
GW-L	groundwater incubated illuminated
GW-O	initial groundwater
HD	water star-grass
HPLC	high performance chromatography
HSD	honest dignificant difference
LS	liquid scintillation counting
MA	parrot-feather
MNX	mono-nitroso-derivative of RDX
NB	nitrobenzene
2NT	2-nitrotoluene
3NT	3-nitrotoluene
4NT	4-nitrotoluene
NT	nitrotoluene
PA	reed canary grass
PP	sago pondweed
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
SC	wool-grass
SED	sediment
SPE	solid phase extraction
TLC	thin layer chromatography
TNB	trinitrobenzene
TNT	2,4,6-trinitritoluene
TNX	tri-nitroso-derivative of RDX
XAD	resin