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“Insensitive Munitions: Environmental Health Criteria, Fate and Transport”

June 2, 2016

SERDP DOD • EPA • DOE | ESTCP

Welcome and Introductions

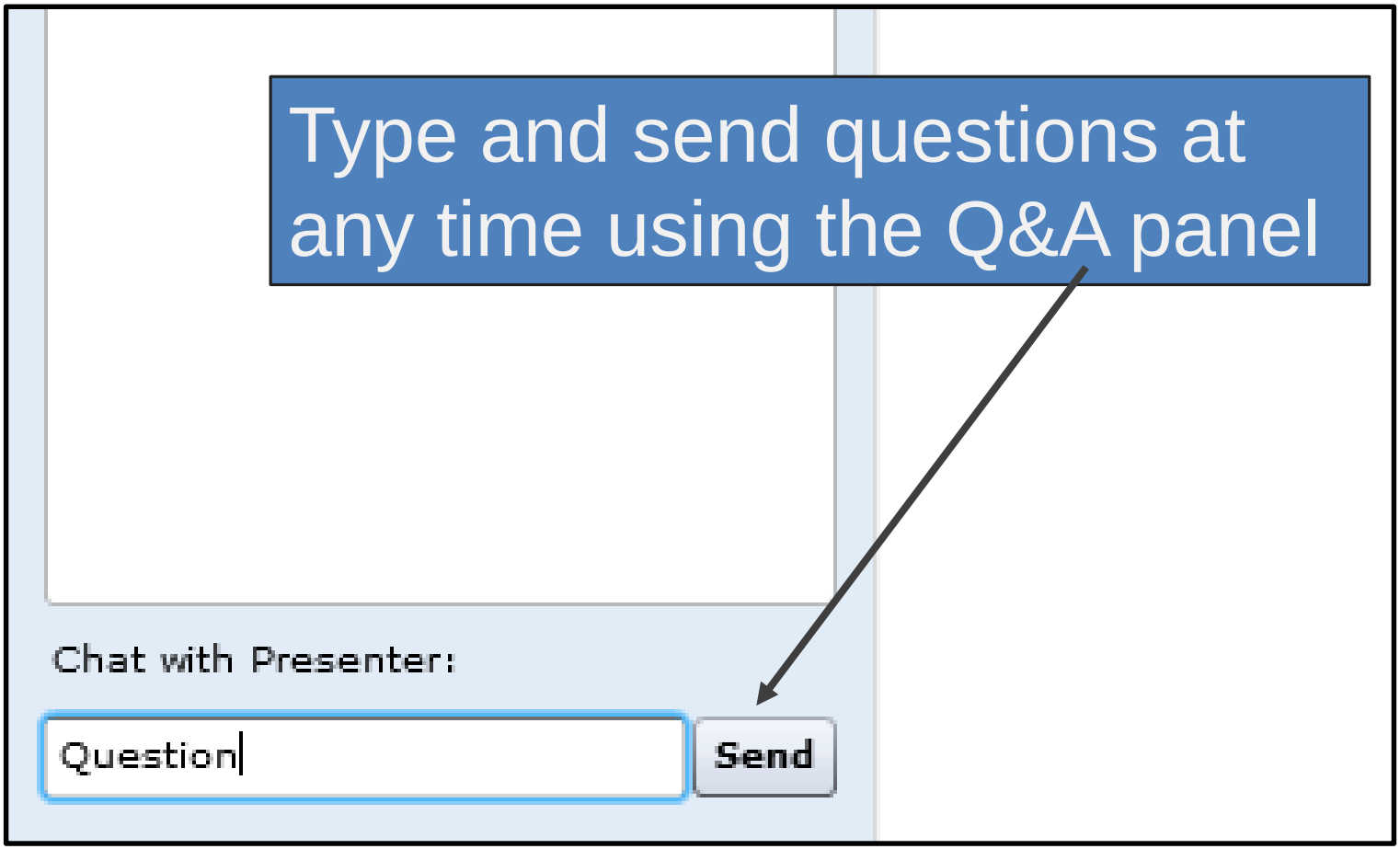
Jennifer Nyman, Ph.D., P.E.
Webinar Facilitator

SERDP DOD • EPA • DOE | ESTCP

Agenda

- **Webinar Logistics**
  Dr. Jennifer Nyman, Geosyntec Consultants (5 minutes)
- **Overview of SERDP and ESTCP**
  Dr. Andrea Leeson, SERDP and ESTCP (5 minutes)
- **Development of Environmental Health Criteria for Insensitive Munitions**
  Dr. Mark Johnson, U.S. Army Public Health Center (15 minutes + Q&A)
- **Dissolution of NTO, DNAN and Insensitive Munitions Formulations, and Their Fates in Soils**
  Dr. Katerina Dontsova, University of Arizona, Tuscon (15 minutes + Q&A)
- **Biodegradation of Insensitive Munition Compound NTO to Mineral Products**
  Dr. Jim Field, University of Arizona, Tuscon (15 minutes + Q&A)
- **Final Q&A session**

How to Ask Questions



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SERDP and ESTCP Overview

Andrea Leeson, Ph.D.
Environmental Restoration
Program Manager

SERDP

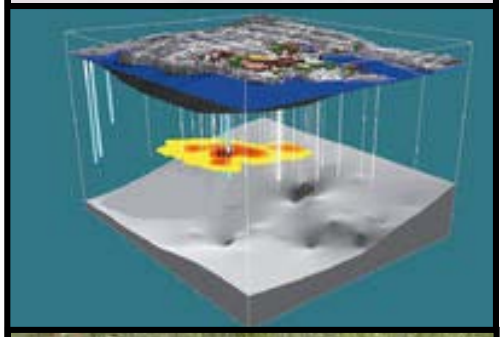
- Strategic Environmental Research and Development Program
- Established by Congress in FY 1991
  - DoD, DOE and EPA partnership
- SERDP is a requirements driven program which identifies high-priority environmental science and technology investment opportunities that address DoD requirements
  - Advanced technology development to address near term needs
  - Fundamental research to impact real world environmental management

ESTCP

- Environmental Security Technology Certification Program
- Demonstrate innovative cost-effective environmental and energy technologies
  - Capitalize on past investments
  - Transition technology out of the lab
- Promote implementation
  - Facilitate regulatory acceptance

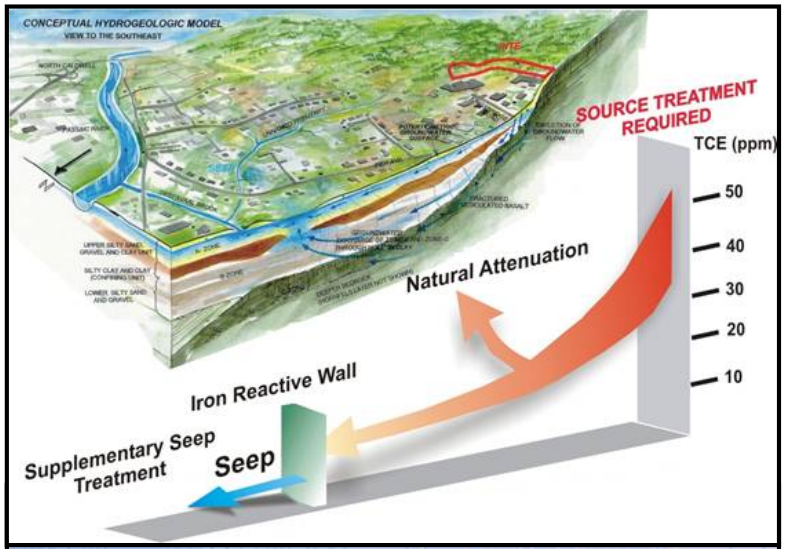
Program Areas

1. Energy and water
2. Environmental restoration
3. Munitions response
4. Resource conservation and climate change
5. Weapons systems and platforms



Environmental Restoration

- Major focus areas
  - Contaminated groundwater
  - Contaminants on ranges
  - Contaminated sediments
  - Wastewater treatment
  - Risk assessment



SERDP and ESTCP Webinar Series

Date	Topic
June 16, 2016	Quality Assurance Project Plan (QAPP) for Advanced Geophysical Classification Investigations – Part 2
June 30, 2016	Geophysics 101: Realistic Expectations for Geophysics When Used for Site Characterization and Remediation Monitoring – Part 1
July 14, 2016	Remote Methods for Water Conservation
July 28, 2016	An Environmentally Acceptable Alternative for Fast Cook-off Testing, Demonstration, Validation and Implementation Efforts
August 11, 2016	TBD
August 25, 2016	Geophysics 101: Realistic Expectations for Geophysics When Used for Site Characterization and Remediation Monitoring – Part 2

Development of Environmental Health Criteria for Insensitive Munitions

Mark Johnson, Ph.D., D.A.B.T.
U.S. Army Public Health Center

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Outline

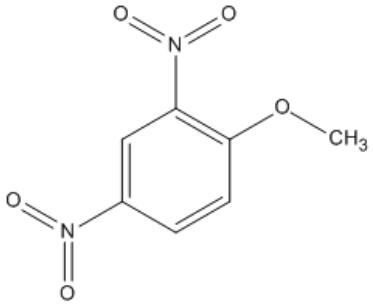
- Problem
- Introduction
- Technical approach
  - DNAN aquatic tests
  - NTO mammal tests
  - NTO avian tests
  - NTO amphibian tests
- Conclusions
- Lessons learned

Problem Statement

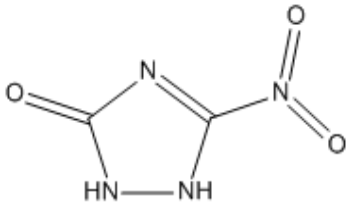
- Rapid development of IMX-101/104*
  - Nitroguanidine
  - 2,4-Dinitroanisol (DNAN)
  - 3-Nitro-1,2,4-triazol-5-one (NTO)
  - RDX (104)*
- Manufacturing halted/delayed
  - Occupational Health
- Environmental consequences
  - Water solubility
  - Toxicology incomplete
  - Sustainability
    - EOD issues
- What is a safe level of exposure?
- **Goal**
  - "Enable developers to better estimate full life-cycle costs, implement engineering controls, and protect the environment, all of which will help sustain range operations and readiness"

Introduction

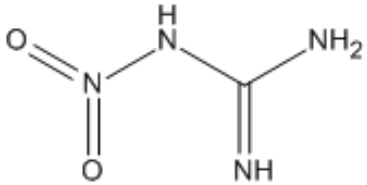
- Dinitroanisole (DNAN)
  - LD50 = 200 mg/kg
  - 14d Oral = 12.5/25 mg/kg-d anemia
  - Water solubility = 297 mg/L



- Nitrotriazolone (NTO)
  - LD50 > 2000 mg/kg
  - 90d Oral – 315 mg/kg-d – ogliospermia
  - Water solubility = 12-17 g/L



- Nitroguanidine (NQ)
  - LD50 > 5000 mg/kg
  - 14d Oral > 1000 mg/kg-d
  - Water solubility = 3.2 g/L



Technical Approach

- Anticipate regulatory needs/requirements/concerns
  - NTO
    - Understand reproductive/developmental toxicology
      - Extended one-generation (rats)
      - Extended one-generation (quail)
      - Developmental Toxicity (frogs)

Develop safe exposure criteria

  - DNAN
    - Aquatic toxicity

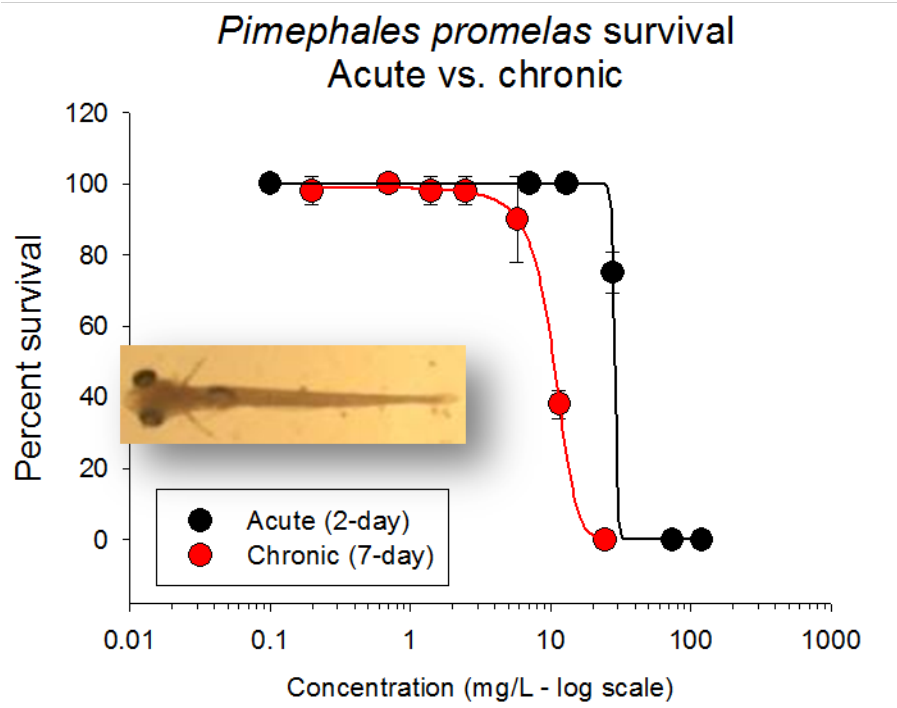
Sensitivity of Test Species to DNAN (Survival)

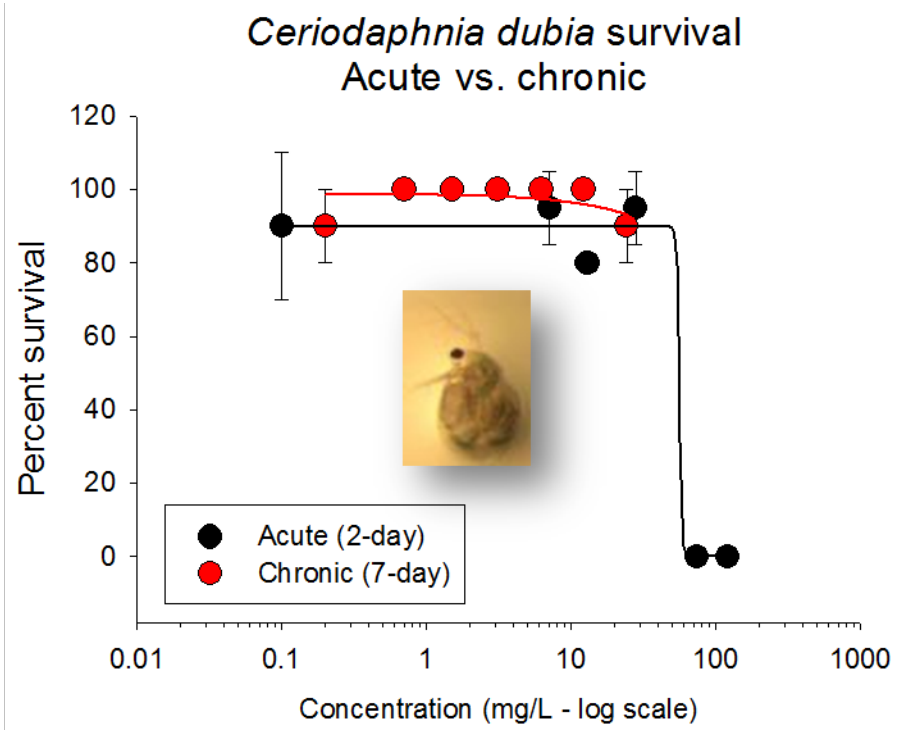
Pimephales promelas

- Sensitivity increased with time

Ceriodaphnia dubia

- No sensitivity increase with exposure time



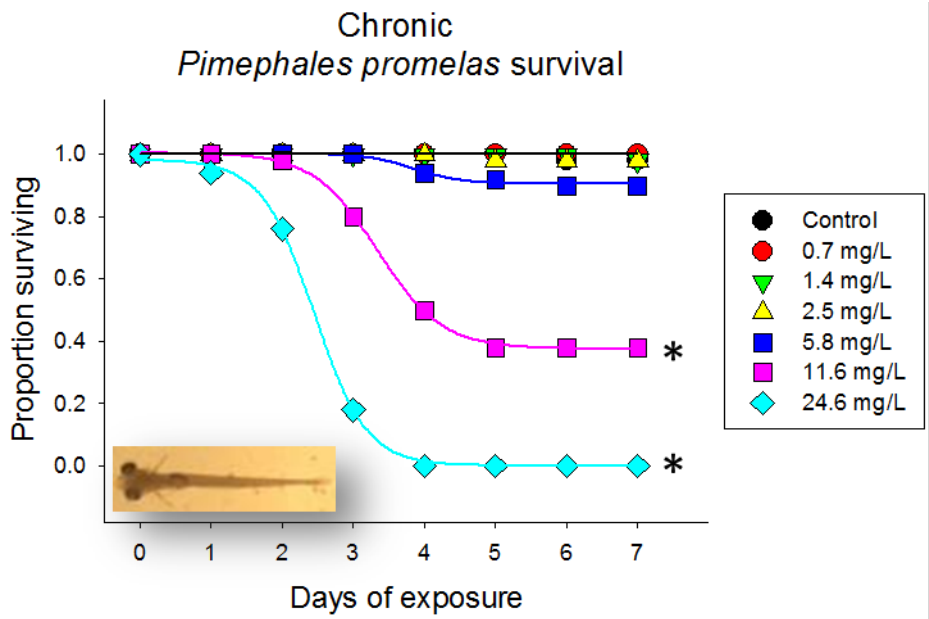


Kennedy et al., 2014

Chronic Toxicity to DNAN

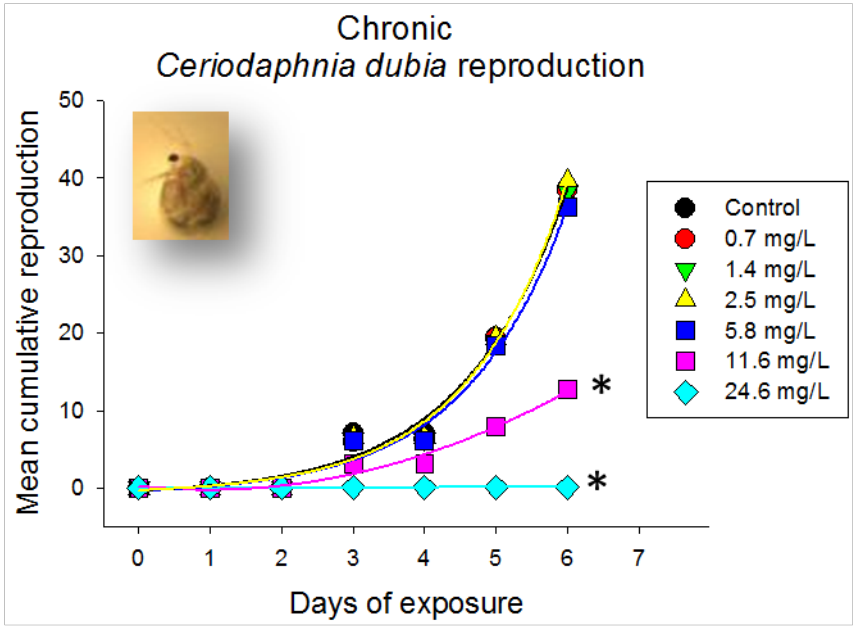
Pimephales promelas

- **Survival**: reduction in top 2 concentrations
- **Growth**: fish ate little when exposed to 11.6 mg/L or greater
  - Lead to significant mortality after 4 days



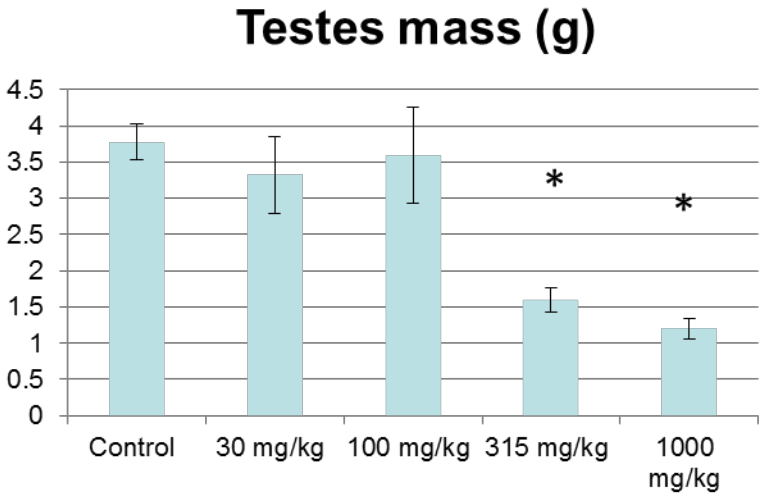
Ceriodaphnia dubia

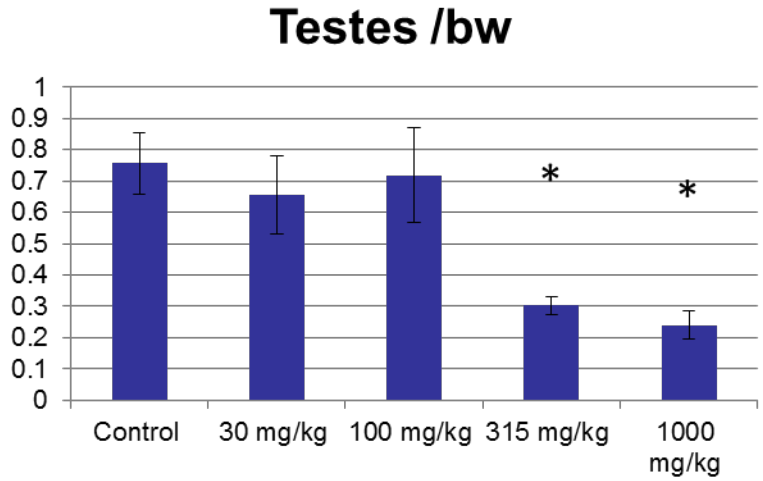
- No survival impact up to 25 mg/L
- Reproduction: reduction at top 2 concentrations

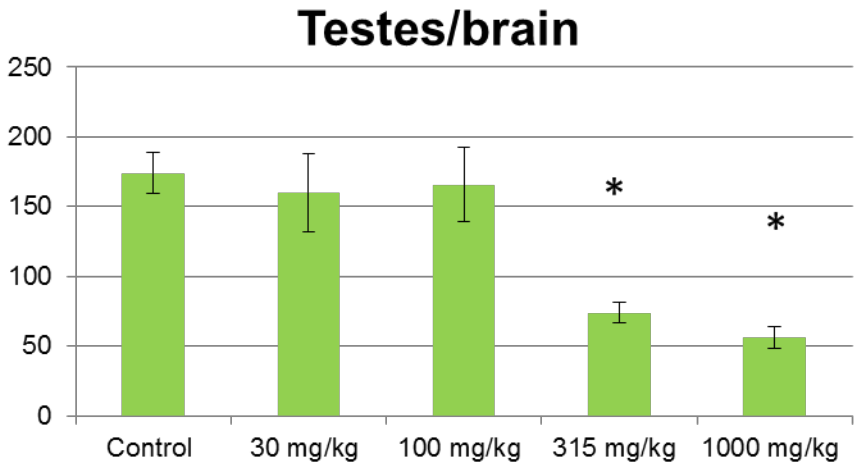


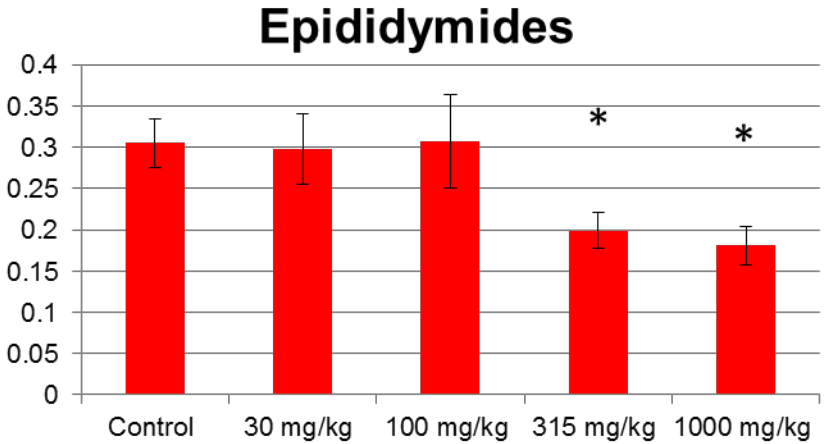
Kennedy et al., 2014

NTO Oral Toxicity in Rats





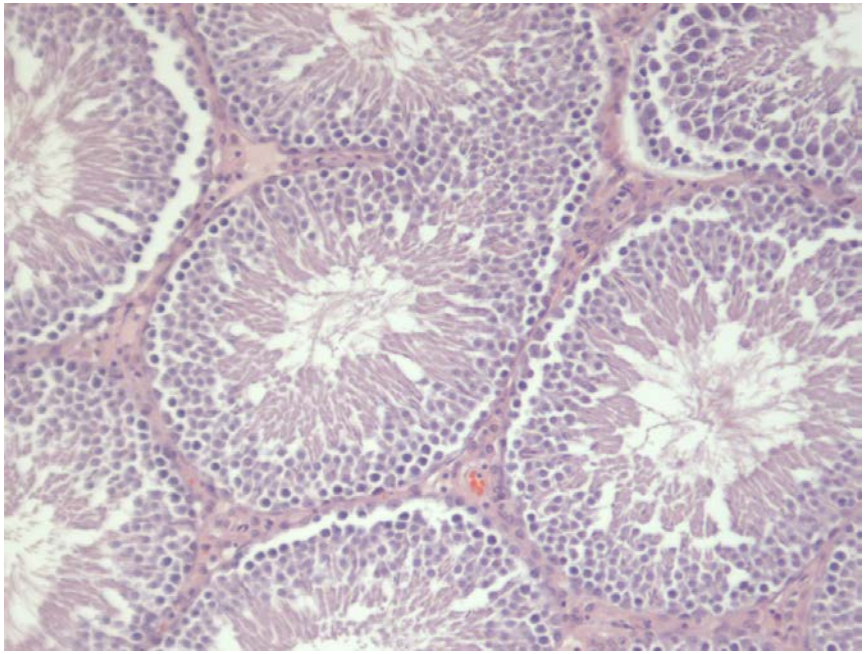




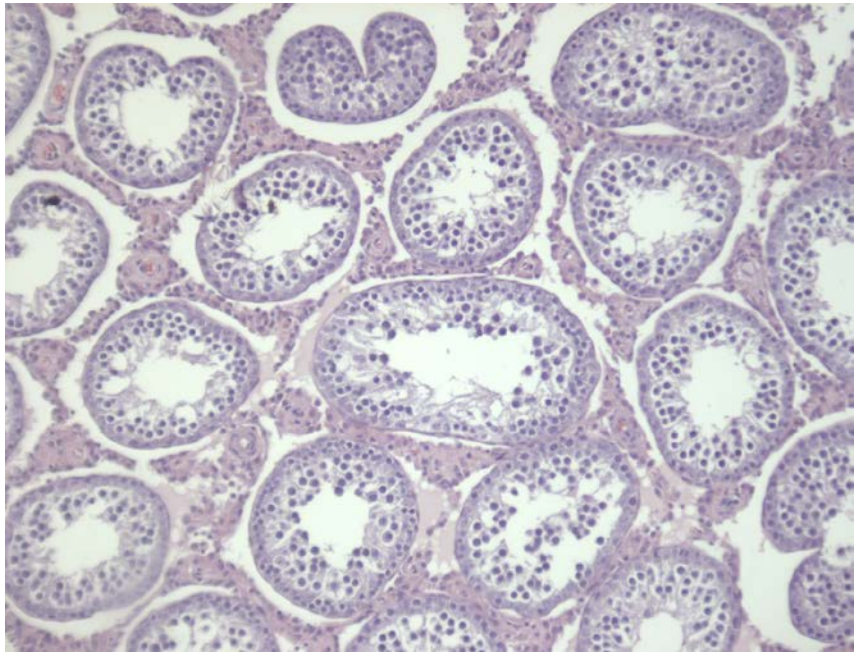
Crouse et al., 2015

NTO Testes Histology

Control 0251



1000 mg/kg-day 0251



In vitro Endocrine Disruption

Assay	NTO Concentration	Results
Estrogen receptor binding ER α (recombinant-human) ER β (recombinant-human)	0.003 - 30 µM 0.0004 - 3.9 µg/mL	Negative No significant interaction
Androgen receptor binding (recombinant-rat)	0.003 - 30 µM 0.0004 - 3.9 µg/mL	Negative No significant interaction
Estrogen receptor α/β transcriptional assay (BG1 cells ovarian-human)	0.02 - 200 µM 0.0025 - 25 µg/mL	Negative No significant interaction
Steroidogenesis Two cell lines H295R (adrenal-human) BLTK1 (Leydig-mouse)	0.03 - 100 µM 0.004 - 13 µg/mL	Negative No significant interaction (testosterone) Follow up with estradiol only if testosterone affected
Aromatase inhibition (cell line microsomes)	0.0001 - 100 µM 1.3×10^{-5} - 13 µg/mL	Negative No significant inhibition

Extended One-Generation Reproductive Assay Task 1

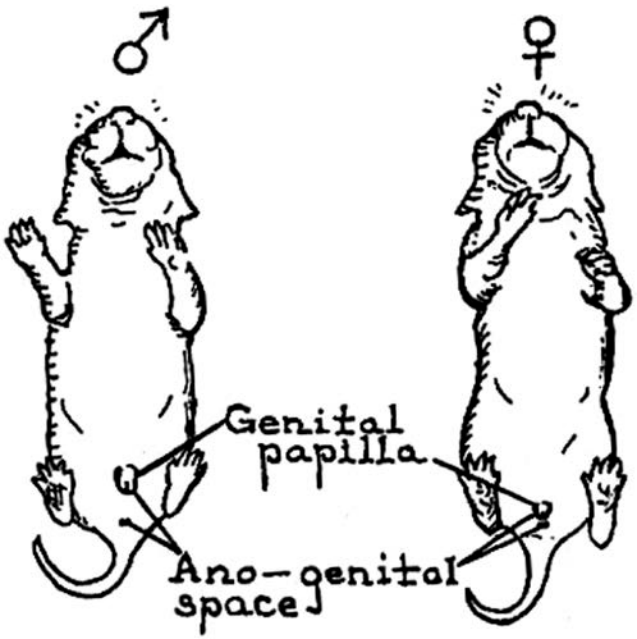
	Pre-mating Exposure	Mating Exposure	Post-Mating Exposure	
P Males	4 weeks	2 weeks	4 weeks	
P Females	2 weeks	2 weeks	Pregnancy: 3 weeks	Lactation: 3 weeks

In-utero	Pre-weaning	F1 Post-weaning Exposure	
		21 days	F1 Females
		32 days	F1 Males
		0 days	Surplus

Lent et al., 2016

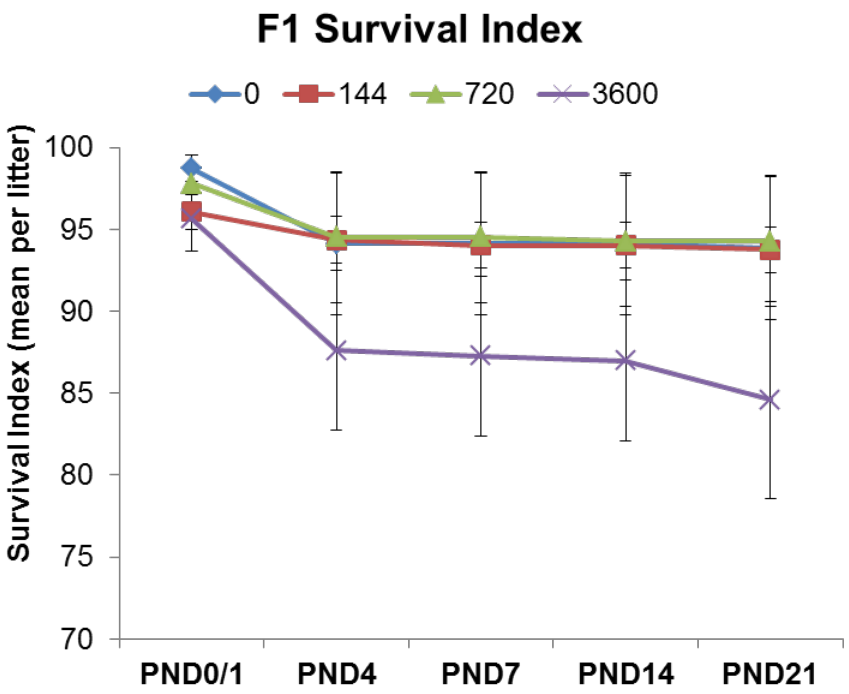
Extended One-Generation Reproductive Assay Task 1

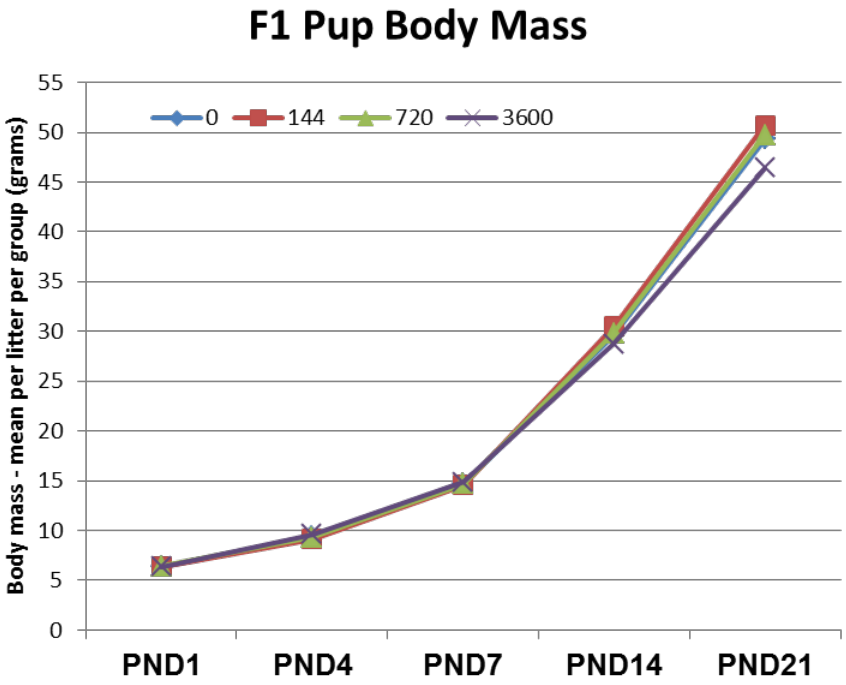
- Clinical observations
- Body weight/food consumption
- Mating and pregnancy
  - Pre-coital interval, mating indices
- Offspring parameters
  - Number and sex of pups, stillbirths, gross abnormalities, etc.
  - Ano-genital distance (AGD), nipple retention, vaginal opening (VO) and preputial separation (PPS)
- Clinical chemistries, thyroid hormones
- Organ weights and histopathology
- Sperm parameters
  - Motility, linearity, flagella speed, count



Lent et al., 2016

Extended One-Generation Reproductive Assay Task 1

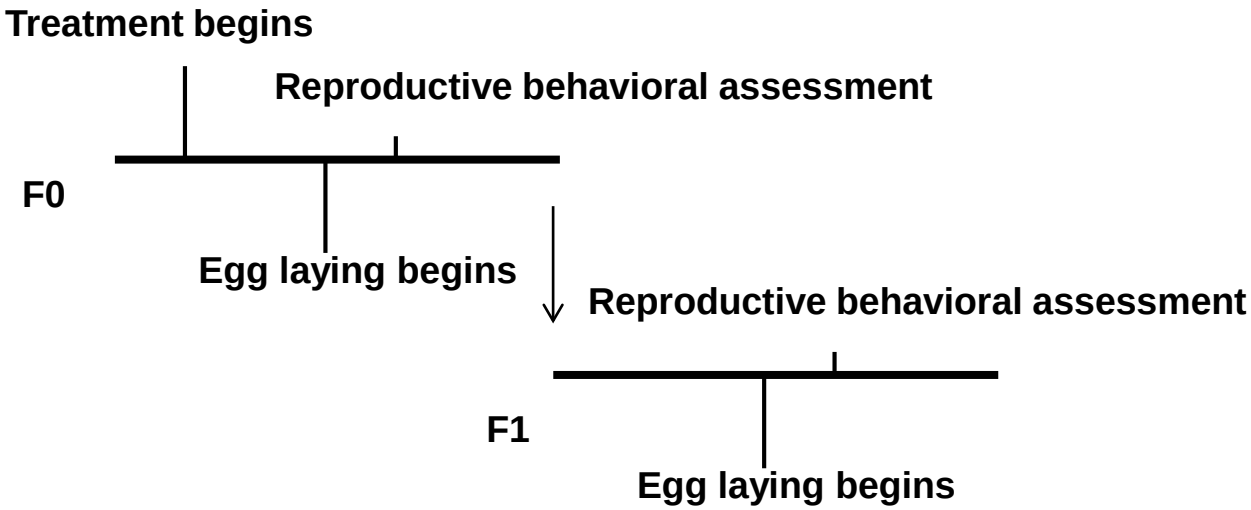




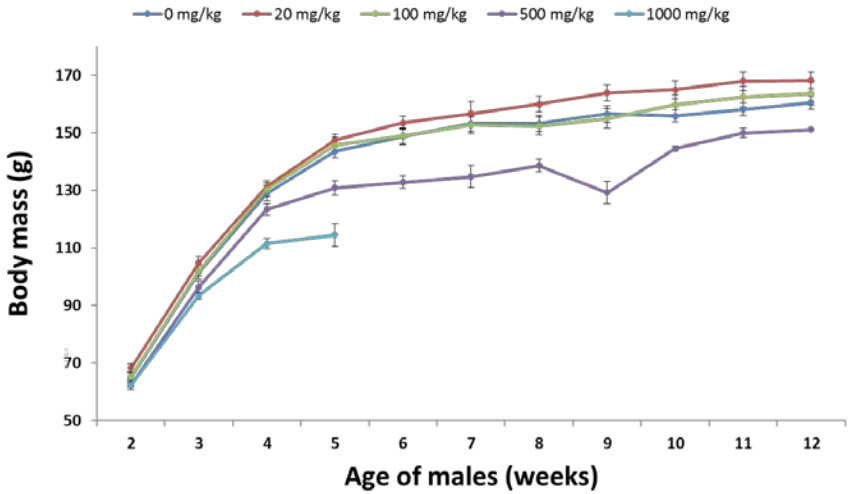
Changes in nipple retention pubertal delays

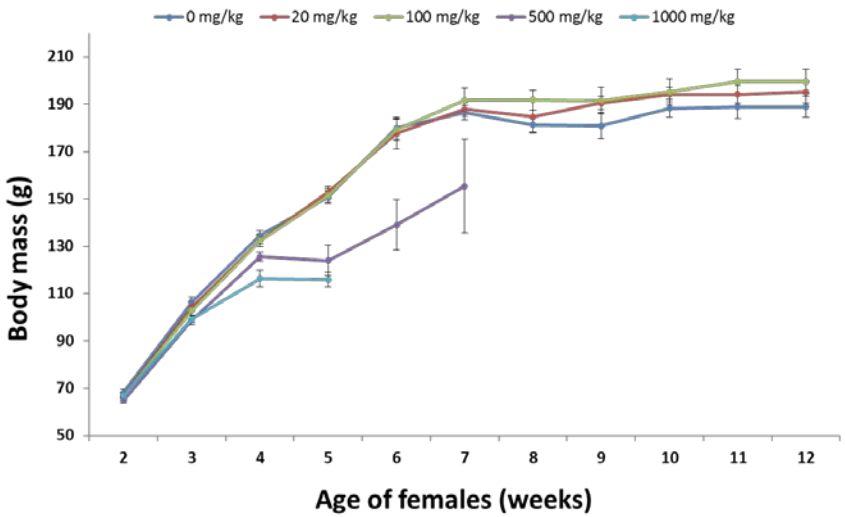
Avian Toxicity
Extended One-Generation (Jackovitz)

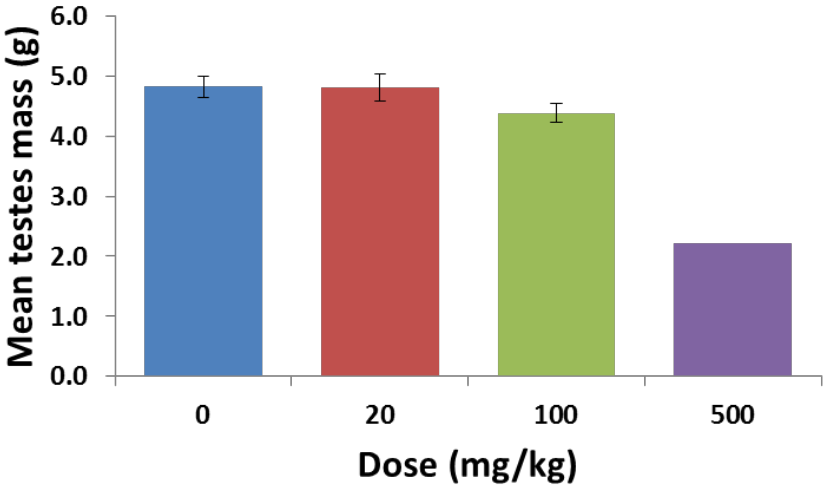
- P (F0) exposed orally at two weeks
- F1 exposed as embryos and orally from hatch throughout study



Avian Toxicity
Extended One-Generation (Jackovitz)







Severe neuromuscular signs and death in 100% of 1000 mg/kg group (N=45) and 98% of 500 mg/kg group (N=42)

Mild neurological signs (e.g., head tilt) in 10% of 100 mg/kg-d animals (N=31)

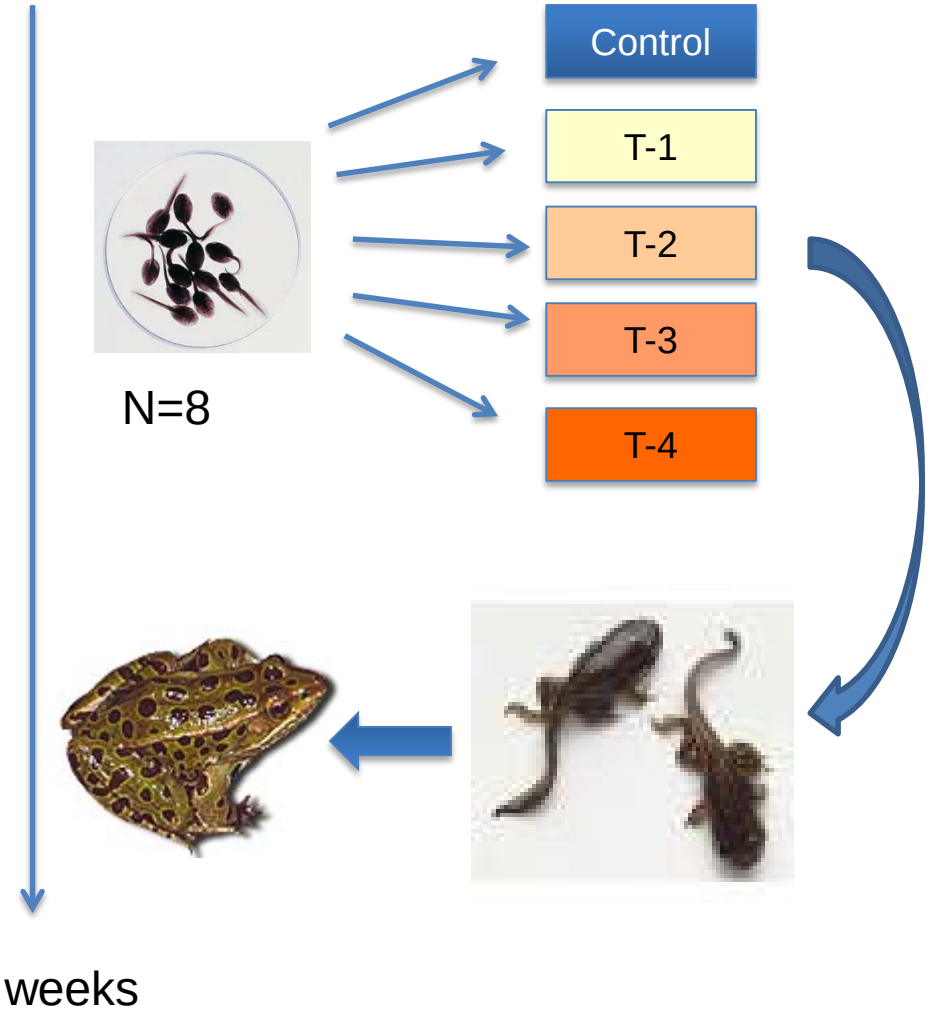
Amphibian Toxicity
Developmental Task 3 (Pillard)

Modified OECD 2591

- Daily renewal
- Four treatments + control, 8 reps
- °C, pH, DO, NH_2
- Mortality, deformities, behavioral

Results

- 8,382 mg/L lethal
- Front limb emergence affected
- 3338 mg/L – reductions in organ mass
- NOAEC – 190 mg/L



Conclusions

- NTO
  - Water solubility: 12-17 g/L
  - High doses causes reduction in sperm production
    - Affects Sertoli cells
  - High doses neurotoxic to birds
- DNAN
  - Toxicity similar to/less than other nitroaromatics

Lessons Learned

- Phased approach useful
- Breadth of ESOH data needed is extensive – need guidance (see ASTM E-2552)
  - Requires holistic view of cradle-to-grave
    - Conception
    - Synthesis/manufacture
    - Production and use
    - Environmental fate and transport
      - Analytical methods – various media/matrixes
    - Peer-review of toxicity-based benchmarks
    - Exposure potential
      - Each phase
        - » Environmental
        - » Occupational (including warfighter)
        - » Demilitarization

- Cost/benefit risk management of options
- Need to begin asking ESOH questions during RDT&E phase

For additional information, please visit
https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminants-on-Ranges/Characterizing-Fate-and-Transport/ER-2223

Speaker Contact Information

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410-436-3980

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Q&A Session 1

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Dissolution of NTO, DNAN and Insensitive Munitions Formulations and their Fates in Soils

Katerina Dontsova, Ph.D.
University of Arizona, Tucson

SERDP DOD • EPA • DOE | ESTCP

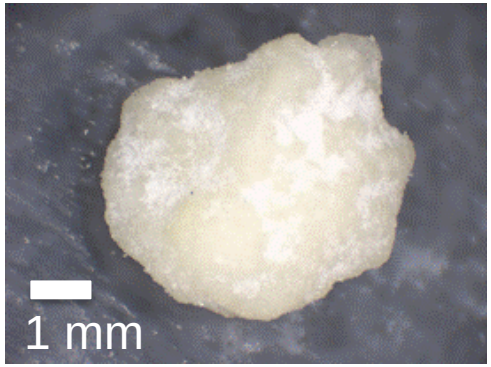
Agenda

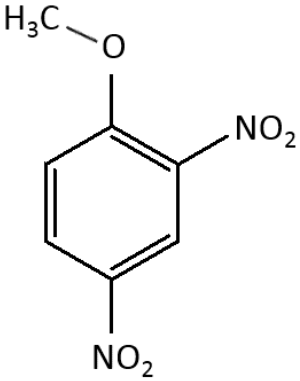
- IM dissolution under controlled conditions
- Outdoor dissolution and phototransformation
- Soil adsorption of NTO and DNAN
- Transport of pure NTO and DNAN in soils
- Dissolution and soil transport of IM formulations

Insensitive Munition Compounds

DNAN

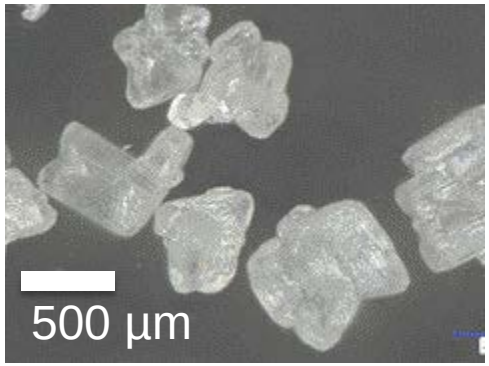
2,4-dinitroanisole

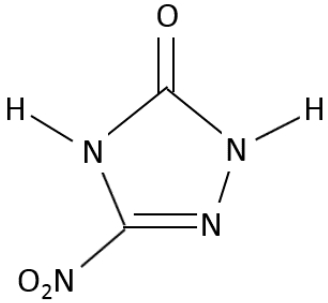




NTO

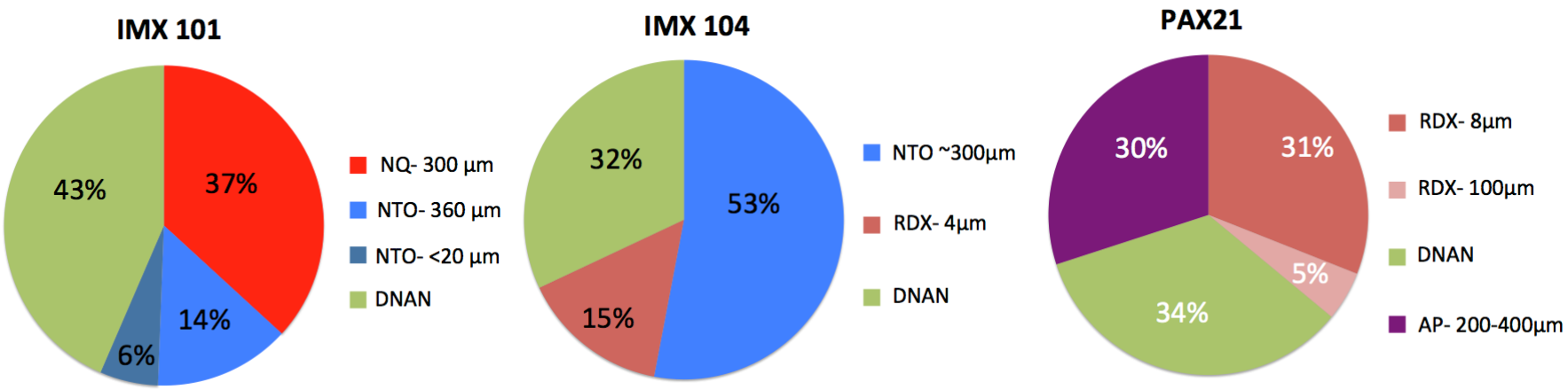
3-nitro-1,2,4-triazol-5-one

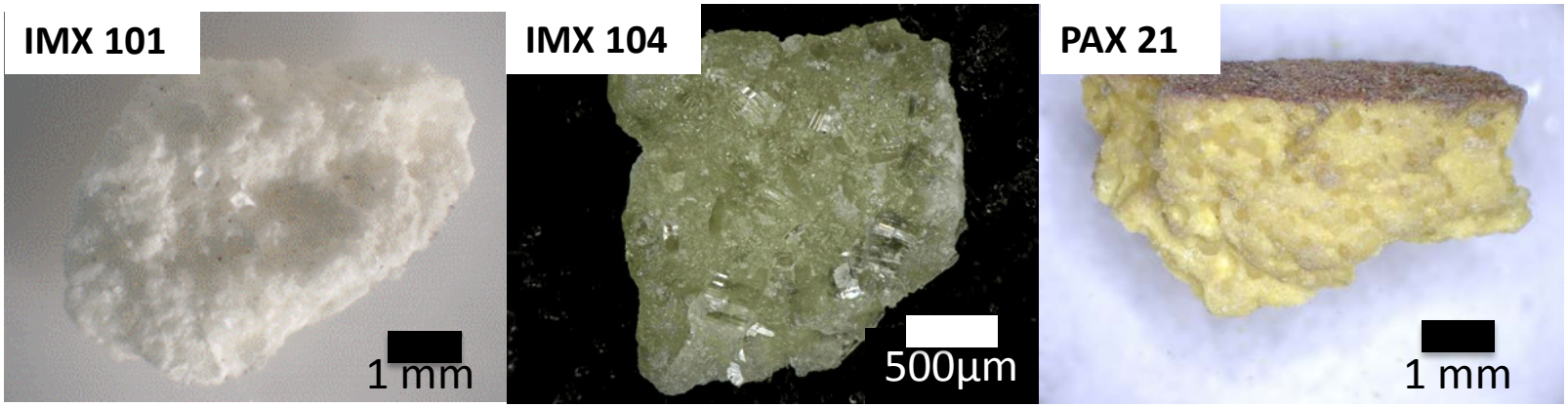




- New insensitive energetics with high solubility, 16.6 g L^{-1} for NTO, 0.3 g L^{-1} for DNAN
- DNAN structure is similar to TNT and DNTs
- NTO is negatively charged at neutral and basic pH

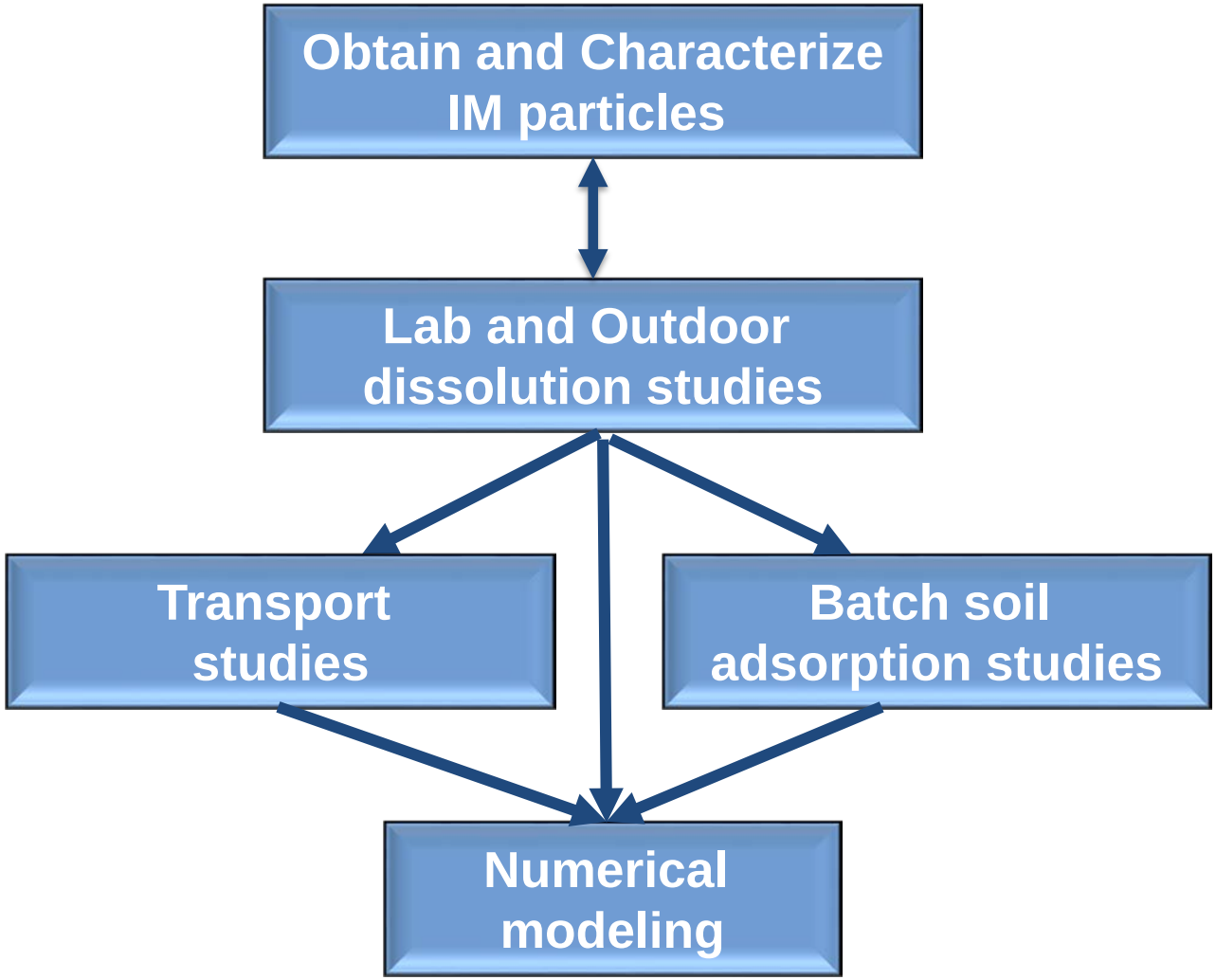
Insensitive Munition Formulations



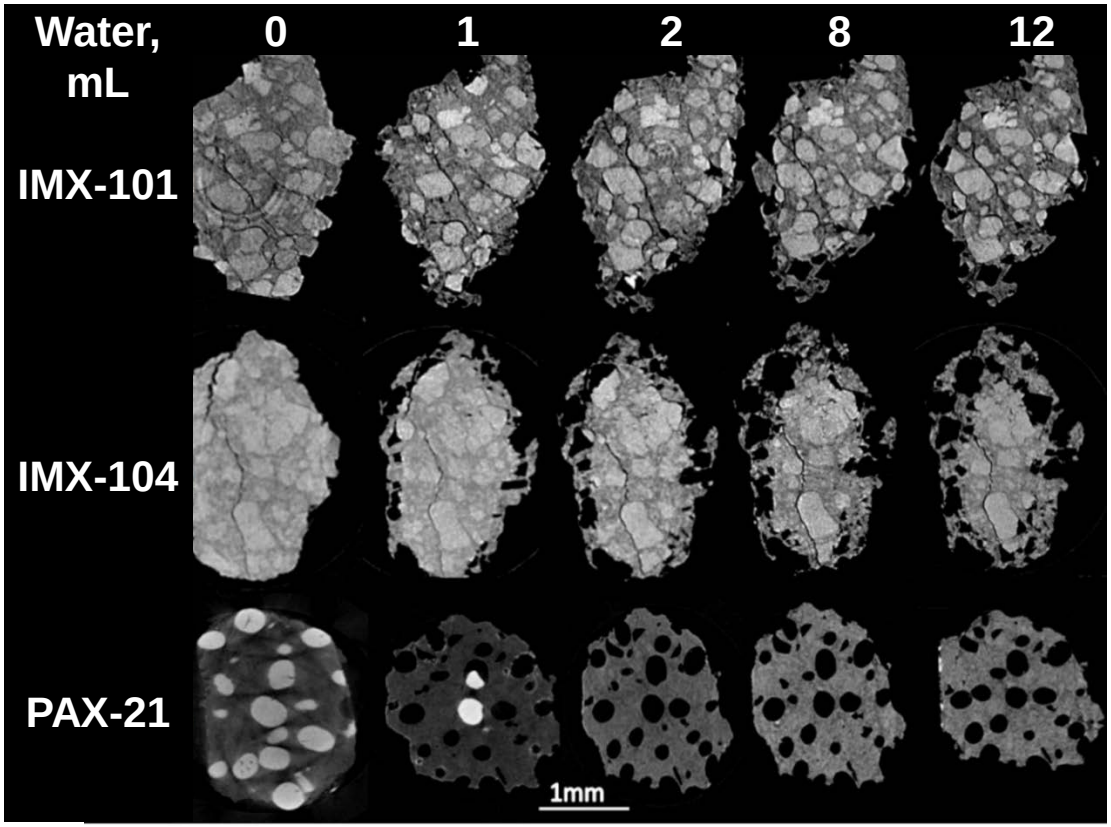


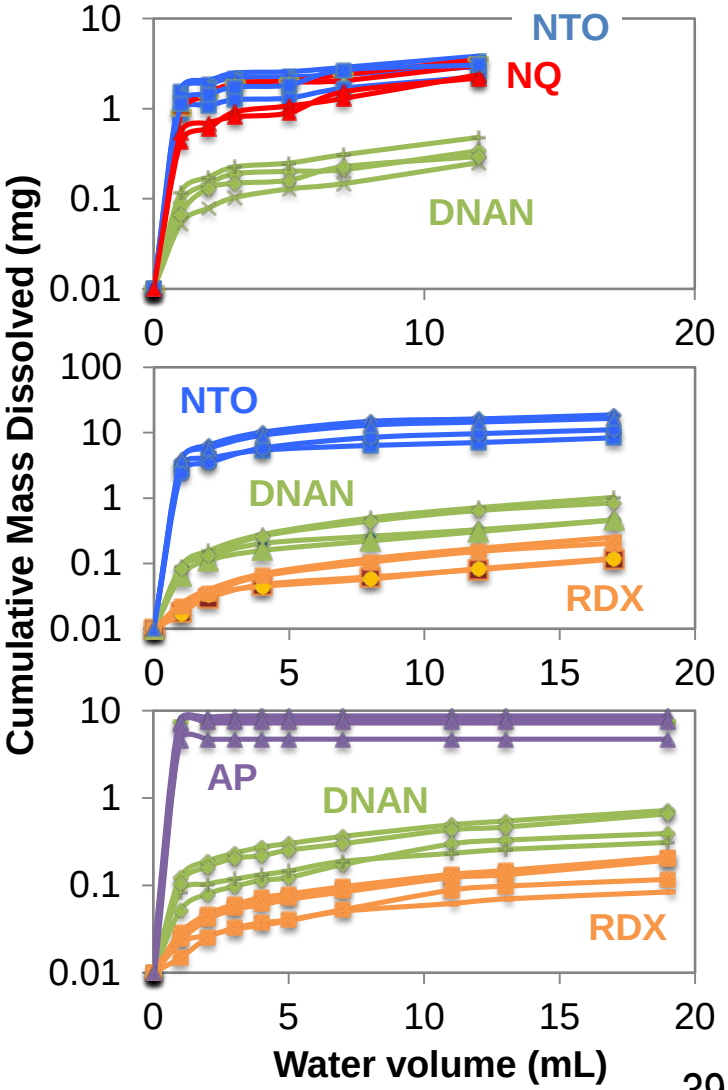
Melt cast with NTO and NQ (IMX-101), NTO and RDX (IMX-104), or AP and RDX (PAX-21) in DNAN matrix

Technical Approach



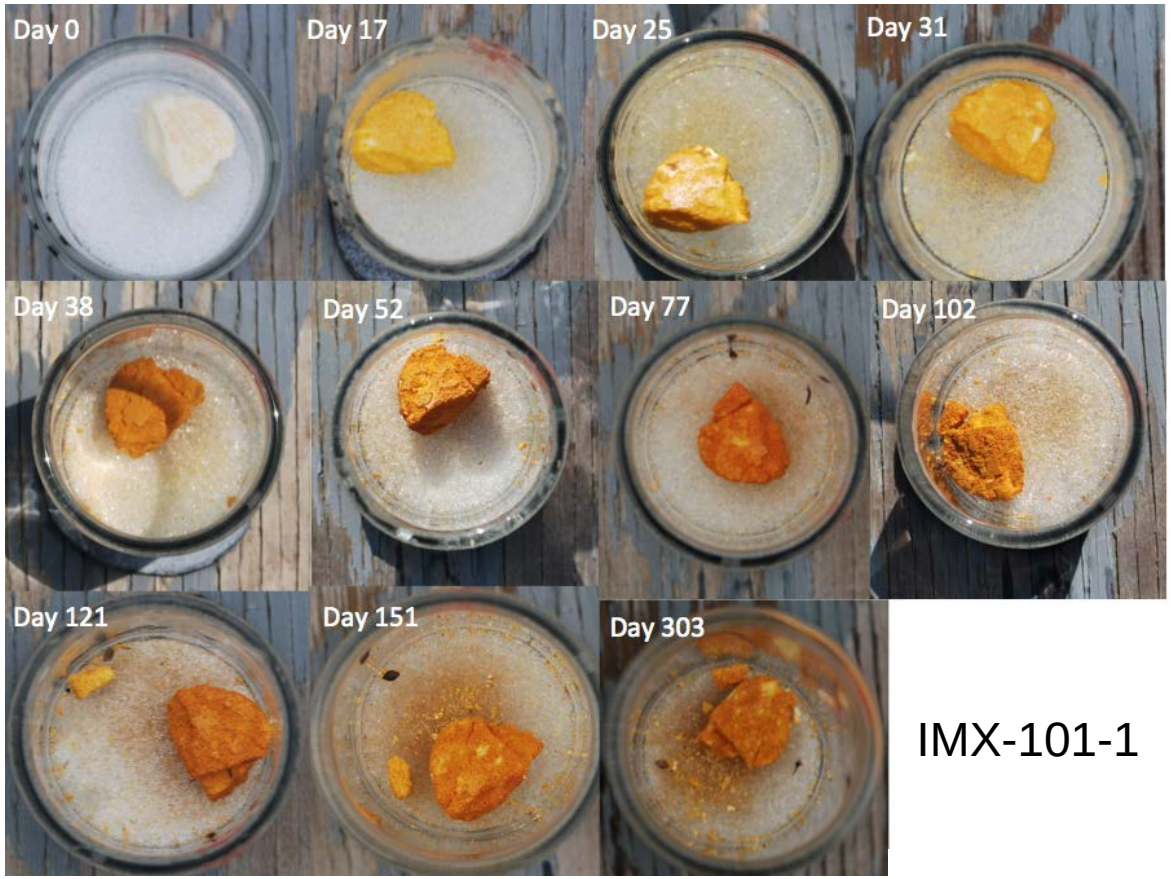
Lab Dissolution





IM components dissolve sequentially according to their solubilities

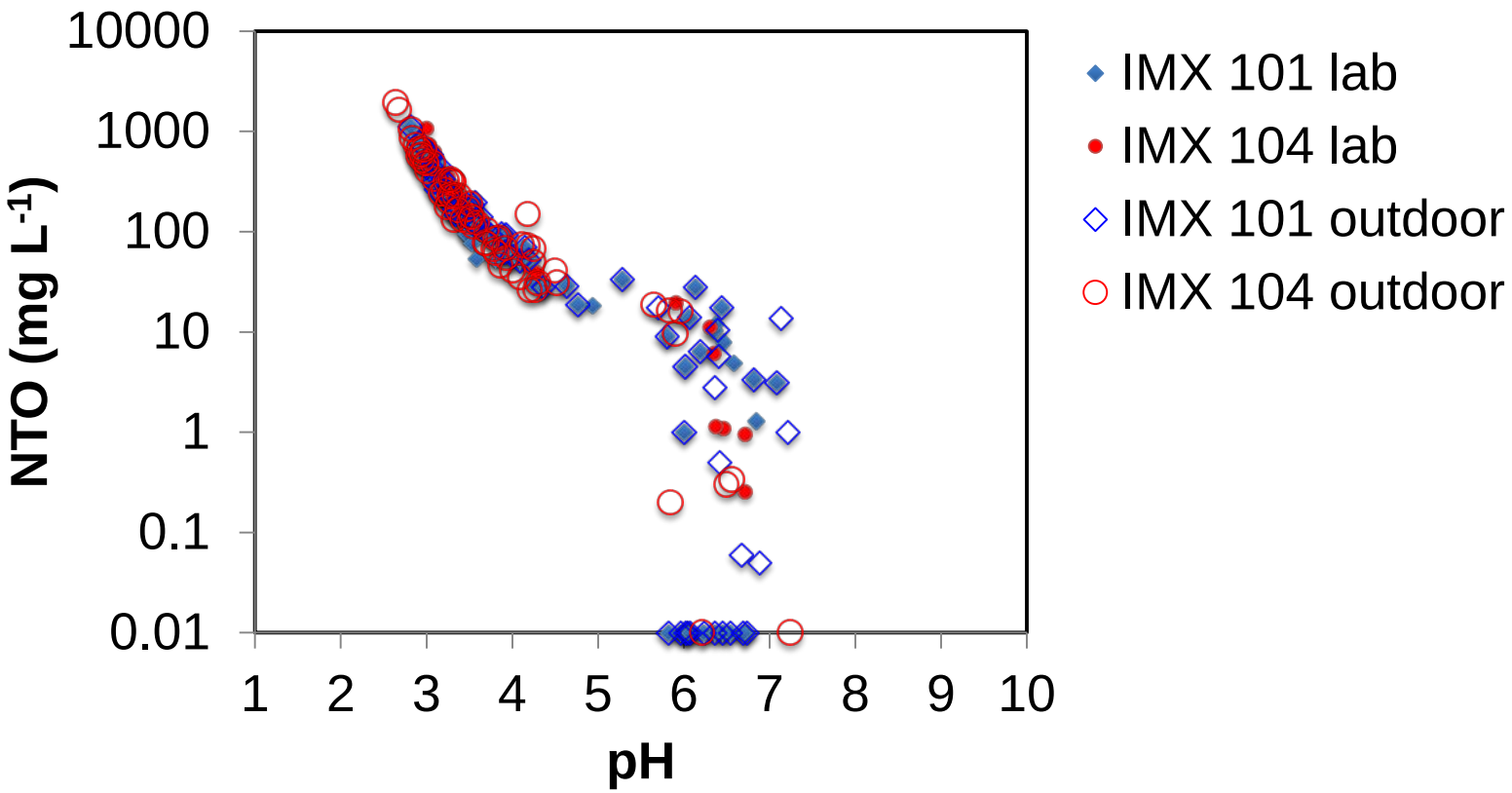
Outdoor Dissolution



IMX-101-1

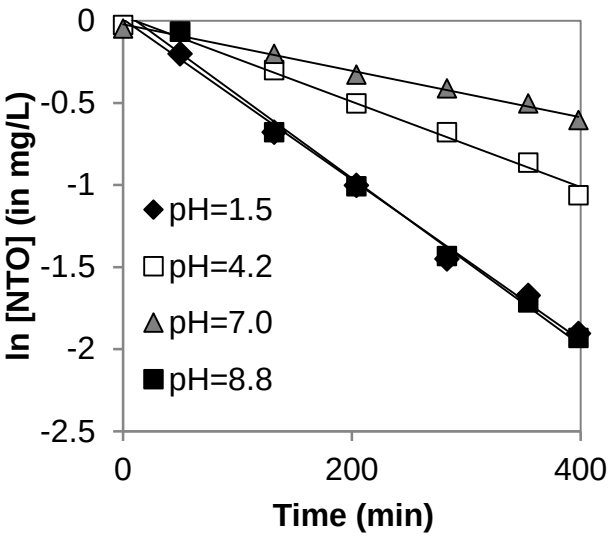
65% was dissolved in two years and 15% of IM mass was lost as a result of phototransformation

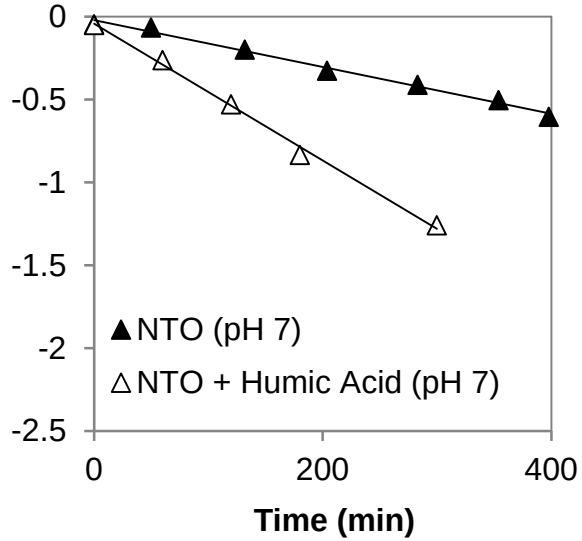
Acidification of Water and Soil

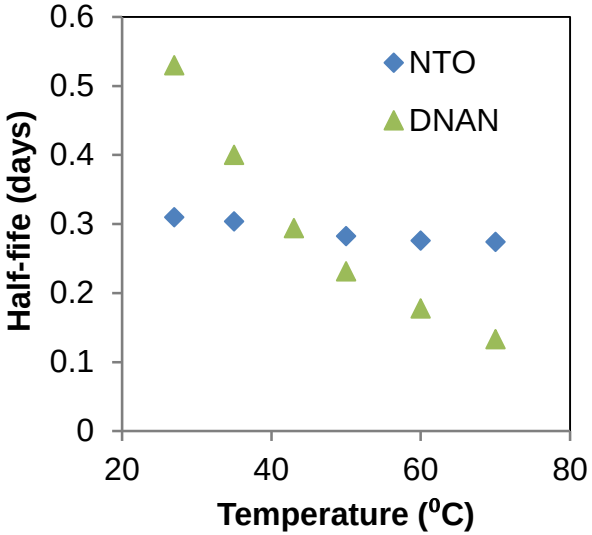


Dissolution of IM formulations resulted in solution with low pH

Phototransformation in Solution

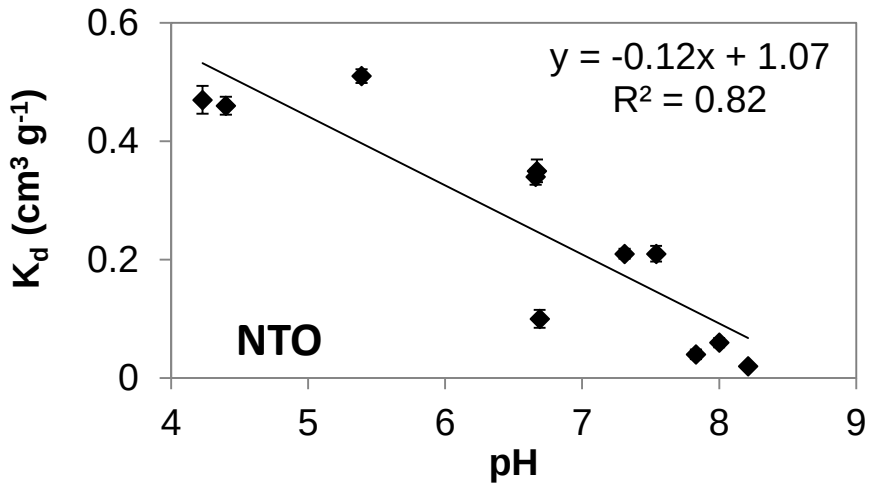


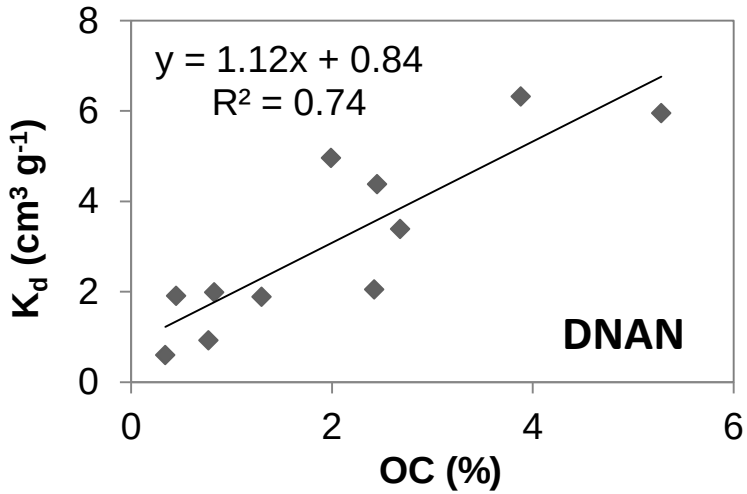


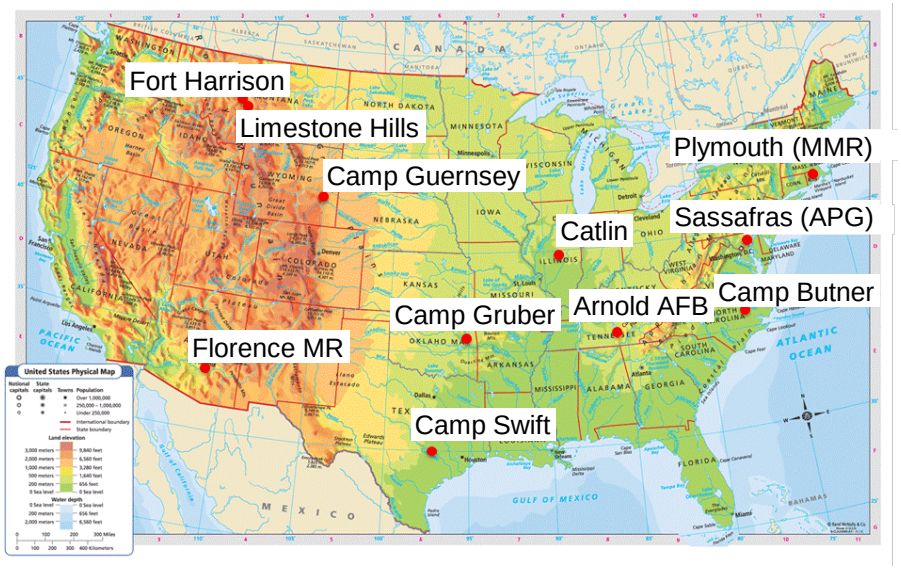


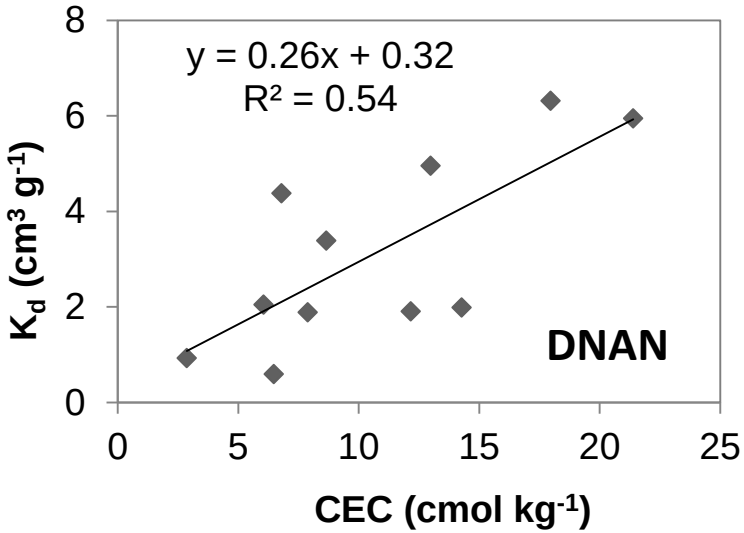
- Both NTO and DNAN experienced phototransformation in solution
- NTO phototransformation was influenced by pH and enhanced in the presence of dissolved organic matter
- DNAN phototransformation rates increased with temperature

Soil Adsorption

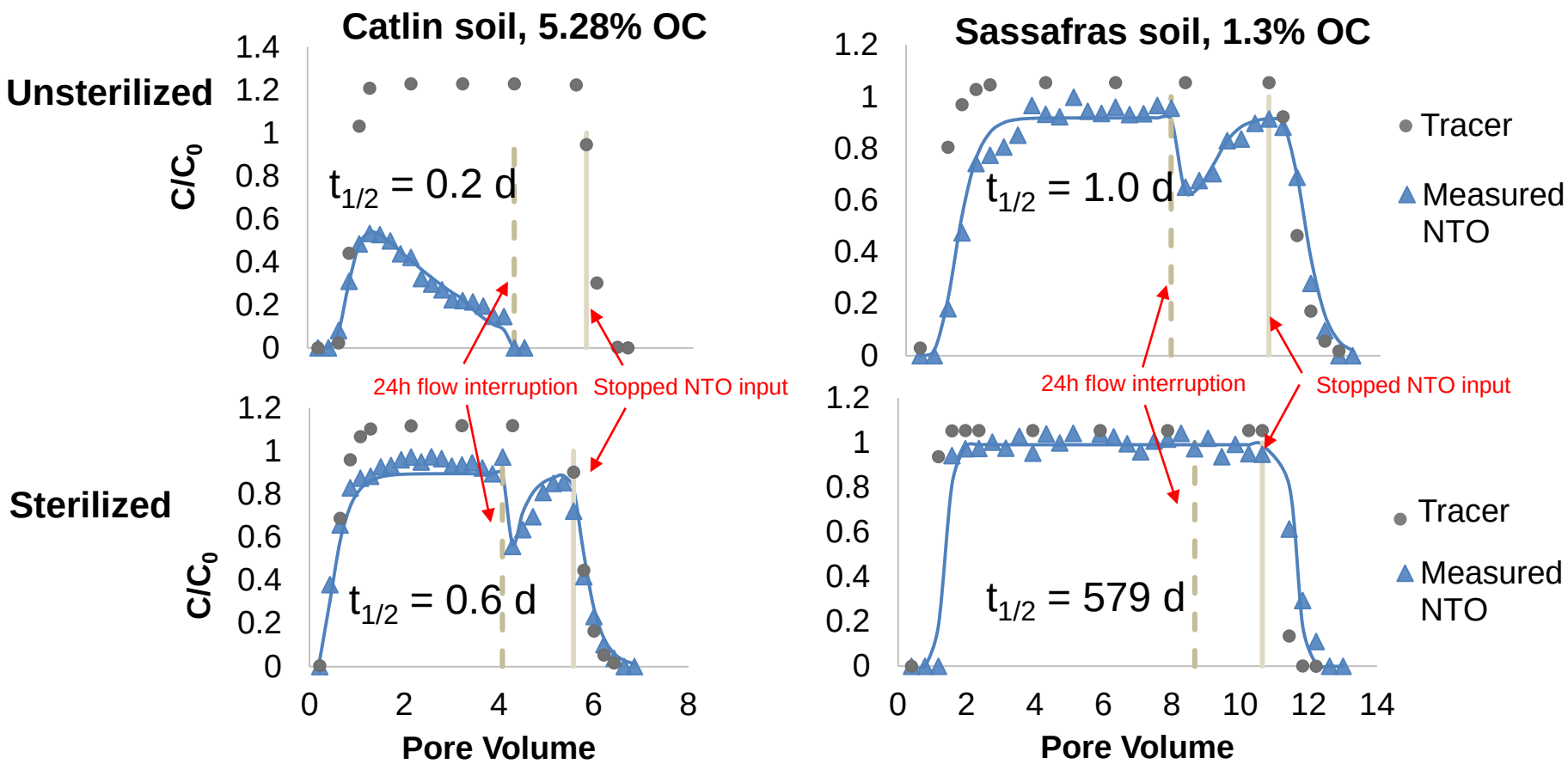






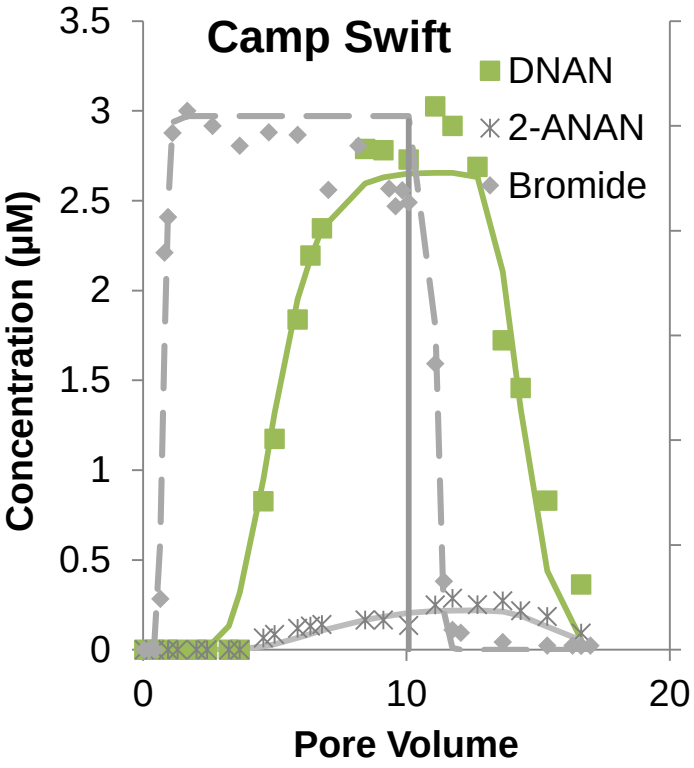


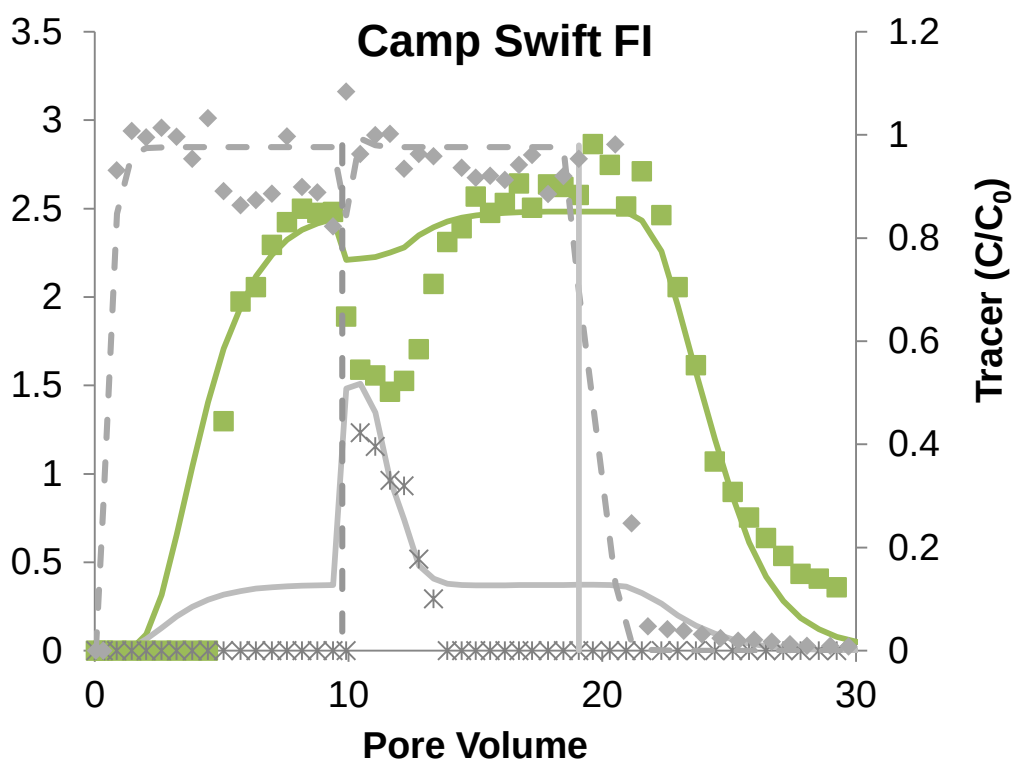
NTO Transport in Soils



Low adsorption; biotic and abiotic transformation, particularly in high OM soils

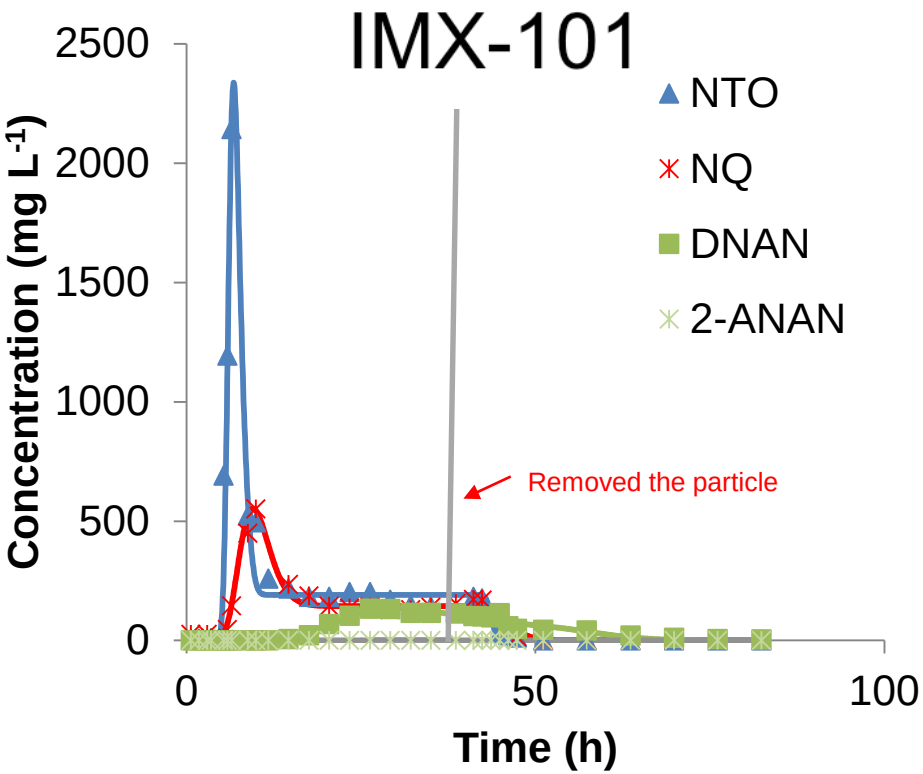
DNAN Transport in Soils

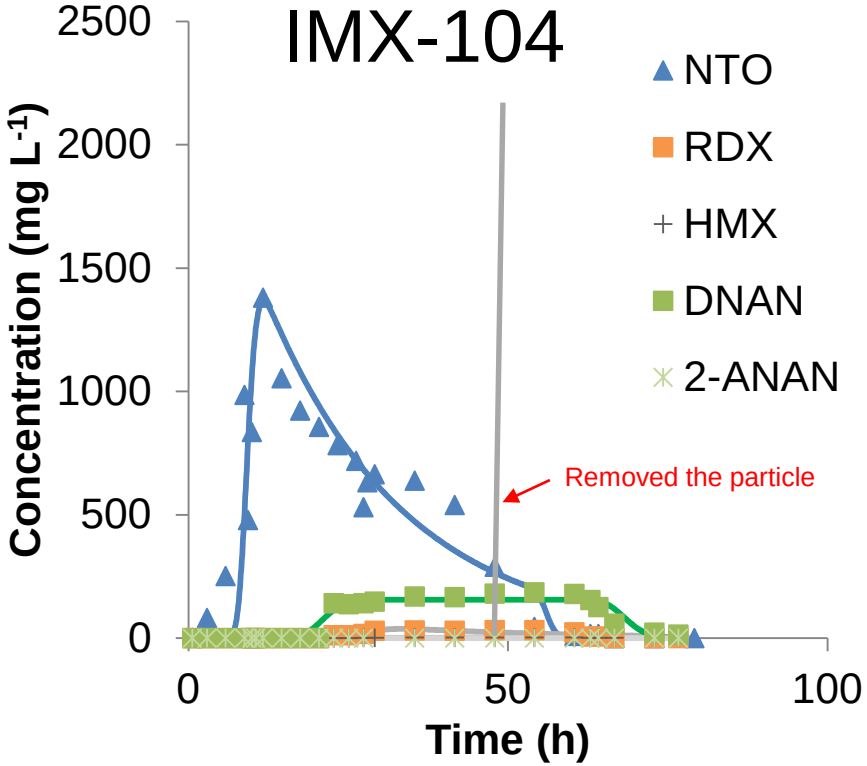




Higher adsorption; biotic and abiotic transformation in high OM soils

Camp Swift Dissolution and Transport





- NTO: large initial concentration decreasing over time
- DNAN: much lower concentration, stable over time

Conclusions

- IM constituents dissolve sequentially as predicted by solubility
- Fast removal of most soluble components results in porous particles that can break up easily
- Photo-transformation of outdoor IM was evident but its contribution is smaller than for traditional explosives due to faster dissolution
- NTO adsorption in soils was very low and depended on soil pH

Conclusions (Cont'd)

- DNAN was being adsorbed with attenuation higher in soils with high OM and clay
- NTO and DNAN were transformed in soils
- High release of NTO and its low attenuation in soils indicate high risk of its transport to the ground and surface waters
- DNAN slower dissolution and TNT-like behavior in soils indicates lower potential for off-site transport

Acknowledgments

- Dr. Susan Taylor, ERDC-CRREL
  - Specialist in dissolution processes in particulate formulations
- Dr. Mark Brusseau, University of Arizona
  - Specialist in fate and transport of organic contaminants
- Dr. Rose Pesce-Rodriguez, Army Research Laboratory
  - Providing expertise in photodegradation of explosives
- Dr. Jiri Simunek, UC Riverside
  - Providing expertise in fate and transport modeling
- Marianne Walsh, ERDC-CRREL
  - Analytical chemist and specialist in energetics identification and quantification
- Noah Mark, University of Arizona
- Jennifer Arthur, University of Arizona

For additional information, please visit
https://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminants-on-Ranges/Characterizing-Fate-and-Transport/ER-2220

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Q&A Session 2

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Biodegradation of Insensitive Munition Compound NTO to Mineral Products

James Field, Ph.D.
University of Arizona, Tuscon

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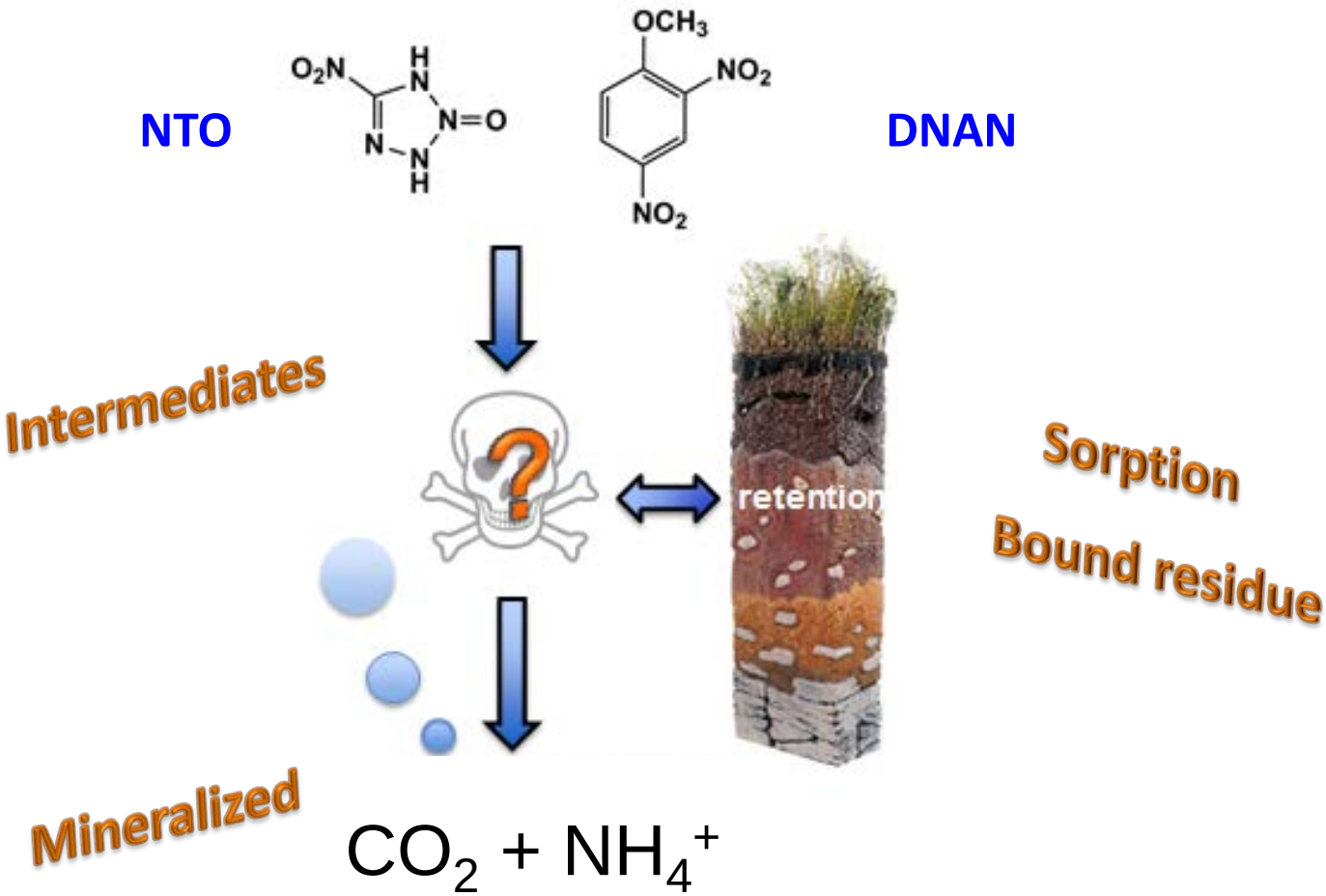
What is the Fate of Insensitive Munitions Compounds (IMC) in Soil?

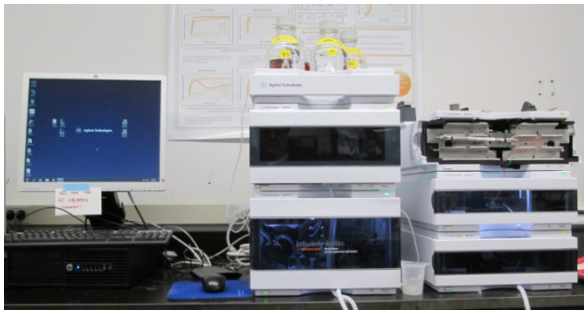


Objectives

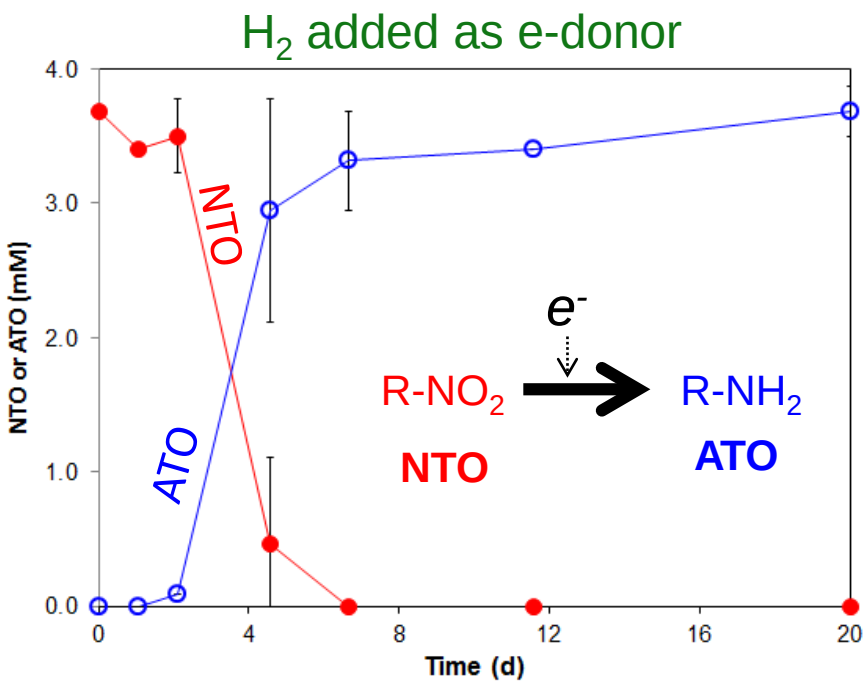
- Biodegradation/biotransformation of NTO in soil?
- Anaerobic – aerobic pathway for NTO?
- Feasibility of NTO biodegradation in bioreactor?
- Microorganisms involved?

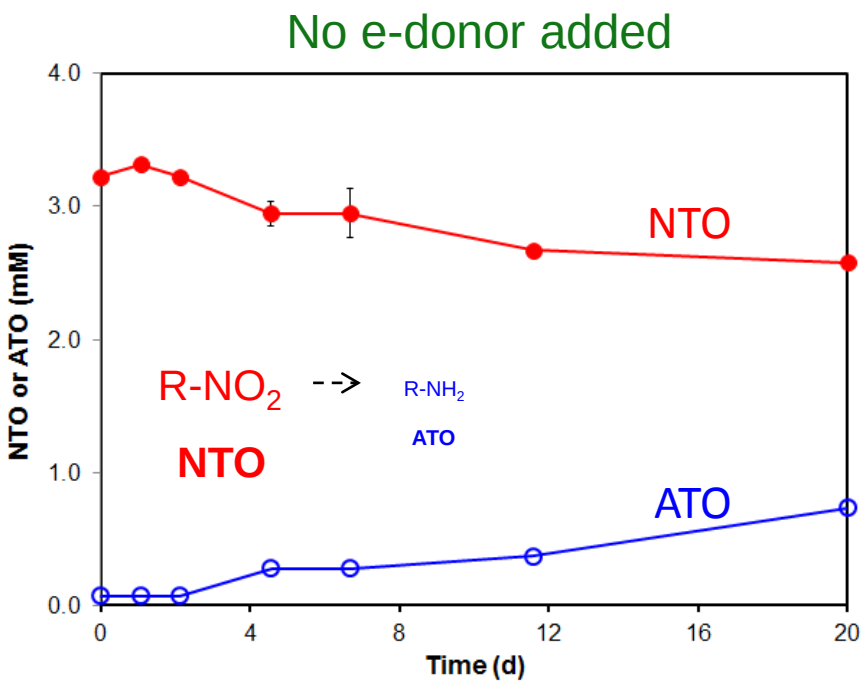
Technical Approach

- Biotransformation NTO
- 160 mL serum flasks
- 5 to 50 g/L soil
- Headspace aerobic (80:20 He:O_2); Headspace anaerobic (80:20 He:CO_2)
- Anaerobic: Add electron donor (H_2 and/or pyruvate)
- Measure metabolites HPLC
- HPLC DAD NTO and ATO
  - Hypercarb column
  - 0.1% TFA buffer/acetonitrile; gradient
  - Detector 340 nm (NTO) and 216.5 nm (ATO)
- Other methods
  - GC-TCD to measure CO_2
  - IC to measure NH_4^+, NO_2^- and NO_3^-



NTO is Reduced to ATO in Soils if Electron-Donating Substrate is Added

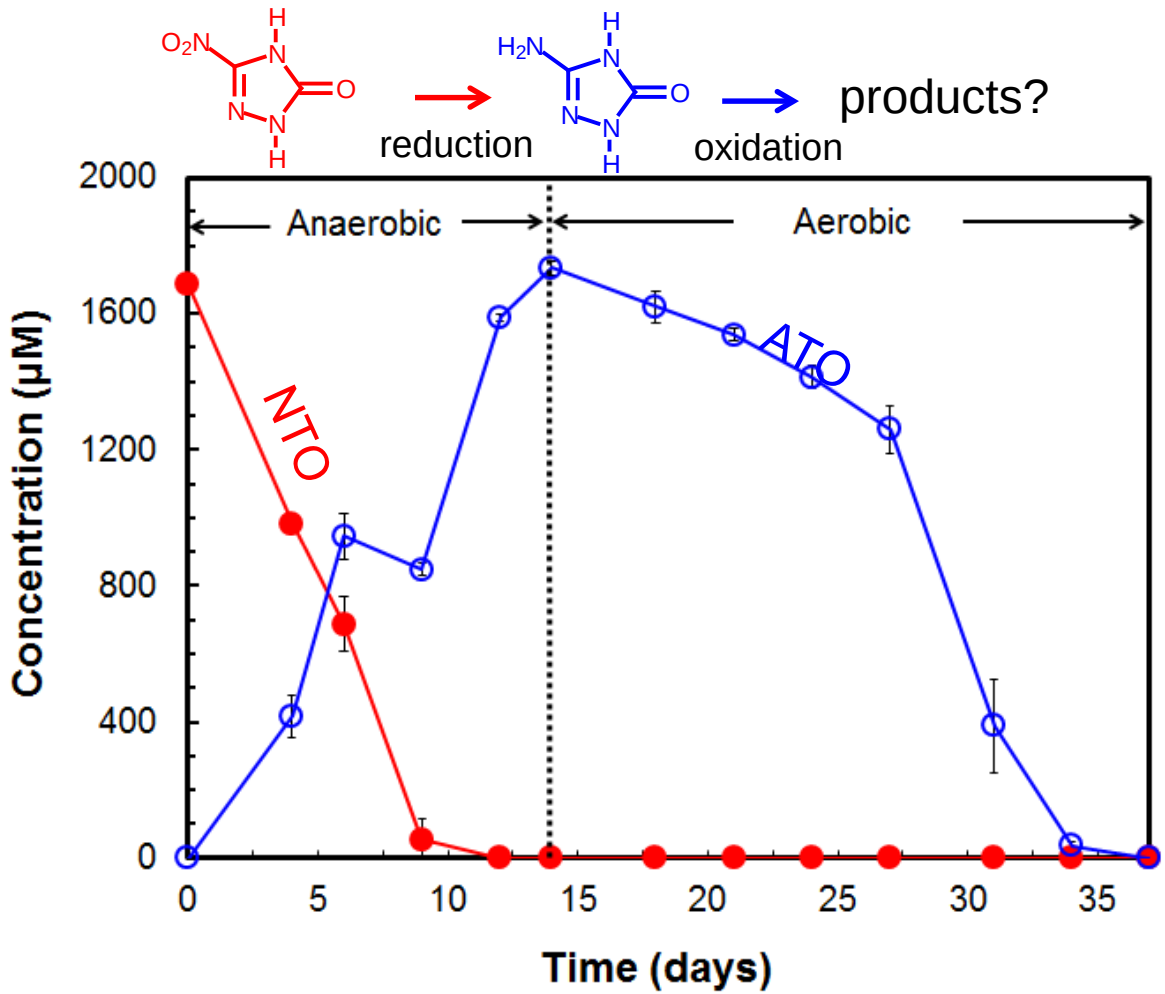




- Anaerobic biotransformation of NTO in soil microcosms
  - NTO incubated with soil (5 g dry wt/L) with H_2 as electron donor

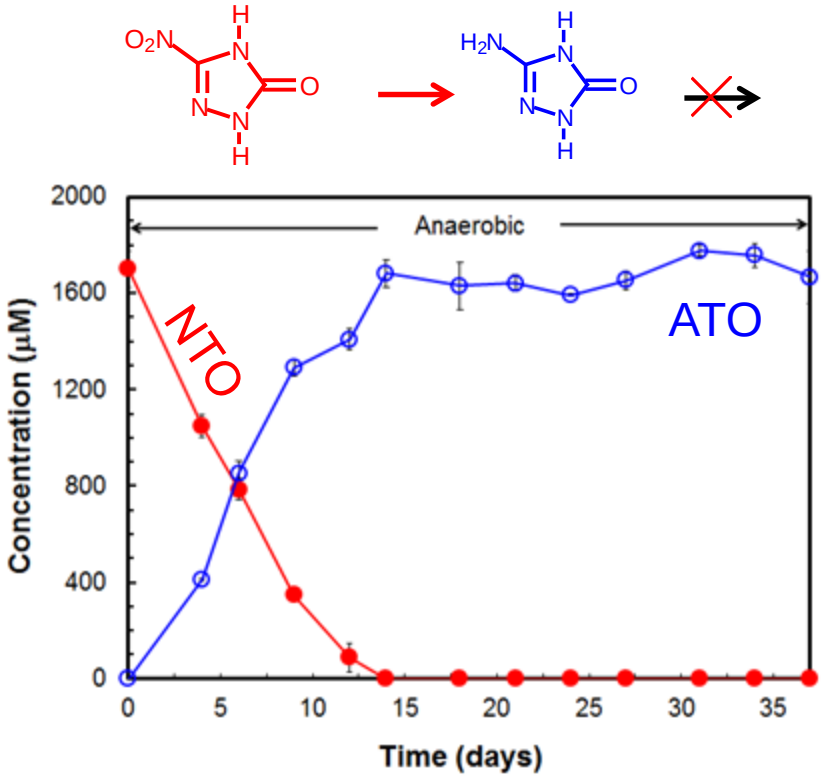
Krzmarzick et al., 2015; Environ. Sci. Technol. 49, 5681−5688

Anaerobic-Aerobic Biodegradation of NTO in Soil

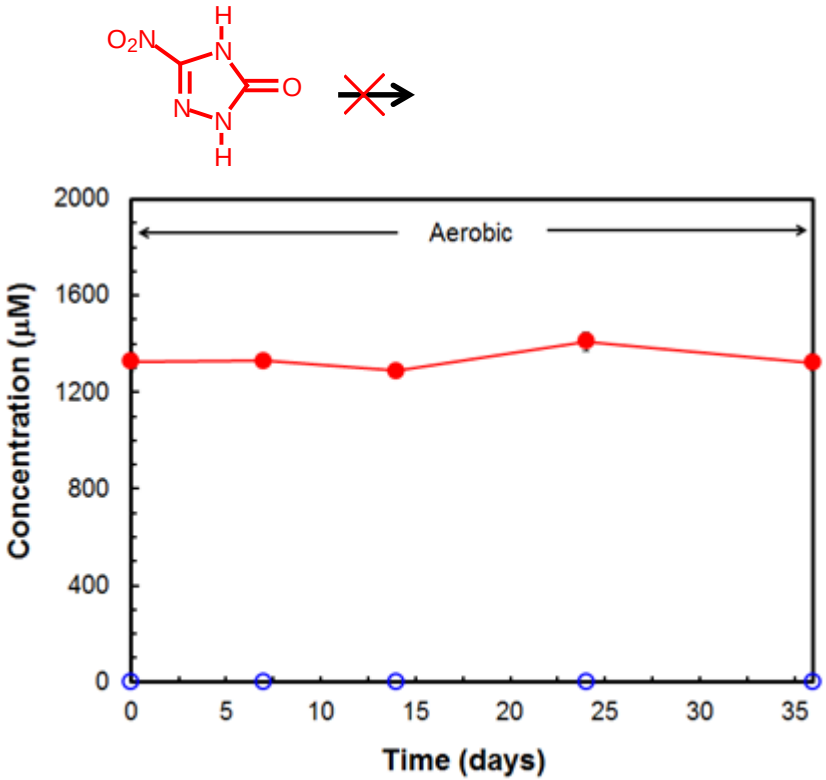


NTO incubated with Gortner soil (10 g dwt/L) and pyruvate (anaerobic stage)

What Happens if Incubation is Anaerobic Only? Or Aerobic Only?

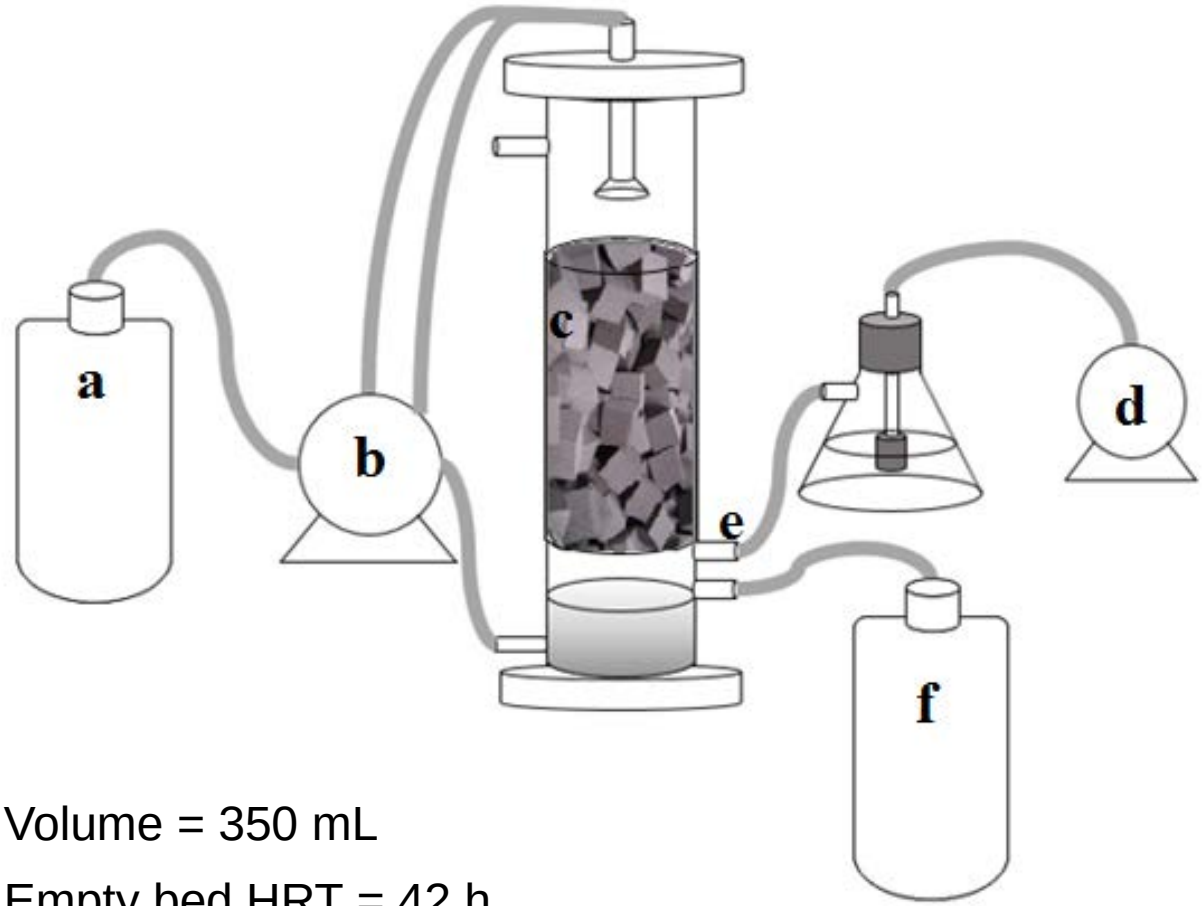


Anaerobic Only



Aerobic Only

Biotrickle Reactor Set-Up



Influent **(a)**,
peristaltic pump **(b)**,
polyurethane foam blocks **(c)**,
air compressor **(d)**,
humid air inlet **(e)**,
effluent **(f)**

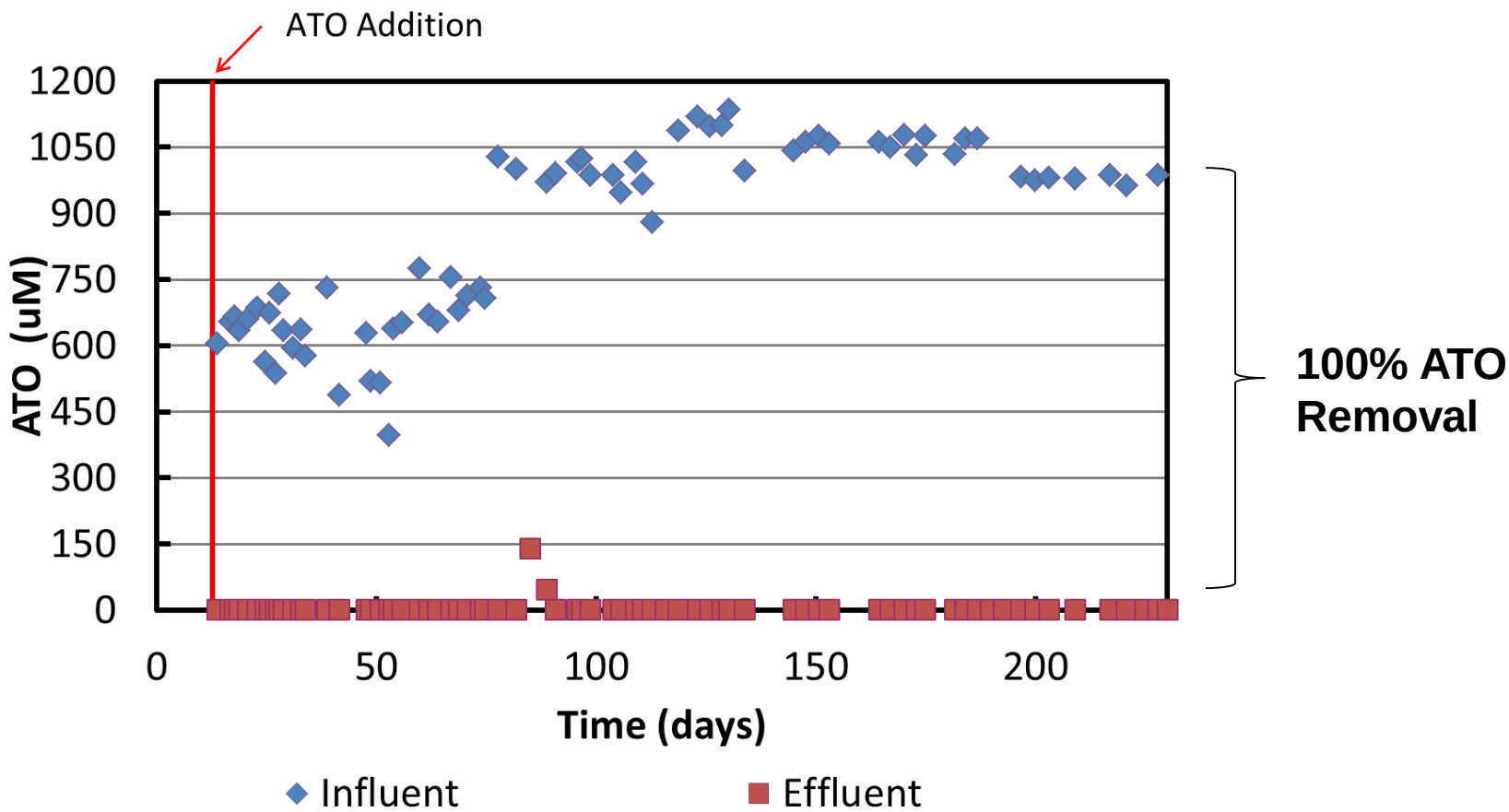
Volume = 350 mL

Empty bed HRT = 42 h

50% volume filled with 1 cm^3 polyurethane foam blocks

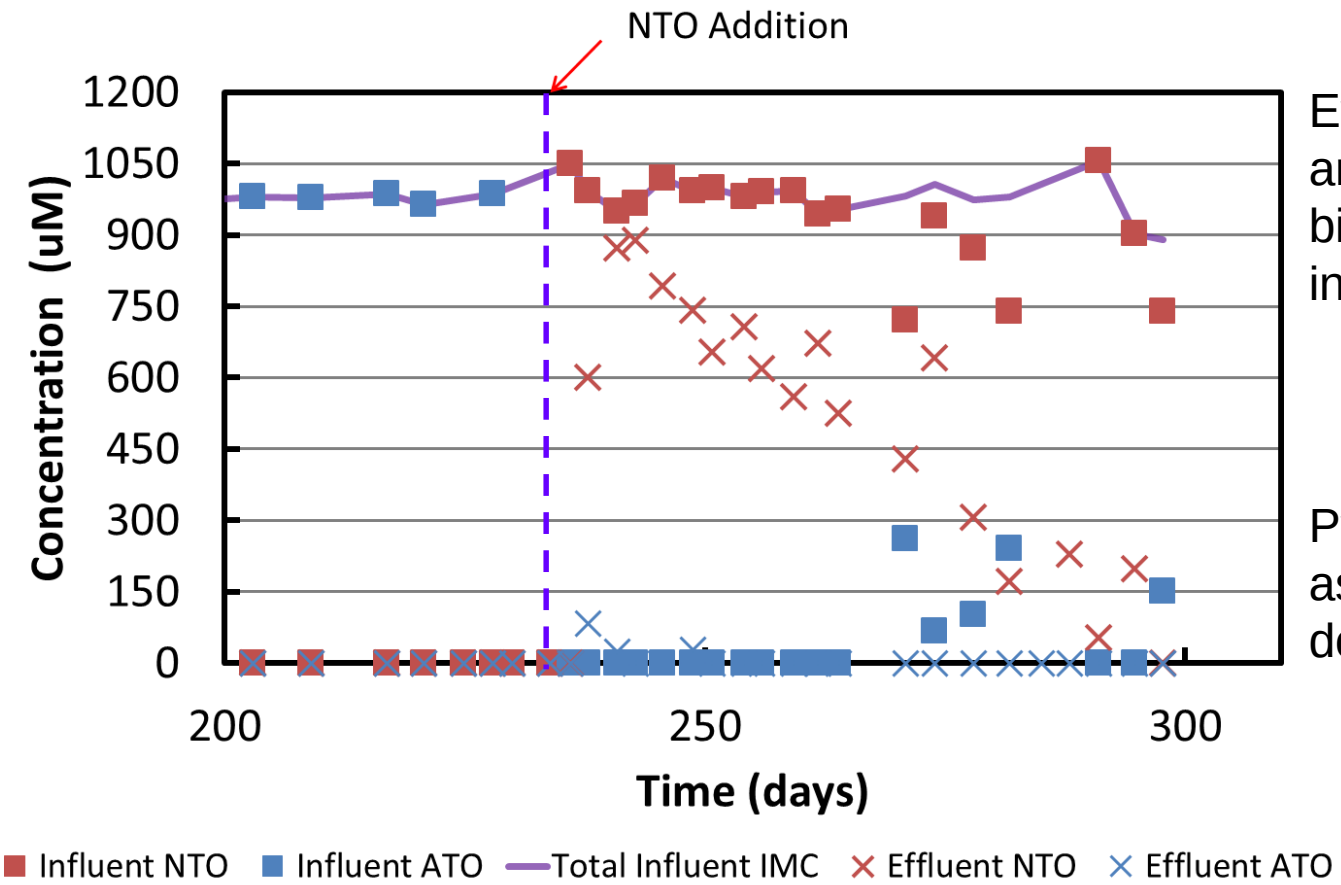
Biotrickle Reactor ATO Biodegradation Period

ATO (as sole C and N source) completely biodegraded in biotrickle reactor



Biotrickle Reactor NTO Biodegradation Period

After acclimation, NTO is degraded, ATO does not accumulate as product

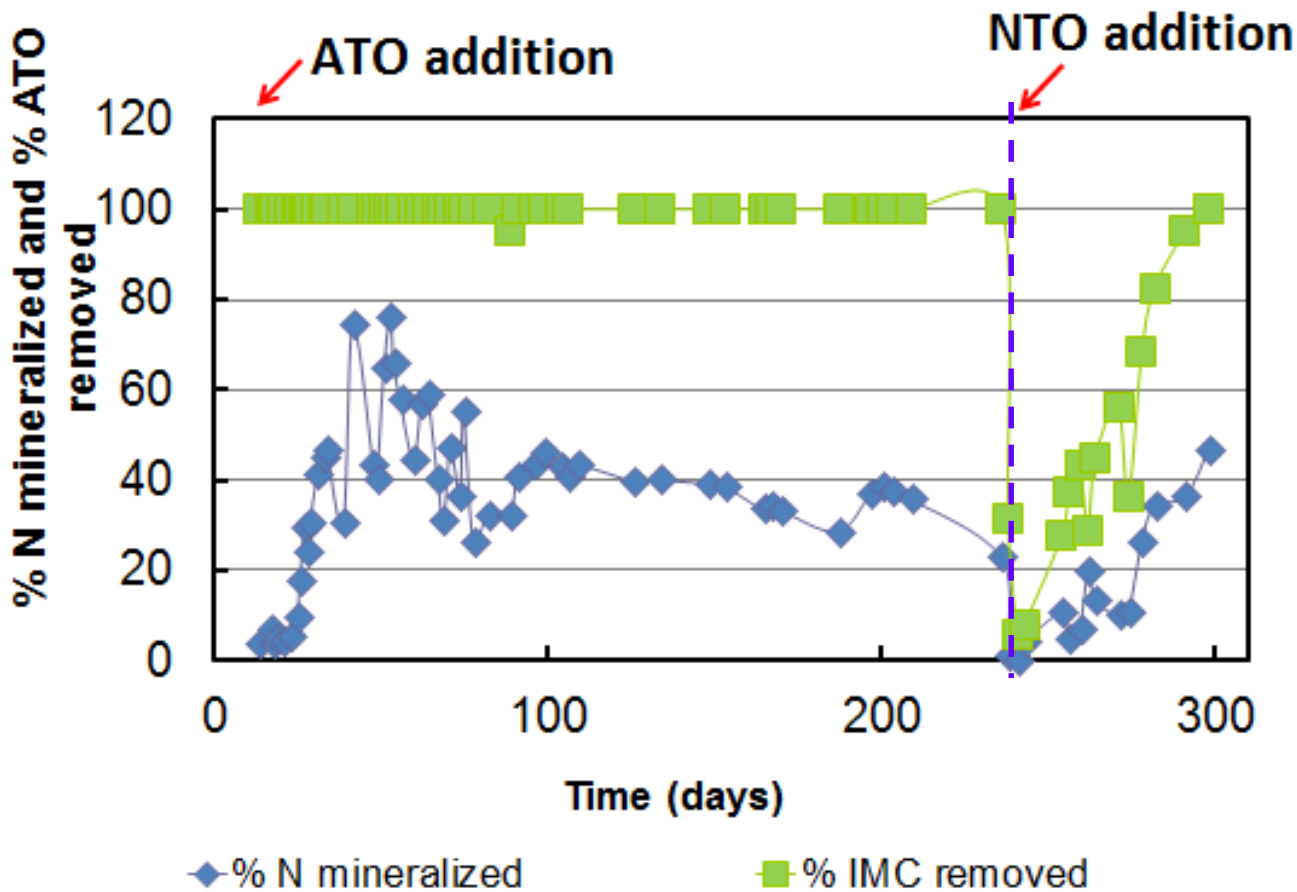


Evidence of anerobic-aerobic biodegradation in foam blocks

Pyruvate used as electron donor (1.8 mM)

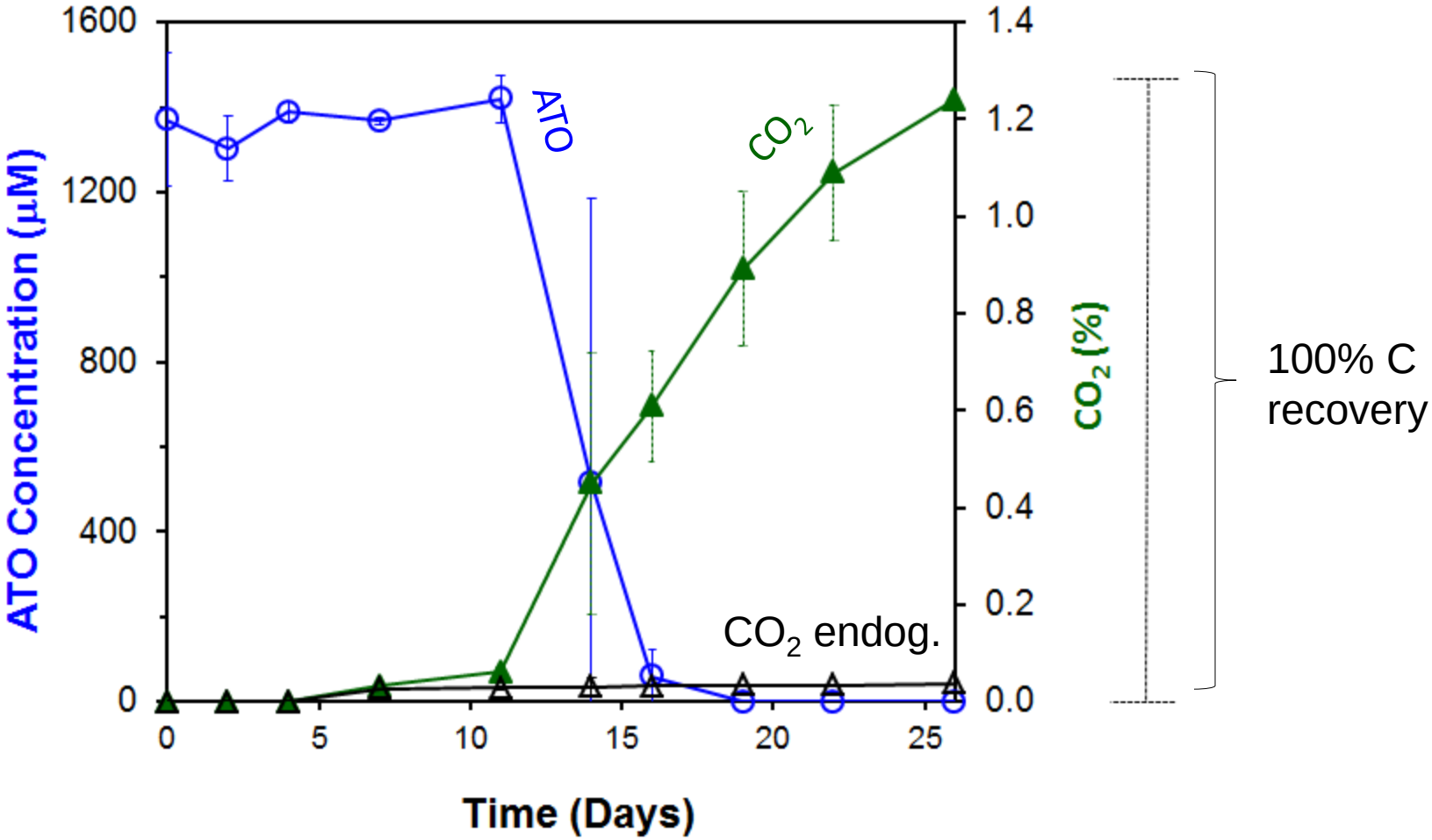
Nitrogen Mineralization in Bioreactor

N in ATO or NTO mineralized by 40-50% to ionic inorganic N species



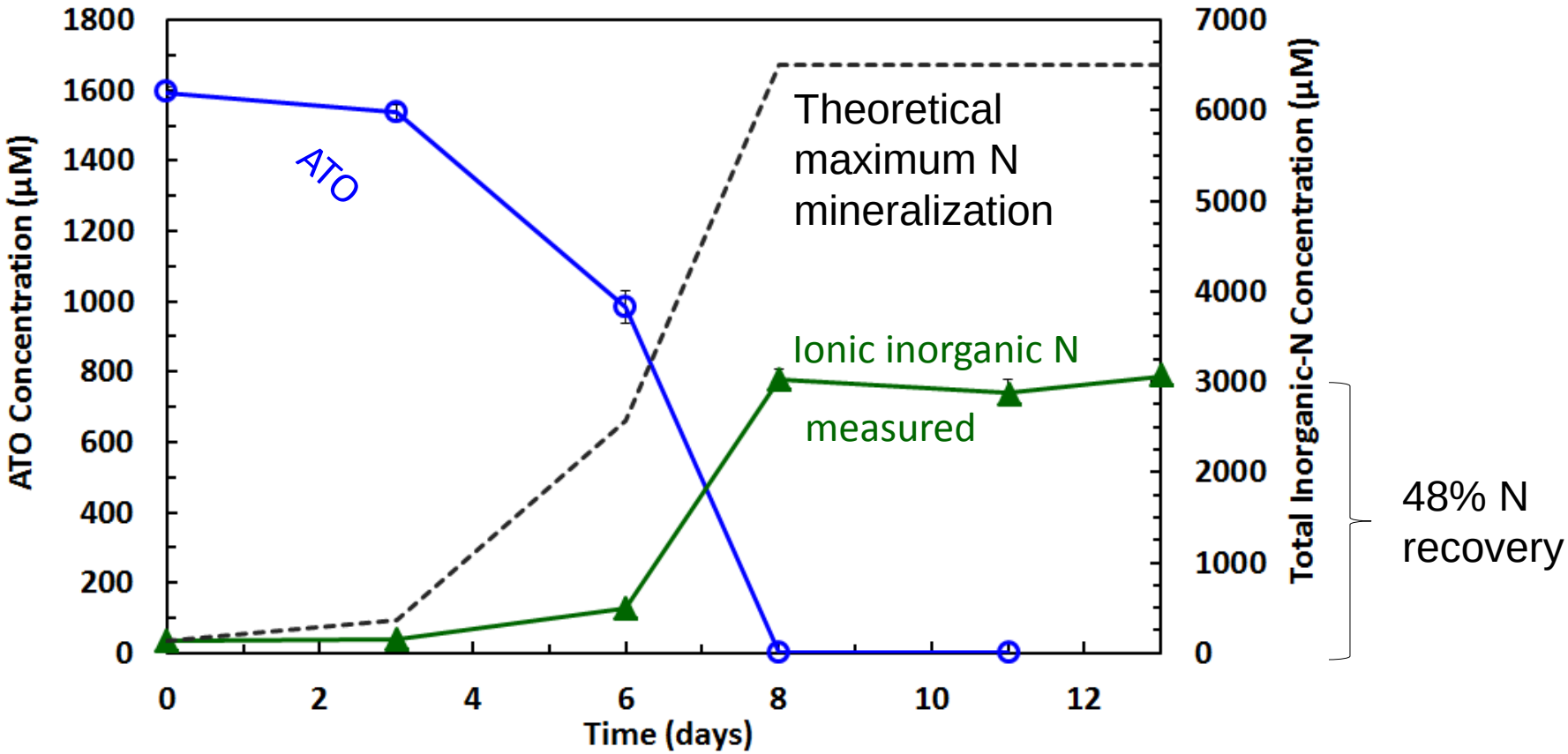
Carbon in ATO 100% Mineralized

Aerobic ATO Biodegrading Enrichment Culture (26th transfer 10% v/v)



Nitrogen in ATO Highly Mineralized

Aerobic ATO Biodegrading Enrichment Culture (28th transfer 10% v/v)



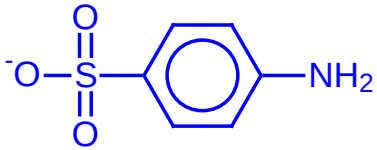
Clone Library of Enrichment Culture Indicates Presence of Aromatic Amine Degraders

Universal Bacterial 16s rRNA genes

6 of 25 clones *Hydrogenophaga* sp. PBC (>99% match)

4 of 25 clones *Ralstonia* sp. KN1 (>99% match)

Co-culture of *Hydrogenophaga* and *Ralstonia* known to biodegrade 4-aminobenzenesulfonate



$\rightarrow NH_4^+ + SO_4^{2-}$

Gan et al., 2011
Chemosphere 82:507-513

2 of 25 clones *Nitrospira* sp. KN1 (>99% match)

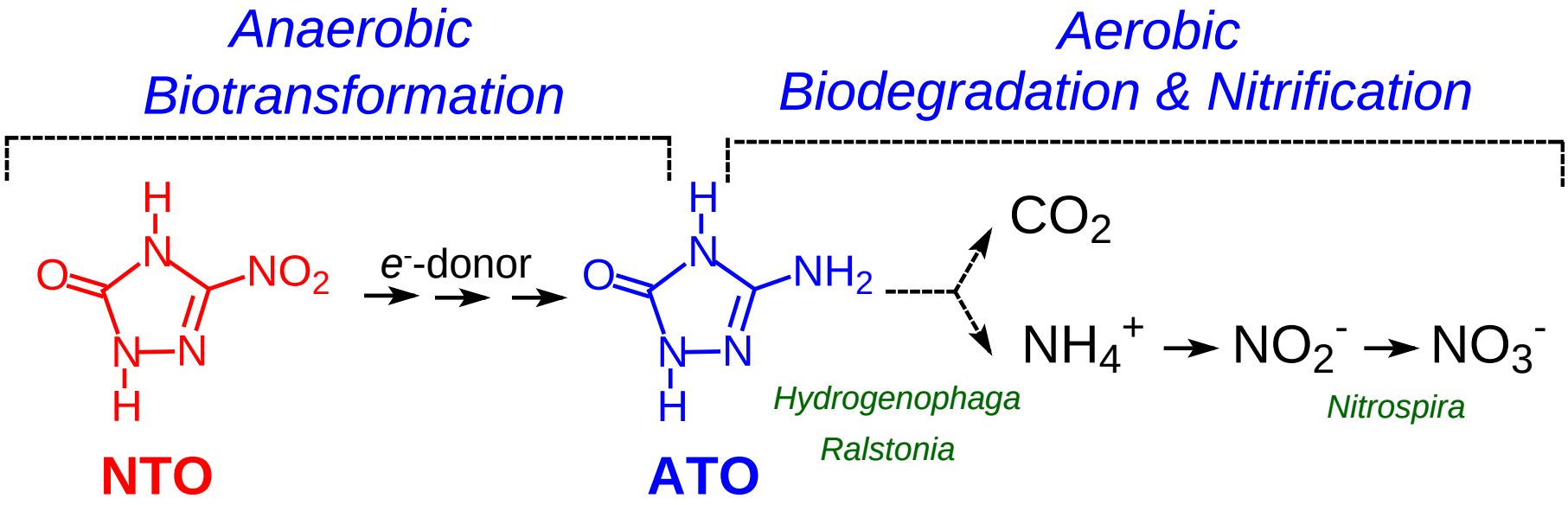
$NO_2^- \rightarrow NO_3^-$

Well known to nitrifying bacterium

Conclusions

- Anaerobic-aerobic biotransformation sequence promotes NTO biodegradation
- Complete mineralization of ATO-C
- Mineralization and nitrification of ATO-N
- *Hydrogenophaga* and *Ralstonia* implicated as suspect responsible ATO degraders
- NTO can be converted to ATO and biodegraded in biotrickle reactor (feasible via anaerobic microniches)

Conclusions (Cont'd)

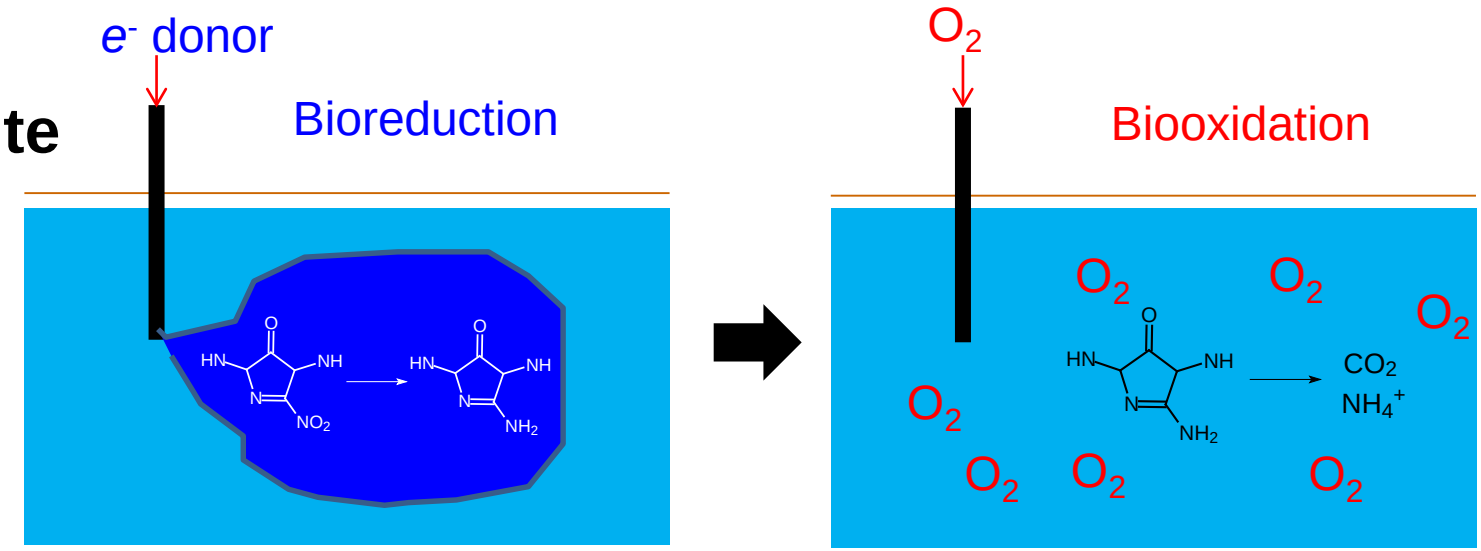


Suggestions to Practitioners

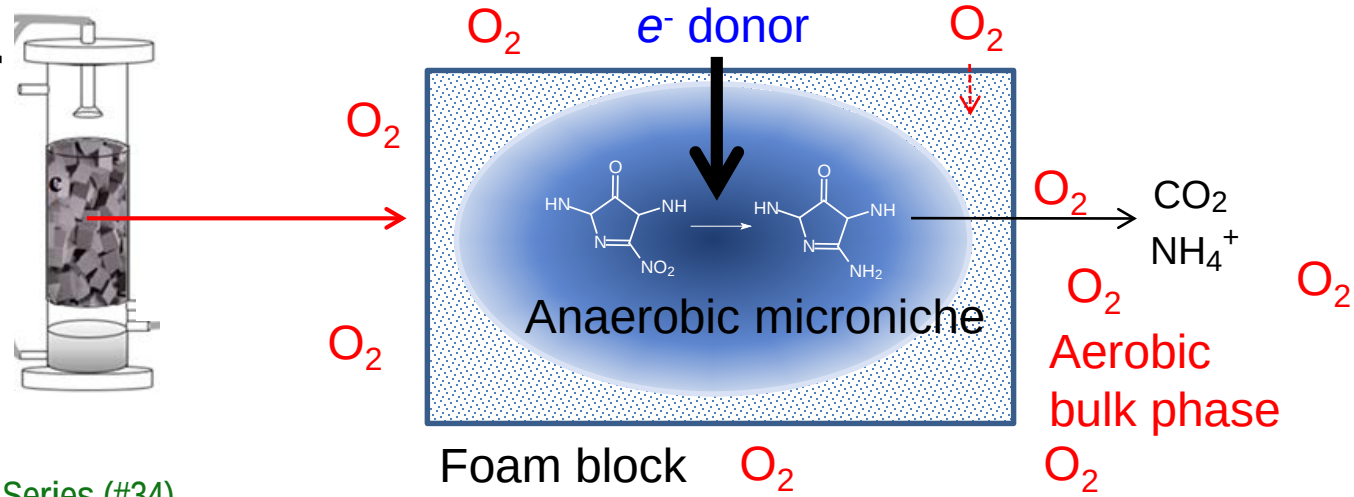
- Add electron-donating substrate to soil to promote anaerobic reduction of NTO
- After reduction event, provide aerobic conditions
- Future goal
  - qPCR primers to detect ATO-degraders at any give site
- Bioaugmentation of ATO-degraders may be required at sites lacking ATO-degraders
- Continuous bioreactors with aerobic and anaerobic microniches can reliably biodegrade NTO

Suggestions to Practitioners (Cont'd)

Field Site



Bioreactor



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https://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminants-on-Ranges/Characterizing-Fate-and-Transport/ER-22212

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http://abrell.faculty.arizona.edu/serdp

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Q&A Session 3

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The next webinar is on June 16, 2016

“Quality Assurance Project Plan (QAPP) for Advanced Geophysical Classification Investigations – Part 2”

Survey Reminder

Please take a moment to complete the survey that will pop up on your screen when the webinar ends

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