

FINAL REPORT

Incorporation Transformation Products into Models of the Environment Fate of Insensitive Munition Constituent

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4. LIST OF ACRONYMS

Acronym	Definition
(I)MCs	(Insensitive) Munition Compounds
ANAN	Aminonitroanisole
BBD	Biocatalysis/Biodegradation Database
COSMO	Conductor-Like Screening (Solvation) Model
CTS	Chemical Transformation Simulator
DNAN	2,4-dinitroanisole
DRDC	Defense Research and Development Canada
EAWAG-BBD	Swiss Federal Institute of Technology, Biocatalysis and Biodegradation Database
EPA-CTS	US Environmental Protection Agency, Contaminant Transformation Simulator
ERDC	Energy Research and Development Center
FRAMES	Framework for Risk Analysis in Multimedia Environmental Systems
GUI	Graphical User Interface
IMC	Insensitive Munitions Constituent
Koc	Organic-Carbon Partition Coefficient
Kow	Octanol-Water Partition Coefficient
LS-MS	Liquid Chromatography, Mass Spectrometry
ML	Machine Learning
NACs	Nitroaromatic Compounds
NQ	Nitroguanidine
NTO	Nitrotriazolone
ODE	Ordinary Differential Equation
ORP	Oxidation Reduction Potential
PChPs	Physicochemical Properties
pp-LFERs	Polyparameter Linear Free Energy Relationships
PPS	Pathway Prediction System
QSARs	Quantitative Structure-Activity Relationship
RAIS	Risk Assessment Information System
RDX	1,3,5-trinitro-1,3,5-triazacyclohexane
RMG	Reaction Mechanism Generator
RP	Reaction Predictor
S	Water Solubility
SPARC	SPARC Performs Automated Reasoning in Chemistry
TNT	2,4,6-trinitrotoluene
TREECS	Training Range Environmental Evaluation and Characterization System

5. KEYWORDS

Insensitive Munition Compounds, Transformation Products, Pathway Prediction, Machine Learning Models, Microcosm Experiments, Physicochemical Properties, pp-LFERs.

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7. ABSTRACT

Introduction and Objectives: A strategic research priority for the U.S. Department of Defense is the development and deployment of munitions formulations based on insensitive munition compounds (IMCs) rather than traditional explosives such as TNT, RDX and HMX. To ensure that these materials do not create new environmental contamination issues, there is a need to understand the environmental fate and the risk associated with the manufacturing and use of the new generation of IMCs before putting these chemicals into widespread use. This project aimed to provide modeling tools that use methods that are versatile, flexible, and robust to predict reaction products, transformation pathways, and fate-determining physicochemical properties of existing as well as candidate/future (I)MCs.

Technical Approach: The methods employed in this project included: (i) determination of IMC transformation pathways and products, (ii) determination of the fate-determining properties of IMC transformation products, and (iii) development of models that describe the fate and transport of IMC transformation products.

Results: For the identification of transformation products (i), data on experimentally observed products and reaction rates were mined from the literature and existing models for the prediction of reaction products and transformation pathways were evaluated. The results from these Tasks guided the development of this project's own machine-learning based predictive model, Arrows.

For the determination of product properties (ii), data on physicochemical properties were collected from the literature and a few models were tested. The data suggest that IMCs and their transformation products have an increased environmental mobility relative to legacy MCs. The models were able to estimate properties of legacy MCs and IMCs with similar molecular structures within reasonable prediction errors, but yielded larger uncertainties for IMCs with unconventional molecular structures.

With respect to the fate and transport models (iii), it was determined that the transformation in surface soils was the most important capability lacking in the pre-existing TREECSTM model framework. The TREECS Soil Surface Contaminant Fate Model was upgraded to include the capability to track mass from parent to daughter species and through multiple succeeding reaction pathways. The capability to transmit fluxes of multiple tracked species to downgradient media (vadose zone, surface water) was also added.

Benefits: The benefits of this project span both specific and general applications. The specific application is enabling DOD training site managers to include consideration of (I)MC transformation and products into the modeling they do for evaluation of exposure assessment scenarios, risk, remediation, etc. General applications are through the advancement of methods for prediction of transformation pathways, chemical properties, and reactive transport.

8. EXECUTIVE SUMMARY

Introduction

Many countries, especially the United States, aims to develop insensitive munitions (ISMs) to replace TNT, RDX and HMX in the manufacturing of munitions. It is imperative, therefore, to understand the environmental fate and the risk associated with the manufacturing and use of the new generation of IMCs before putting these chemicals into widespread use. This project aimed to provide modeling tools that use methods that are versatile, flexible, and robust to predict reaction products, transformation pathways, and fate-determining physicochemical properties of existing as well as candidate/future (I)MCs.

Technical Objectives

To provide a complete assessment of the potential environmental effects of insensitive munitions constituents (IMCs), it is necessary to consider the *transformation products* and their fate and effects. The transformation of the highest priority IMCs—DNAN and NTO—has only recently been subjected to detailed investigation, so little is known about their transformation products, and even less is known about the fate and effects of these products. The currently available data on the environmental fate determining properties of IMC degradation products are limited, and rely heavily on estimation methods, most of which have not been validated for compounds of this type.

The overall goal of this project is to lay the foundation for comprehensive assessment of the fate and effects of IMC degradation products on training ranges and other DOD sites. The scope includes three major research objectives: (i) determination of IMC transformation pathways and products, (ii) determination of the fate determining properties of IMC transformation products, and (iii) development of models that describe the fate and transport of IMC transformation products. By integrating these three objectives into one concerted and coordinated multi-investigator effort, we also expect to provide assessment of the consistency and accuracy of the information on all aspects of this problem, thereby increasing confidence in the reliability of the modeling results.

Technical Approach

This study focused on the transformation of two major IMCs (DNAN and NTO), but the models were designed to accommodate a range of (I)MC parent compounds and daughter projects. The models include all of the fate-determining processes

(volatilization, sorption, etc.), but with emphasis on transformation, the relative rates of competing transformation pathways, and the resulting variation in intermediate/products concentrations as a function of space and time.

The approach juxtaposes experimental and modeling Tasks within each Objective, so that each can benefit from the results of the other, resulting in the highest confidence in the overall outcome. Objective 1 (Product/Pathway Identification) adapted and used state-of-the-art algorithms for predicting contaminant transformation, with calibration and validation with experimental data obtained with bench-scale microcosm experiments. Objective 2 adapted and used the most promising algorithms for prediction of contaminant fate determining properties, with calibration and validation against new and previously measured property data. Objective 3 developed and tested a numerical (kinetic) model for IMC transformation and link this kinetic/reaction model to the leading reactive-transport model for characterizing DOD training sites with respect to (I)MC fate and effects.

Results and Analysis

Objective 1—Identification of Transformation Products:

Develop a comprehensive assessment of the major products from degradation of IMCs. The overall approach was to integrate and reconcile experimentally measured data with predictive model results.

The capabilities of a set of existing models for the prediction of transformation products were assessed for IMCs, including DNAN and NTO. The results of this comparative analysis revealed that almost all the models predicted the well-known, expected nitro-reduction products, i.e., amino-containing daughter products, primarily because those are the main/only components of their experimentally measured calibration sets. Only the more mature models, however, provided estimates beyond this first generation/level of transformation. These were the models with a larger extent/history of their calibration databases and/or with presence of an interconnexion system across their transformation libraries that triggered a reaction likelihood/prioritization scheme.

Using the findings above, a semiquantitative metric of evaluation was created for the identification of components and capabilities that should be incorporated into models currently under development, including a diverse and interconnected set of transformation libraries, and a robust reaction prioritization scheme. These criteria have now been implemented in a predictive model already under development by this group,

Arrows. This model not only contains a fairly diverse chemical reaction database but using cutting-edge computational tools it allows for the learning of chemistry across its database so that reaction functions can be created to identify future transformation reactions. In addition, Arrows uses a purely quantitative method based on computed reaction energies to prioritize likely transformation pathways. This predictive model, therefore, complements the efforts of the existing tools delivering a comprehensive network of estimated transformation products.

Objective 2—Determination of Product Properties:

Gather data on the fate/effects determining properties of the compounds. As in Objective 1, the overall approach was to integrate and reconcile predictive model results with experimentally measured data.

Three existing molecular-structure-based prediction models were used for the estimation of physico-chemical properties (PChPs) of (I)MCs and their transformation products. These predictions were compared against a set of experimentally measured aqueous solubilities (*S*) and octanol-water partition coefficients (*K_{ow}*) compiled from the literature for about 40 (I)MCs including their transformation products. All three models predicted the data with reasonable estimation errors for most of the compounds except for nitroamines, which have molecular structures that are largely foreign to the mainly nitroaromatic training sets of the tested models.

Both predictions and measured data, nonetheless, indicated that aliphatic IMCs (e.g., NTO) tend to be significantly more water soluble than legacy nitroaromatic MCs (e.g., TNT). Similarly, that reduction products (mainly aromatic amines) tend to be more soluble and less lipophilic than their parent compounds. These characteristics are indicative of poor sorption to soil and significant presence in the aqueous phase, which raise concerns for increased environmental mobility relative to parent compounds.

Objective 3—Development of Transformation Models:

Develop models of IMC fate and transport for risk assessment and site management (i.e., TREECS). The overall approach was to provide a validated framework for modeling IMC transformation that is integrated into TREECS or other site management tools.

A transformation capability feature was implemented into the surface soil contaminant fate model (SSCFM) in TREECS™. When this feature is used, additional chemical parameters that characterize transformation process are required for the model inputs. The enhanced soil model generates yearly or daily fate and export fluxes, as well as an additional transformation flux. Testing of transformation capability in SSCFM with

daily hydrology was performed by applying the model to a small arms firing range located at FLW, MO and running the same site conditions conducted previously for RDX and Pb. This POC application showed that the enhanced SSCFM performed well for tracking transformation flux from one MC to its daughter products and computing soil concentrations and transport and reaction process fluxes of daughter products. The SSCFM skill in predictions of field soil concentrations and transformation fluxes of MC in the AOI has not been validated due to a lack of field data at this time. The need remains to develop a controlled field case study utilizing well characterized MC loading to an AOI and measured multi-media concentrations in soils, groundwater, and surface water to validate the processes and parameterization of the enhanced SSCFM and test the skill of TREECS to track transformations through multi-media pathways.

Implications for Future Research and Benefits

The benefits of this project span both specific and general applications. The specific applications are to enable DOD training site managers to include consideration of (I)MC transformation and products into the modeling they do for evaluation of exposure assessment scenarios, risk, remediation, etc. For this type of application, technology transfer occurs mainly through integration directly into the TREECS (and its successor the Fate and Risk Evaluation System for Contaminants, FRESCO). Additional, general applications of the results from this project are through advancement of methods for prediction of transformation pathways, chemical properties, and reactive-transport models that can be adapted for assessing the environmental fate of other classes of contaminants. The application of methods developed in this project certainly can be applied to other MCs, but might also be extended to chlorinated solvents, or even emerging contaminant classes such as polyfluorinated alkyl substances, in future work.

9. OBJECTIVES

The original, general objective of this project was to provide a more comprehensive basis for assessing and managing the environmental risks of IMCs by extending current fate and transport models for DOD sites to include the products of IMC transformation. The scope of research needed to reach this goal was organized into three major objectives:

- **Objective 1—Identification of Transformation Products.** Starting with the project team's extensive experience with munition constituent fate and remediation, this objective developed a comprehensive assessment of the major products from degradation of IMCs. The overall approach was to integrate and reconcile experimentally measured data with predictive model results, in order to provide greater confidence in both (and improved models for predicting degradation pathways for contaminants, in general).
- **Objective 2—Determination of Product Properties.** Many of the degradation products identified in Objective 1 are not well characterized in terms of environmental fate or potential effects. Therefore, this objective focused on gathering data on the fate/effects determining properties of the compounds. As in Objective 1, the overall approach was to integrate and reconcile predictive model results with experimentally measured data, in order to provide greater confidence in the overall reliability of recommended property values.
- **Objective 3—Development of Transformation Models.** The transformation pathways and products determined in Objective 1 and fate-determining properties of transformation products determined in Objective 2 provided the background necessary to incorporate transformation into models of the fate, transport, and risk of IMCs. In this objective, the approach emphasized collaboration with existing efforts to develop models of IMC fate and transport for risk assessment and site management (i.e., TREECS). The overall approach was to provide a validated framework for modeling IMC transformation that is integrated into TREECS or other site management tools.

Each objective was pursued through three or more tasks, which are described below, in Section 11 on Methods and Results.

10. BACKGROUND

10.1. Insensitive Munitions Constituents

In the past, the defense industry has focused on the use of explosives such as RDX, HMX, and TNT, but handling and use of these chemicals is associated with high risk of unwanted detonation (Beauregard, 2011). Recently, there has been in many countries, especially the United States, to develop new IMCs to replace TNT, RDX and HMX in the manufacturing of munitions formulations (Oxley et al., 2016; Sikder & Sikder, 2004; Talawar et al., 2007). Several of these IMC—including 3-nitro-1,2,4-triazole-5-one (NTO) and dinitroanisole (DNAN) (**Figure 1**)—are already being used by the munitions industries to develop new insensitive formulations for DOD applications. Examples of these formulations include IMX 101 (DNAN, NTO, NQ) and IMX 104 (DNAN, NTO, RDX).

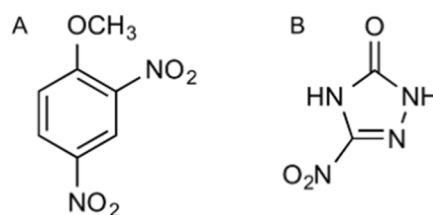


Figure 1. Structures of two characteristic IMCs: (A) 2,4-dinitroanisole (DNAN) and (B) 3-Nitro-1,2,4-triazol-5-one (NTO).

Based on past experience of the environmental and health risks associated with the use of the traditional explosives RDX, HMX and TNT (Gong et al., 2001; Lachance et al., 1999; Savard et al., 2010), it is imperative to understand the environmental fate and the risk associated with the manufacturing and use of the new generation of IMs before putting these chemicals into widespread use. In general, little information is available on the environmental fate, transport, and transformation pathways of these emerging IMCs (Dontsova et al., 2014; Fida et al., 2014; Hawari et al., 2015; Le Campion et al., 1999; Olivares et al., 2013) and even less is known about the environmental implications of their transformation products.

10.2. Pathways and Products of IMC Transformation

In many respects, the pathways and products of IMC transformation should be analogous to what is well established from years of research on conventional munitions constituents (TNT, RDX etc.) and this is generally supported by the data currently

available for DNAN and NTO (summarized in the description of **Task 1.2** in **Section 11.1.2**). However, these data reflect only a limited range of conditions, so it is not yet known how IMC transformation products vary across the full range of relevant environmental conditions. In time, this uncertainty will be alleviated as new measured data become available from on-going and future laboratory and field studies (presumably, including projects funded under ERSON-17-03). However, reliance entirely on experimentally determined products is not an efficient way to characterize the full network of potential degradation pathways.

A complementary approach to experimental determination of transformation pathways is to predict transformation pathways using empirical or theoretical models of chemical structure vs. reactivity. A variety of such models have been developed in recent decades, including the EPA-CTS¹ and EAWAG-BBD², and these are utilized in **Task 1.1**. In general, these models use chemical rules to generate a network of reaction steps that determine the individual intermediates and products, and overall pathways of transformation. To illustrate this, we used 2-nitroanisole and the EAWAG-BBD web interface to generate the predicted network shown in **Figure 2**.

Note that we originally intended to use 2,4-nitroanisole (DNAN), but the website generated an error because too many reaction pathways are predicted. This illustrates the limitations to existing models and demonstrates why further development is needed that is specifically directed toward predicting the products of IMC degradation. Furthermore, these models have the fundamental limitation that they only can predict reactions they have been trained to predict. This training requires rules developed and coded by experts, currently with privileged access to proprietary software. These restrictions are a major obstacle to improvement, validation, and acceptance of models that predict contaminant transformation pathways.

The ultimate solution to these problems will require (i) a more fully in silico (ab initio) approach to pathway determination, (ii) integration of pathway modeling with prediction of the relative favorability of competing pathways, and (iii) implementation with open-source software. Ideally, models of this caliber could predict the identity and concentrations of the major transformation intermediates and products, even over a range of reaction conditions. Currently, this is not practical because the necessary data to

¹ <https://www.epa.gov/nerl/> (Part of the National Exposure Research Laboratory's modeling System)

² <http://eawag-bbd.ethz.ch/predict/> (Biocatalysis/Biodegradation Database Pathway Prediction System)

parameterize the models is not available and the required computational resources are too expensive.

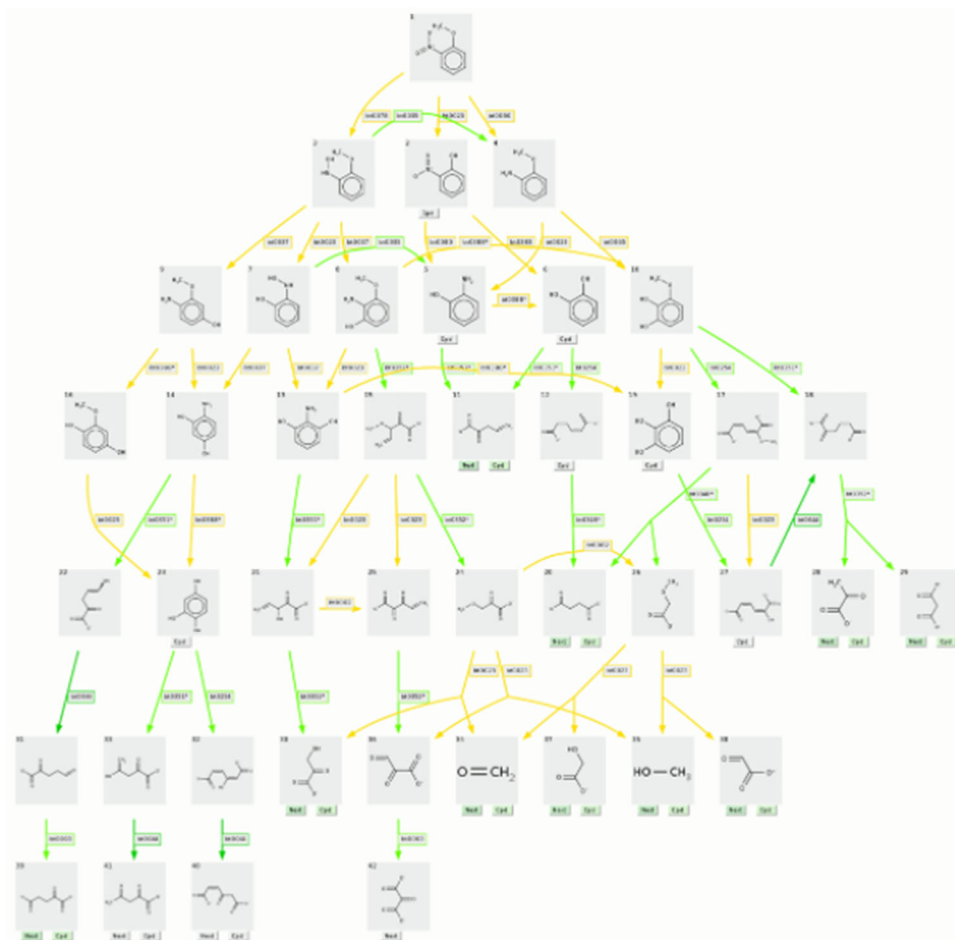


Figure 2. Sample reaction scheme for 2-nitroanisole predicted using the EAWAG-BBD Pathway Prediction System (PPS). Green Arrows indicate most likely reactions; yellow Arrows are less likely. To enlarge, go [here](#).

The most efficient approach to overcoming these challenges is not brute-force fully in silico computation, or determination of pathway models entirely from experimental results, but rather is a combination of empirical and theoretical methods that include quantitative structure-activity relationships (QSARs), combinatorial, and machine learning methods (Tratnyek et al., 2017). This would be a natural extension of the results of our previous SERDP project in this area (ER-1734) and is the approach we take in **Task 1.1** of this project.

10.3. Fate/Effects Determining Properties of IMCs

Once the network of pathways and products for IMC transformation are established, the next step in defining the fate and effects of IMCs and their degradation products is determination of “fate-determining properties” of each species. These properties include physico-chemical characteristics like solubility, Henry’s law constant, partition coefficient to soil organic matter, etc. For the major IMCs parent compounds (DNAN, NTO), some data for these properties have recently become available. For example, several of these studies have provided data on transformation pathways and kinetics (Perreault et al., 2012; Perreault et al., 2011; Salter-Blanc et al., 2015; Salter-Blanc et al., 2013; Sviatenko et al., 2014)

With respect to the daughter products from transformation of the parent IMCs, only a few studies have characterized them in any detail (Hawari et al., 2015; Salter-Blanc et al., 2016), and measured data on their fate-determining physico-chemical properties are very limited (more detail on the existing data are given under **Task 2.1**). Some of the data gaps may gradually be filled by research projects funded by SERDP, but other data gaps will persist until there is sufficient information on these compounds to develop predictive models—such as quantitative structure-activity relationships (QSARs)—for their properties. Currently, the only example of QSARs for predicting properties of energetic compound degradation products is for oxidation of aromatic amines by manganese oxide (MnO_2), which was **Task 4** of our SERDP project on predicting degradation kinetics of the IMC *parent* compounds. **Figure 3** from that work (Salter-Blanc et al., 2016) illustrates the opportunities and challenges that need to be addressed. It compares QSARs for a range of environmentally relevant oxidants, and shows that heterogeneous oxidants (mainly MnO_2 in soil) follow a trend that is much steeper than homogeneous oxidants (including various reactive oxygen species). This result could be the starting point for a general model that can provide the data to predict oxidative coupling (and immobilization) of the reduction products of energetic compounds, but further development is needed before models like that can be linked to contaminant fate models like TREECS. Overcoming this challenge was a focus of **Task 2.3**.

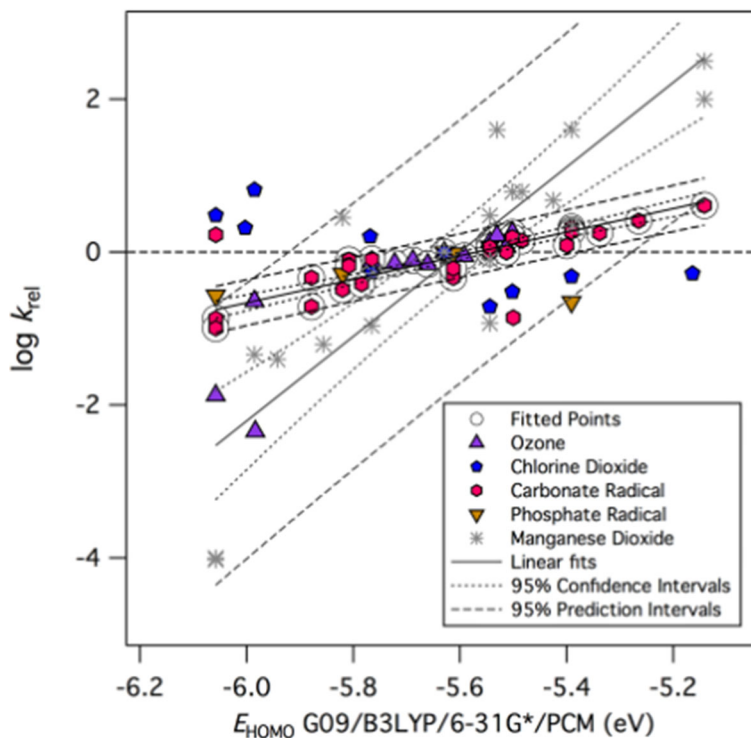


Figure 3. Example of QSAR meta-analysis for prediction of transformation product properties. All data are for aromatic amines, including model compounds and major nitro reduction products of energetic compounds. The rate constants (k_{rel}) are all normalized to 4-chloroaniline, in order to allow comparison among different oxidants and experimental conditions. Figure from [16].

10.4. Fate/Transport Models for Site Management

A wide range of models have been developed to describe contaminant fate, transport, exposure, and risk in all sorts of environmental media. Application of these models early in the development process for new materials is a major element of green (sustainable) engineering (Anastas & Warner, 1998). Considerable effort has been invested to achieve this goal as part of on-going efforts to develop new insensitive formulations of energetic materials for munitions, the most significant of which is the Training Range Environmental Evaluation and Characterization System (TREECS)³ developed by the U.S. Army Corps of Engineers. TREECS is a flexible, modular code that was designed to accommodate existing and future resources such as databases of chemical, munitions, and site characteristics. For example, TREECS utilizes other models for comprehensive risk

³ <https://treecs.el.erdc.dren.mil> (Training Range Environmental Evaluation and Characterization System)

assessment, such as ORNL's RAIS⁴ and PNNL's FRAMES⁵. All TREECS developments described herein have also been incorporated into TREECS successor, the Fate and Risk Evaluation System for Contaminants (FRESCO), and at the time of this report FRESCO v1.1 is available by email request to Dr. Ryder (jodi.l.ryder@usace.army.mil).

While the on-going efforts to link EPA-CTS with TREECS will strengthen the chemical information that TREECS has to model IMC fate and transport, and TREECS was designed to accommodate the capability to model contaminant transformation, the kinetic model and data for this have not been available and the capability has not been fully implemented. Therefore, additional development is needed before TREECS can be used to address the fate and effects of transformation products. In addition to the systematic and comprehensive definition of reaction pathways (**Objective 1**), and identification of validated chemical fate-determining properties (**Objective 2**), considerable effort is needed to determine how IMC transformation should be modeled and how the transformation model should be integrated into the reactive-transport framework provided by TREECS (**Objective 3**).

⁴ <http://rais.ornl.gov/> (Risk Assessment Information System)

⁵ <http://mepas.pnnl.gov/framesv1/> (Framework for Risk Analysis in Multimedia Environmental Systems).

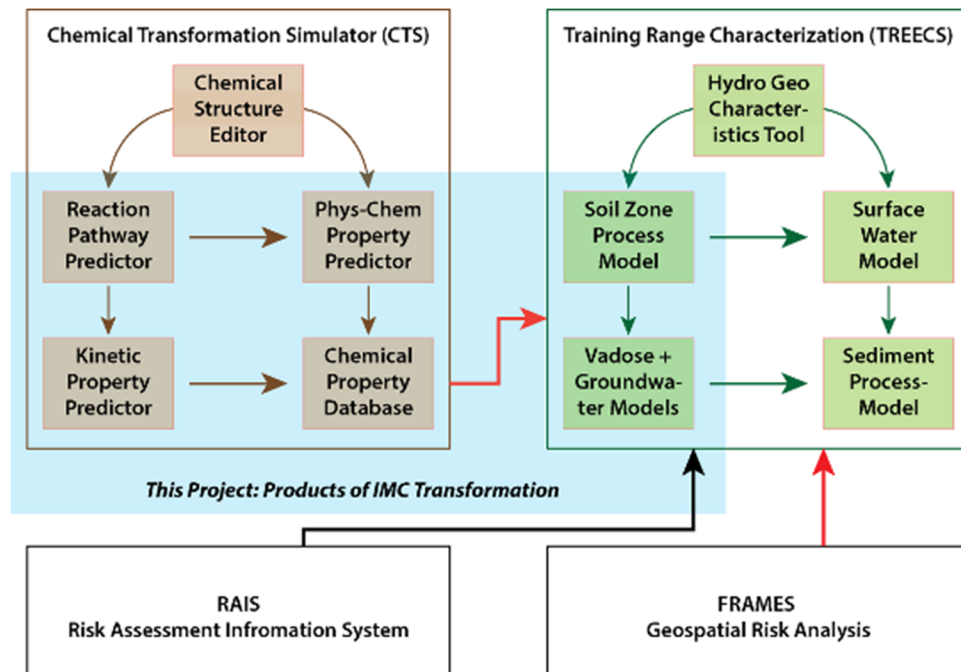


Figure 4. Schematic representation of the relationship between relevant modeling efforts. The red Arrows indicate connections already under development. The area shaded in blue is the scope of the proposed new project.

11. METHODS AND RESULTS

11.1. Objective 1—Identification of Transformation Products

This objective was meant to provide a comprehensive assessment of the major products from degradation of IMCs. The overall approach was to integrate and reconcile predictive model results with experimentally measured data, in order to provide a greater level of confidence in both. This objective included three specific tasks.

11.1.1. Pathway Prediction Models (Task 1.1)

11.1.1.1. Models Developed by Others

While there are similarities in the pathways and products of IMC transformation to those well-established from years of research on conventional munitions constituents (TNT, RDX etc.) with this generally supported by the data currently available for DNAN and NTO (summarized in the description of **Task 1.2, Section 11.1.2.1**), these data reflect only a limited range of conditions, so it is not yet known how IMC transformation products vary across the full range of relevant environmental conditions. In order to efficiently characterize the full network of potential degradation pathways, a complementary approach to experimental determination is to predict transformation products and pathways using empirical or theoretical models of chemical structure vs. reactivity. A variety of such models exist, including U.S. EPA's Chemical Transformation Simulator (United States Environmental Protection Agency's *CTS*), EAWAG's Pathway Prediction System (Swiss Federal Institute of Aquatic Science and Technology's *PPS*) and *enviPath*, UCI's Reaction Predictor (University of California Irvine's *RP*), and PNNL EMSL *Arrows* (Pacific Northwest National Laboratory, Environmental Molecular Sciences Laboratory), which were evaluated in this task. These models use chemical rules, machine learning, or a combination of both (i.e., hybrid) to generate a network of reaction steps that determine the individual intermediates and products, and overall pathways of transformation. **Table 1** shows a comparison of the prediction models across different descriptive aspects including prediction methods and capabilities.

Table 1. Comparison of pathway/product prediction models.

	CTS: Chemical Transformation Simulator	PPS: Pathway Prediction System	Arrows
Developers/ Administrators	E. J. Weber et al. U.S. Environmental Protection Agency, Center for Environmental Modeling and Measurements (Athens, GA, USA).	K. Fenner et al. Swiss Federal Institute of Aquatic Science and Technology, Eawag (Dübendorf, Switzerland).	E. J. Bylaska, Pacific Northwest National Laboratory, PNNL; Environmental Molecular Sciences Laboratory, EMSL (Richland, WA, USA).
History/ Maturity	Development began 2015. First released for public access in February 2020 at https://qed.epacdx.net/cts/	- Began as UM-BBD in 1995. Available for public access at Eawag website for nearly 10 yr at http://eawag-bbd.ethz.ch/predict/	Publicly accessible since August 2017 via the web application EMSLArrows at https://Arrows.emsl.pnnl.gov/api/
Prediction Method	- Execution of reaction libraries manually encoded as general rules determined by expert analysis of experimental data. - Reactions encoded using ChemAxon's Chemical Terms Language and SMARTS Reaction SMILES. - Pathway prioritization: None. User selects prediction pathway, available based on environmental conditions. - Reaction prioritization: None. All available reaction mechanisms are rendered. - Pathway and reaction prioritization: By the manual assignment of likelihoods based on experimental data. User selects reaction library based on reaction system of interest.	- Execution of rules describing biotransformation reactions originally compiled from experimental data collected in BBD. - Reactions encoded using ChemAxon's Chemical Terms Language and SMARTS Reaction SMILES. - Pathway and reaction prioritization: By likelihoods manually assigned by two or more biodegradation experts. Additionally, since 2008 through relative reasoning rules between pairs of biotransformation rules.	- Execution of manually-encoded and machine-learned rules for general classes of organic chemical reactions. - Chemical reaction prediction is based on using canonical reaction hashes. These hashes are similar functionality to molecular and reaction fingerprints used in chemical databases and cheminformatics. By making a database of reaction hashes from a set of balanced chemical reactions, it is possible to generate future chemical reactions by filtering the reaction hash to reduce how far it spans the chemical graph from the reaction center. - Pathway prioritization: None. User determines prediction pathway based on reaction reactants submitted. - Reaction prioritization: Based on relative values of quantum chemically calculated reaction energies.

Pathways/Products Prediction Capabilities	- Transformation products for well-characterized reaction pathways: - Abiotic hydrolysis - Abiotic reduction - Photolysis - Mammalian metabolism (developed by ChemAxon)	- Transformation pathways and products for: - (An)aerobic microbial biodegradation reactions. - Breadth of reaction database covers most enzymatic pathways, many of which can also take place abiotically. - Reaction trees built over multiple generations (with user options to decide on trimming criteria).	- Transformation products for: - Alkaline hydrolysis - Reaction energies for mechanisms including: - Reduction/oxidation half-reactions - (Nucleophilic) substitution - Elimination
Operational Features	- Web-based, open-access. - Single chemical and batch mode submission. - User has choice of generating PDF, HTML, or CSV reports.	- Web-based, open-access. - Single chemical submission by default or batch mode submission available with administrator access (with additional option to switch off relative reasoning). - User has choice of generating PDF, HTML, or CSV reports.	- Web application and rest APIs - Reaction energies calculated with levels of theory, basis set, etc. that are available in NWChem. 2D, 3D, spreadsheet, and reaction input options - Multistage reaction trees can be calculated by using the “Reaction Predictor – (Beta Version)” tab in the web-based periodic and molecular builders” web application.
Other Prediction Capabilities	- Reaction site selectivity, chemical speciation effects (microspecies distribution from pKa’s). - Measured/estimated physico-chemical properties (e.g., water solubility, octanol-water partition coefficient, bioconcentration factor) from EPISuite, OPERA, TEST, and ChemAxon.	Biotransformation rules linked to database reactions, which in turn are linked to information on enzymes known to catalyze the respective reaction	- Molecular orbital energies, enthalpies, free energies, solvation energies, NMR, IR, EXAFS, spectroscopy - Multiple solvents - Free energy simulations of reaction pathways - Calculation queues - Extensive workflow capabilities to carry out complex molecular simulations, e.g., hybrid AIMD and molecular dynamics free energy simulations, AIMD-EXAFS.

CTS makes rule(knowledge)-based predictions for abiotic pathways, namely reduction, alkaline hydrolysis, and photolysis, using Chemaxon syntax; it also renders physicochemical properties (PChPs) (Tebes-Stevens et al., 2017). In contrast, PPS is built for biotic pathways and uses a prioritization scheme based on both knowledge rules and experimentally observed reactions, it also uses Chemaxon syntax (Gao et al., 2011). enviPath is the machine-learning-based extension of PPS, which uses a relative reasoning model that applies ML to limit the combinatorial explosion resulting from rapid growth of complexity of pathways and products (Wicker et al., 2010; Wicker et al., 2015). RP is fully machine-learning-based and it is built mainly for abiotic pathways considering inter- and intra-molecular elementary reactions that are analyzed as interactions between electron rich and electron depleted molecular sites (Fooshee et al., 2018).

In order to avoid duplication of efforts by developing a new model that overlooks previously curated databases of pathways and neglects to provide features that supplement those of existing predictive tools, each of the aforementioned models was examined and evaluated. A semiquantitative performance metric that includes criteria such as diversity of pathways and accessibility and ease of use was developed in this Task. Using the results of this evaluation as guidelines, a new model, Arrows, is being developed. Arrows is ML-based and consists of a combination of NWChem, SQL (Structured Query Language) and NoSQL computational chemistry software and databases. It predicts reaction/transformation products and calculates reaction thermodynamics for the predicted reactions.

Arrows has specifically been structured to tackle fundamental limitations of the existing models such as the inability to predict reactions they have not been trained for, by implementing ML, and the inefficiency of prioritization schemes that are based on occurrence frequency and/or experts' knowledge, by using thermodynamic favorability from computed reaction energetics instead.

Four existing models were examined for the prediction of reaction products and transformation pathways of (I)MCs from molecular structure. **PPS** is robust in the accuracy of predictions as its estimations are prioritized based on a database of experimentally observed reactions. In particular, it forecasts reductive transformations ($\text{NO}_2 \rightarrow \text{NH}_2$) for nitroaromatic (I)MCs (e.g., DNAN) in the first prediction level, as seen in **Figure 5**, which are by far one of the most environmentally-relevant reaction types for these compounds. For aliphatic (I)MCs (e.g., NQ), however, these products are not predicted, despite experimental evidence of their occurrence. This could be due to either or both: (i) lack of experimental reactions that include reductive transformations

for aliphatic (I)MCs in PPS' database, which would give said type of transformation a low priority and hence prevent reduction products from appearing in the output, or (ii) the $-NO_2$ reduction rule simply being set only for aromatic compounds. Using a test user mode, access to which was granted to this group by PPS's developers, this model was also run with the prioritization function (i.e., relative reasoning) turned off, but reduction products for aliphatic (I)MCs were still not predicted. This is a common downside of rule-based prediction tools, if the immediate vicinity of the reactive site in the queried compound is not an exact match to that of the one encoded in the rules database, said rule will be automatically disregarded.

enviPath, PPS' ML-based extension, is intended to incorporate all the features of PPS while using a relative reasoning model(s) that applies machine learning to prioritize what reactions are most likely to take place. Given that **enviPath**, a web-based tool, is still in very early stages of development relative to PPS, a few issues that need improvement arise when using it. Some of the user interface glitches include significant decrease in the number of predicted transformation levels relative to PPS which generates deep (> 6 level depths) and broad transformation trees (**Figure 5** vs. **Figure 6**), graphical disconnection between clearly linked transformation products in some pathways (**Figure 7**), and unresponsiveness and multiple broken links. In time and with continuous optimizations and updates, migration of data and features from PPS is expected to make **enviPath** fully functional.

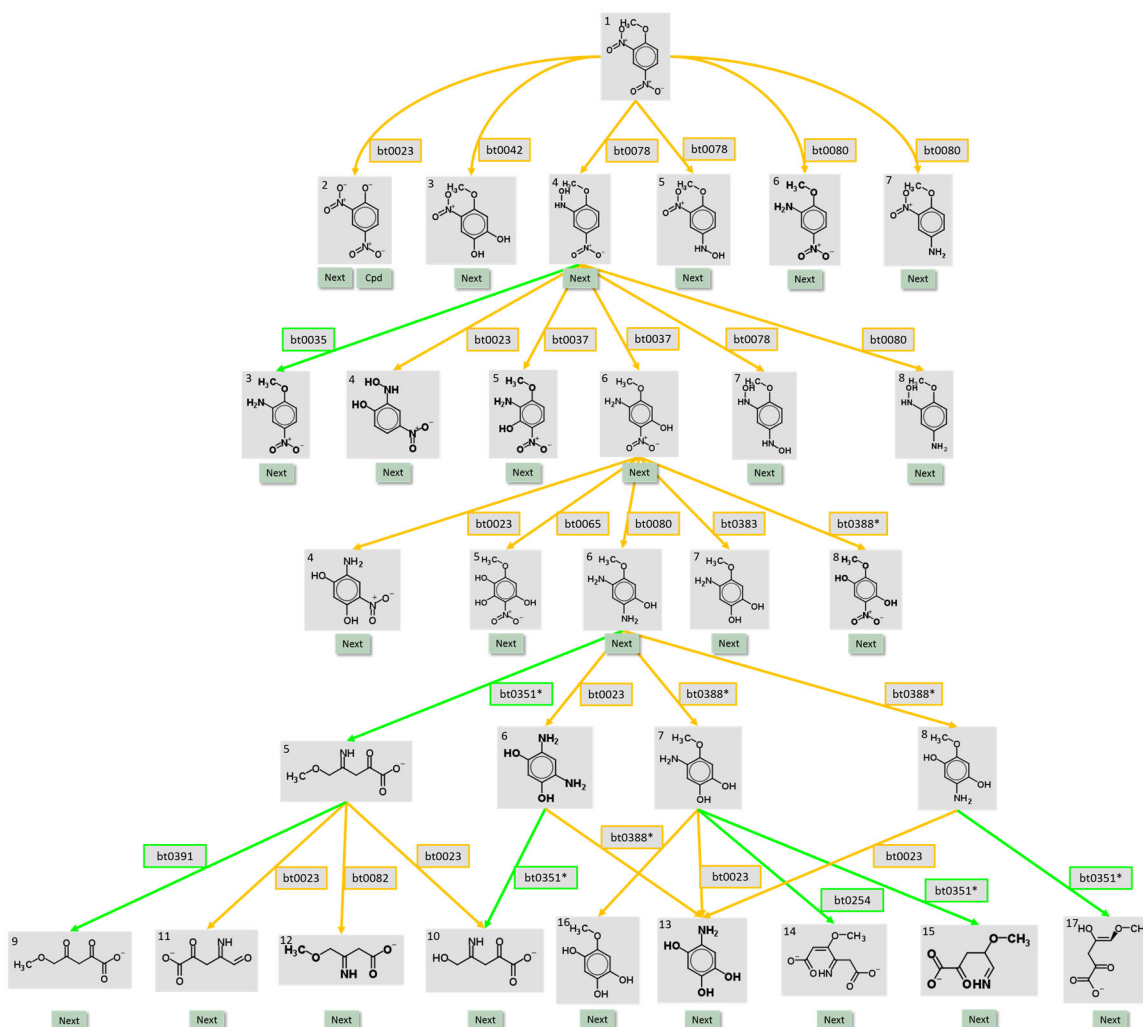


Figure 5. Sample reaction scheme for IMC DNAN predicted using EAWAG's PPS (Pathway Prediction System). Green Arrows indicate most likely reactions; yellow Arrows are less likely. To enlarge, go [here](#).

At a similar development stage, CTS has recently been released after its peer review process, a prerequisite for its public release. Unlike PPS or enviPath, CTS is designed for specific reaction mechanisms rather than a conglomerate of diverse potential degradation routes. The most complete reaction rules libraries incorporated into CTS thus far are for reduction and alkaline hydrolysis, which are considered to be reaction mechanisms of significant environmental importance. Through a request to CTS' developers, an output file with predictions was obtained for some of the (I)MCs. Predictions consisted of MS Word graphical outputs for reduction reactions and a MS Excel spreadsheet with PChPs. CTS was able to predict all the corresponding reduction products for traditional-like (I)MCs such as DNAN and TNT (**Figure 8**). Analogously to PPS, however, no reaction

rules were triggered for aliphatic (I)MCs like NQ, which once more highlights the limitations of rule-based models.

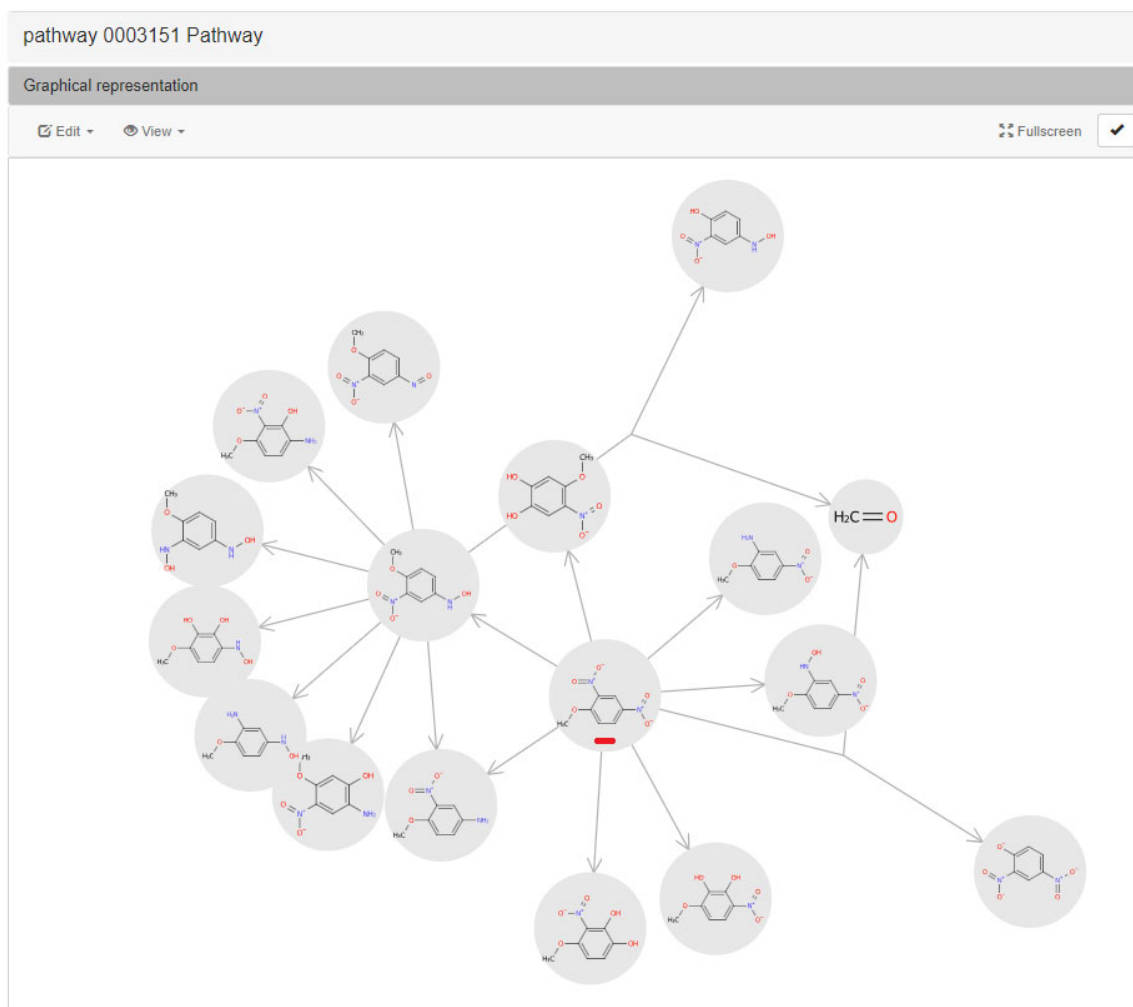


Figure 6. Degradation pathway for IMC DNAN (molecule with added red underline) predicted by EAWAG's envPath. Only a maximum of two depth levels are predicted. To enlarge, go [here](#).

With regard to PChPs, CTS does not generate predictions per se but rather produces an output table with a compilation of experimental and estimated values by other models such as U.S. EPA's EPI Suite and ChemAxon (see **Task 2.2, Section 11.2.2**) for properties including water solubility, Henry's law constant, and octanol-water partition coefficient (K_{ow}).

To note, is that none of these three pathway models predicts intermediate products (e.g., $-NO$ and $-NHOH$, in the reduction of $-NO_2$ to $-NH_2$), which are not only important

for a complete understanding of the transformation mechanisms but also for accuracy of predictions when either thermodynamic or kinetic barriers hinder the completion of the reaction to the end product.

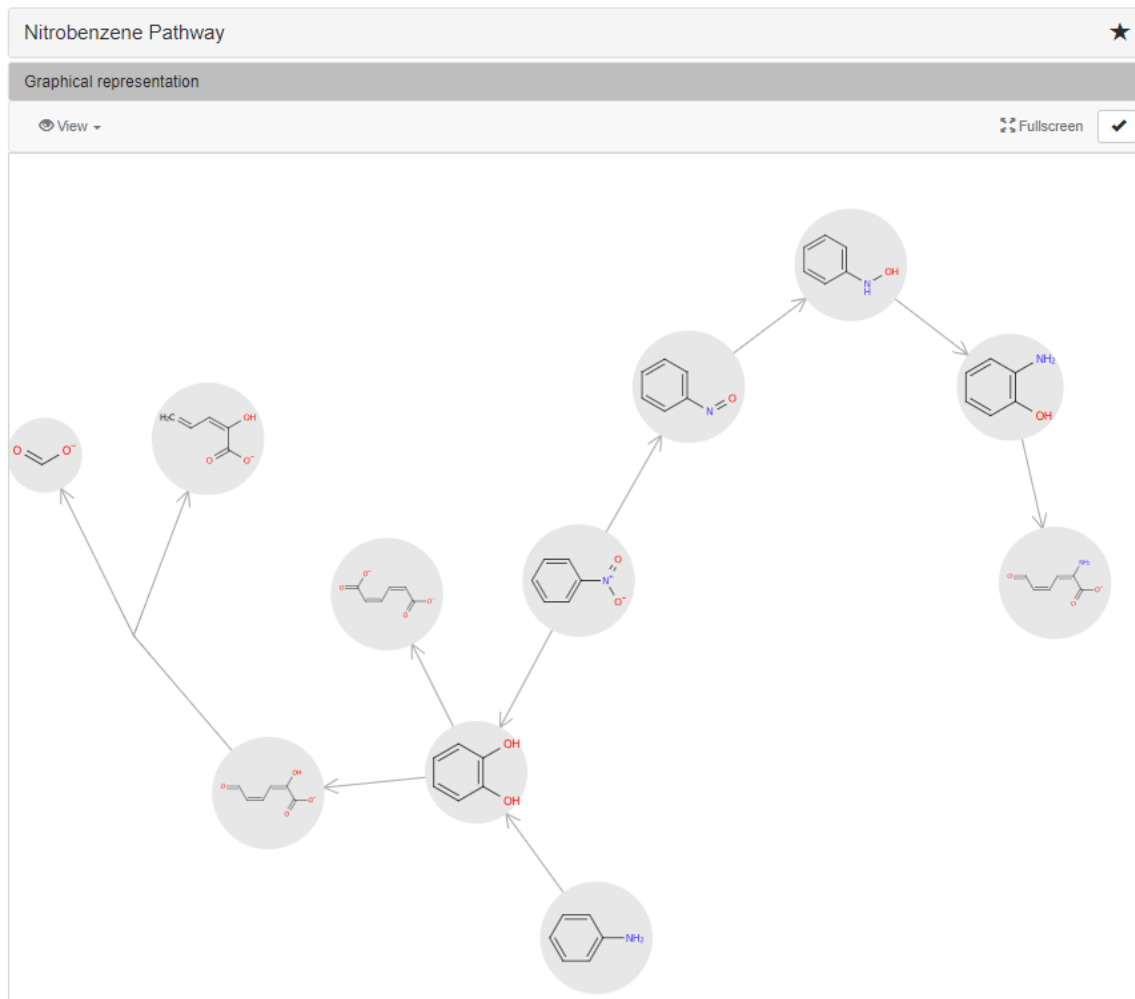


Figure 7. Degradation pathway for MC-like Nitrobenzene (NB, center) predicted by EAWAG's enviPath. The well-known reduction product of NB, Aniline (bottom), is not connected to NB and none of the intermediate products of such reduction reaction are predicted. To enlarge, go [here](#).

In contrast, UCI's **RP**, developed and maintained by the Institute for Genomics and Bioinformatics, produces an explicit output that includes arrow-pushing depictions representing electron flows for predicted elementary reactions (**Figure 9**), which is a useful feature and a step further in reaction mechanisms insight relative to the other models. RP predicts reaction outcomes and mechanisms using ML. The model requires

the input of molecules that will react with the compound of interest (e.g., explicit alkaline hydrolysis: $\text{OH}^- + \text{DNAN}$), so unlike EAWAG's PPS this model does not predict potential pathways starting from a parent compound molecule, unless a "target" molecule (i.e., a likely transformation product) is provided as an input. The latter feature has not been fully evaluated yet. The analysis of RP's results for alkaline hydrolysis of DNAN include similar products to those predicted by **Arrows** for the same reaction mechanism (see discussion on Arrows below). However, the model does not seem to have been calibrated for (I)MCs, in particular for reduction reactions, one of the most environmentally-relevant transformation mechanisms for these compounds, which return either error messages or no predicted products.

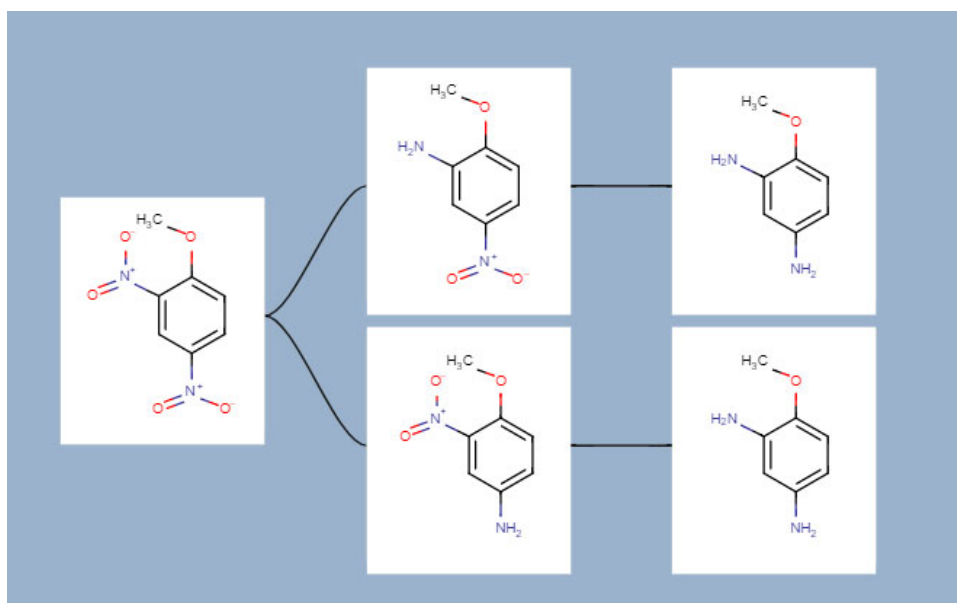


Figure 8. Predictions by U.S. EPA's CTS (Chemical Transformation Simulator) for the reduction of IMC DNAN.

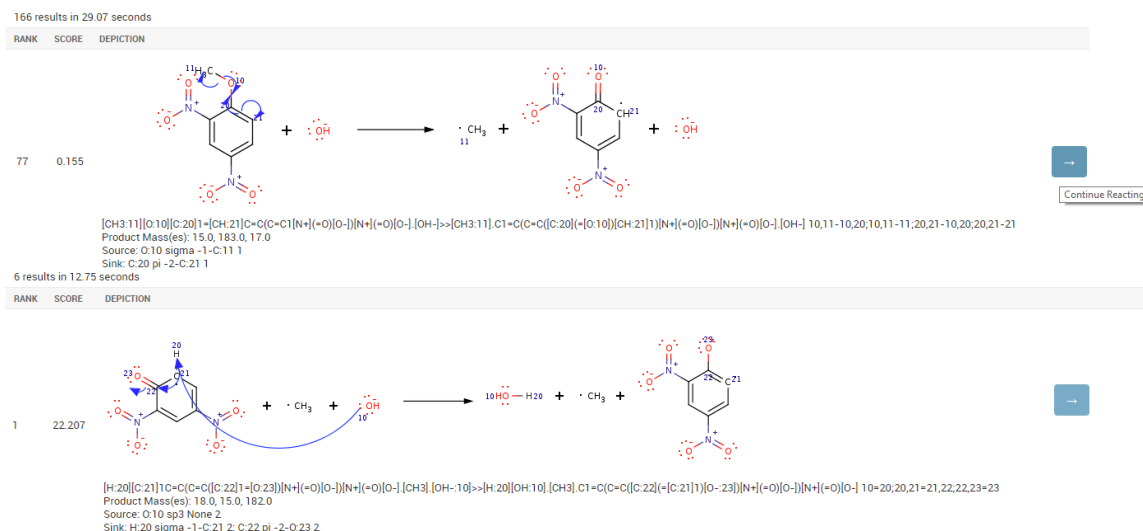


Figure 9. Sample of predicted products by UCI's RP (Reaction Predictor) for alkaline hydrolysis of IMC DNAN. Top panel is reaction ranked #77 of 166, which is a transformation product similar to those predicted by Arrows (see **Figure 11**). Bottom panel is a sample of the predicted reactions when requesting to "Continue Reacting" at reaction #77. To enlarge, go [here](#).

11.1.1.2. Model Developed by this Group: Arrows

In order to provide a predictive tool that supplies solutions to the limitations of the models thus far evaluated, **Arrows**, originally created at PNNL's EMSL, has been developed in this task. Arrows is an open-source web-based computational chemistry tool that uses NWChem molecular modeling software to implement a robust and diverse set of molecular theories that can estimate the thermodynamics and kinetics of molecules and materials. The goal of Arrows, however, is to provide users with a tool that is simple to use so that the inherent complexity of codes and molecular modeling software can be circumvented. To that end, Arrows has been setup so that a user can simply submit a chemical reaction or molecule directly into the Arrows web application and then an output file is returned back with thermodynamic or reaction progress (kinetics) results (**Figure 10**).

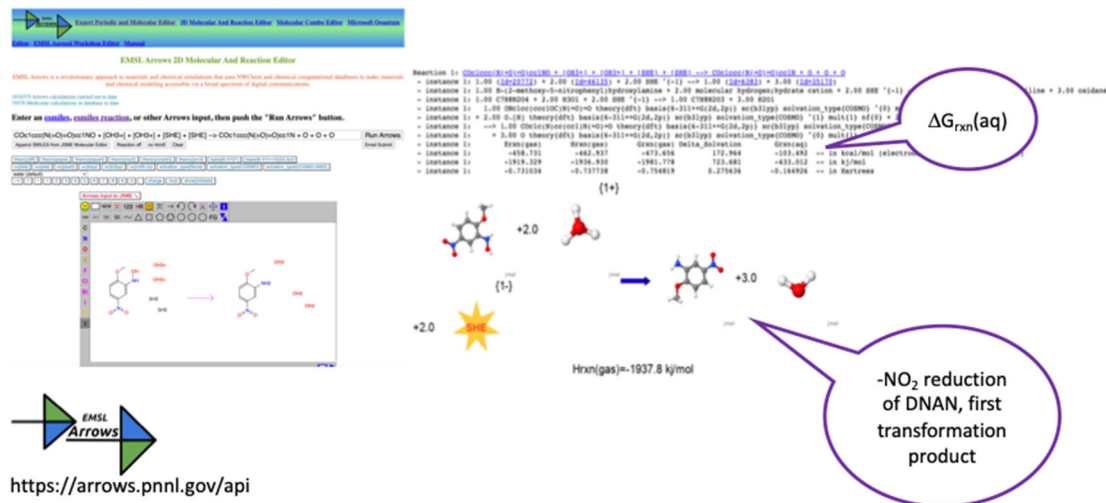


Figure 10. Image capture of using Arrows for a reaction energy calculation request.

Arrows parses the input and then searches the database for the compounds in the reactions. If a compound is not there, an NWChem calculation is setup and submitted to calculate it. Once the calculation is finished the results are entered into the database and then outputs are displayed/e-mailed back; a recent update includes the development of interfaces to spreadsheets, aimed at rendering a manageable/flexible output format. This whole process is completely automated.

One of the recent major developments in Arrows is canonical chemical reaction hashes, designed to allow the learning of chemistry. A hash defines the bonding near the reaction atoms. A compiler has been installed to generate reaction hash functions for any type of reaction. The database of reactions is used to generate hashes that can be employed to classify future chemical reactions, find products given the reactants, and define NEB (nudged elastic band) pathways (method for finding minimum energy paths).

With the purpose of increasing the size and diversity of the database of reactions, Arrows is being trained with the transformation reactions predicted by PPS (making use of the test user mode obtained for that model) and the knowledge rules embedded in CTS libraries (obtained through personal communication with the developers), mainly focusing on environmentally-relevant reaction mechanisms such as alkaline hydrolysis and reduction. To this point, Arrows has learned to predict reaction products of alkaline hydrolysis for (I)MCs, an example output is presented in **Figure 11**, without its estimates being dictated by knowledge-rules or prioritized by frequency of experimentally observed

reactions. Instead, Arrows has identified reaction patterns within the database of reactions and has prioritized those based on computed reaction energetics.

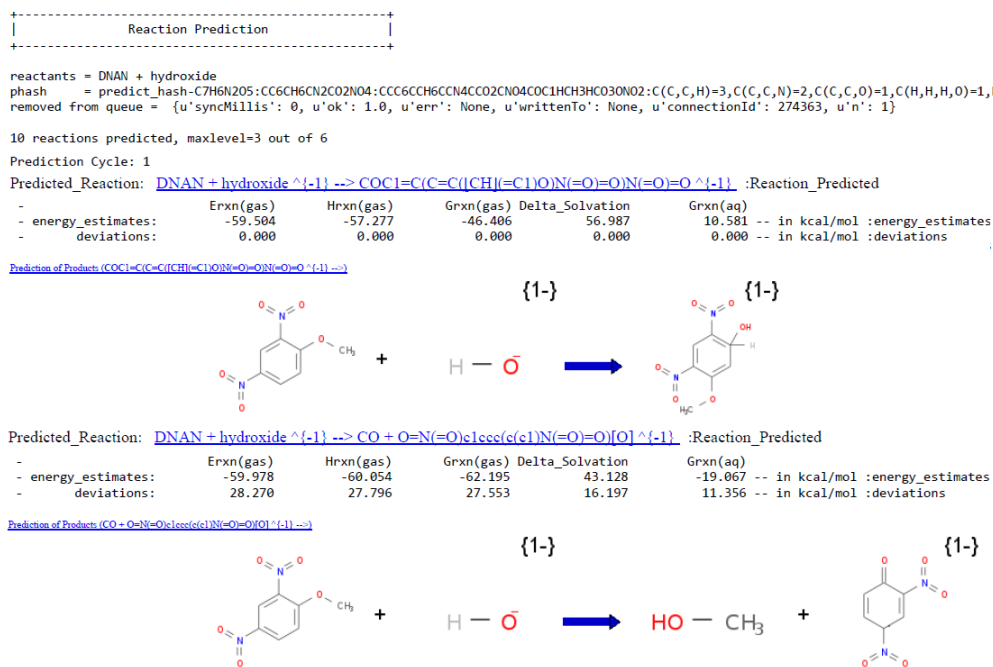


Figure 11. Sample predicted products for alkaline hydrolysis of IMC DNAN by EMSL's Arrows. To enlarge, go [here](#).

Arrows has calculated reaction energetics for the reductive transformations of ten (I)MCs parent compounds. The computations yielded aqueous phase reaction free energies ($\Delta G_{\text{rxn}}(\text{aq})$) for the stepwise reduction (i.e., transfer of $2e^- + 2H^+$: $-\text{NO}_2 \rightarrow -\text{NO} \rightarrow -\text{NHOH} \rightarrow -\text{NH}_2$) of the $-\text{NO}_2$ functional group(s) present in (I)MCs. The compounds include nitroaromatics (e.g., DNAN, TNT) and cyclic and acyclic nitramines (e.g., RDX, NQ). Calculations were performed using DFT (density functional theory) and SMD (solvation model density) with four different exchange correlation functionals, a sample graphical representation of the results is shown as a reaction coordinate diagram in **Figure 12**.

Differences in reaction energies among the different correlation functionals were small, maximum differences for single reactions were between 14 and 26 kcal/mol. No significant differences in $\Delta G_{\text{rxn}}(\text{aq})$ as a function of the position of the $-\text{NO}_2$ group (i.e., *ortho*-, *para*-) for NACs, but larger discrepancies on those were found for the nitramines (**Figure 12**). All the reactions were exergonic, however, the first reduction step (i.e., $-\text{NO}_2 \rightarrow -\text{NO}$) is more favorable for the nitramines than the nitroaromatics while the

second step (i.e., $-\text{NO} \rightarrow -\text{NHOH}$) is significantly less favorable for the nitramines. This might help explain experimental observations for nitramines where no further reduction products are observed after the nitroso ($-\text{NO}$) product is formed.

In summary, the progress on Arrows concerning (I)MCs includes added capabilities for the prediction of reaction products for alkaline hydrolysis, enlargement and diversification of its training database, and performance of computations to estimate reaction energetics for both hydrolysis and reduction. In the works is the automation of reaction progress calculations using transition state theory to generate reaction kinetics. Addition of capabilities to predict transformation pathways using only the parent compound as input (i.e., no reactants), analogous to the scheme in PPS and enviPath, and employing ML methods is the next step in Arrows development.

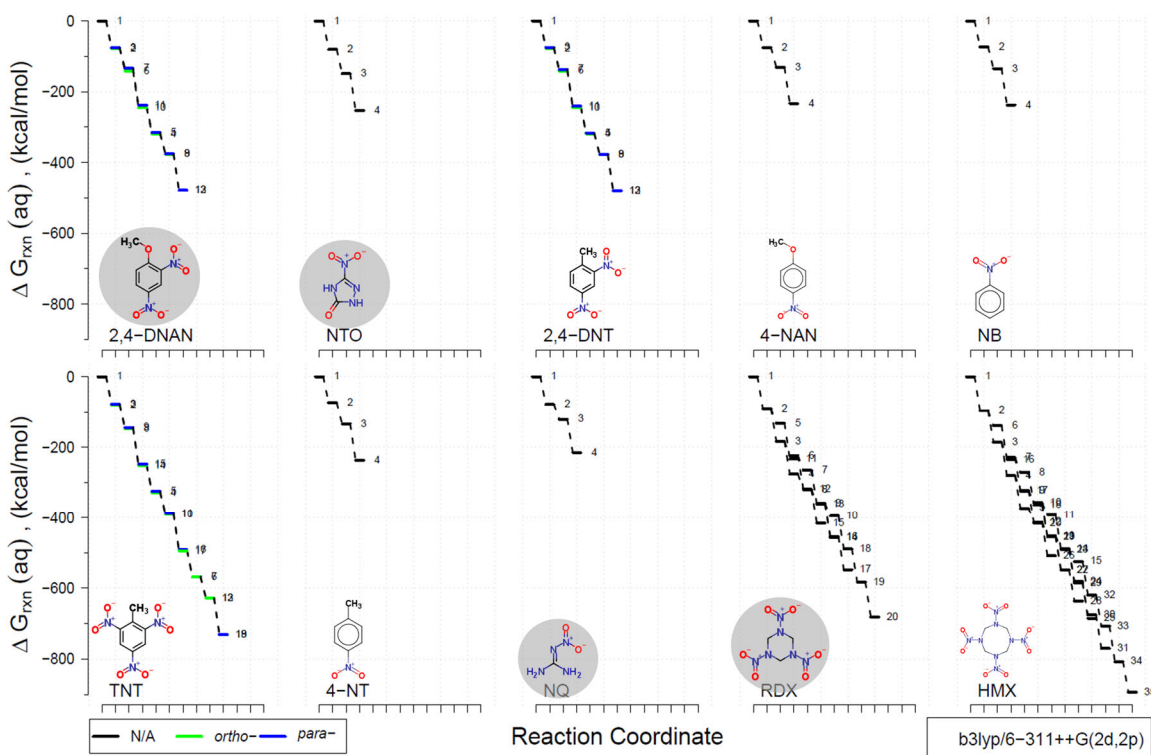


Figure 12. Computed aqueous Gibbs free energies of reaction ($\Delta G_{\text{rxn}}(\text{aq})$) using Arrows for the stepwise reductive transformation of (I)MCs using DFT (density functional theory) with b3lyp exchange correlation functional. Numbers are compound identifiers that follow the sequence of appearance in the transformation pathway (e.g., 1. nitrobenzene, 2. nitrosobenzene, 3. hydroxylaminobenzene, and 4. aniline). Compounds in a gray shade are those included in insensitive munition formulations (e.g., IMX-101).

11.1.1.2.1. *Reaction hashes in Arrows*

By making a database of reaction hashes from a set of balanced chemical reactions, it is possible to generate future chemical reactions by filtering the reaction hash to reduce how far it spans the chemical graph from the reaction center. Recently several groups have proposed algorithms for predicting chemical reactivity based on molecular fingerprints. Following this trend, we have implemented chemical reaction prediction capabilities in Arrows. Reaction predictions can be calculated by using the "Reaction Predictor - (Beta Version)" tab in the web-based periodic and molecular builders" web application (<https://arrows.emsl.pnnl.gov/api/periodic>), or by entering into the Arrows input the reactants followed by the reaction arrow "-->", e.g., "TNT + hydroxide -->".

In principle, the reaction energies of the predicted reactions can be calculated directly with Arrows system, however, this is slow when the molecules in the predicted reaction are not in the Arrows database; and they need to be calculated. Instead, we developed an algorithm inspired by the "Chinese Room Argument" thought experiment to calculate the energies for the predicted reactions. The algorithm is basically as follows: i) calculate the reaction hash for the predicted reaction; ii) fetch the reactions from the (reaction) database having the same reaction hash up to the requested neighbor level; iii) return the average of the reaction energies of the fetched reactions. In **Figure 13**, semi-quantitative results are shown for the hydrolysis reactions of TNT using the reaction energy estimates algorithm. As can be seen in the correlation plot, the errors are quite large and uncorrelated at the neighbor level = 1, and then steadily improves as the neighbor level is increased.

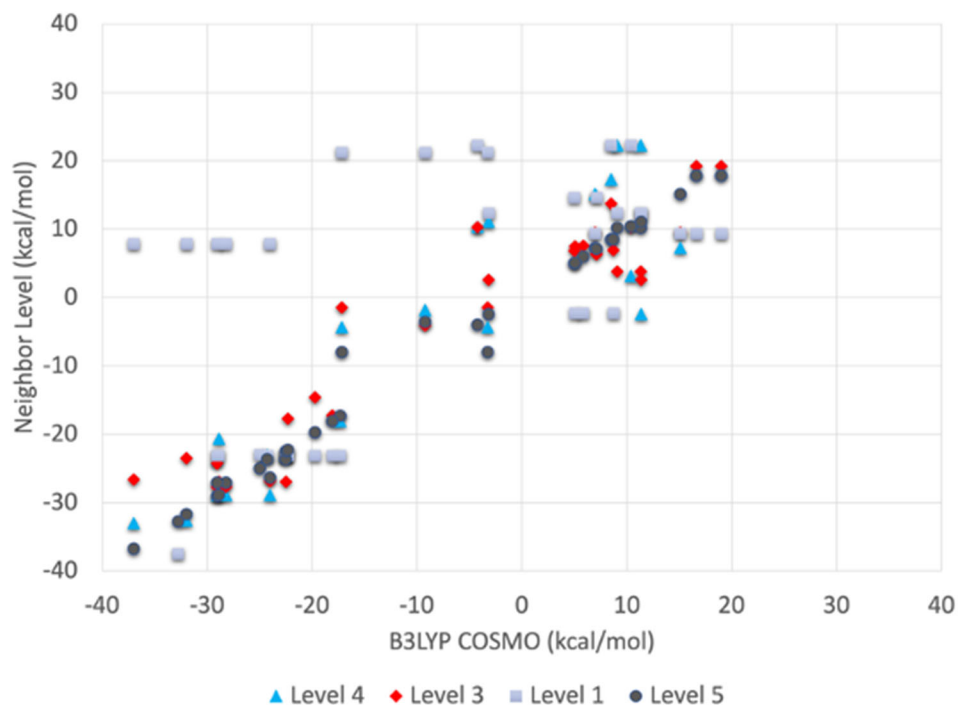


Figure 13. Correlation plot between reaction estimates at different reaction hash levels versus B3LYP COSMO energies for hydrolysis reactions of TNT and its metabolites.

11.1.2. Microcosm Experiments (Task 1.2)

11.1.2.1. Data Mining

We conducted a literature review and data mining process on degradation products of (I)MCs and MC-like compounds (e.g., nitrobenzene). A dataset of 39 compounds was compiled of which 13 are parent compounds and the rest are their corresponding transformation products as listed in **Table 2**. The mechanisms in the dataset include reduction, hydrolysis, and oxidation, and the parent compounds include MCs that are main components of IM formulations, such as DNAN, NTO, and NQ. The corresponding transformation rates are listed in **Table 3** in **Task 3.3 (Section 11.3.3.1)**.

11.1.2.2. Experimental Setup

Based on the data gaps identified in the data mining exercise above, we outlined and set underway the testing of an experimental configuration for the systematic assessment of the transformation of (I)MCs. The first step was to synthesize prior and general considerations on design of microcosms for bench scale testing of contaminant transformation by environmentally-sources materials. We developed this into a white-

paper to satisfy the corresponding Go/No-Go decision. The white paper is included in the Appendix (Section 14.3.1).

Then, reactors were set with a common soil matrix (Sassafras Sandy Loam, SSL, a soil that has been used in several other SERDP-funded projects with (I)MCs, e.g., ER-1254, ER-1416, ER-1688, and ER-1210) but under different redox conditions, this would allow the identification of a variety of transformation products while keeping other “static” physicochemical properties (mineral composition, organic matter content, etc.). The experimental scheme aimed at achieving reducing conditions during an incubation period through promotion of microbial activity. To that end, the setup included batch reactors containing SSL in an electrolyte solution with lactate and subsequent spikes of one of: (i) NO_3^- , (ii) SO_4^{2-} , and (iii) Fe^{2+} . After an incubation period, each of the reactors was expected to represent a different redox environment.

The oxidation reduction potential (ORP) and pH of the systems were monitored periodically throughout the incubation period. This was done using methods adapted from those developed for ER-2617 (Di Toro and Chiu) and ER-2621 (Johnson and Tratnyek). If stable redox conditions were achieved, this type of testing would be followed by withdrawing aliquots of the soil suspensions for consecutive (I)MCs reduction kinetic experiments. We planned to provide a tested methodology to validate model predictions with respect to (I)MCs degradation pathways without requiring an excessively large number of experiments.

Table 2. List of (I)MCs and MC-like parent compounds and their transformation products.

In-house ID	Chem. Abbrev.	Parent: 1 Product: 0	Parent In-house ID	IMC: 1	Transformation	IUPAC Name	CAS
1	DNAN	1	0	1	NA	1-methoxy-2,4-dinitrobenzene	119-27-7
2	2-ANAN	0	1	0	Reduction	2-methoxy-5-nitroaniline	99-59-2
3	4-ANAN	0	1	0	Reduction	4-methoxy-3-nitroaniline	577-72-0
4	DAAN	0	1	0	Reduction	4-methoxy-1,3-benzenediamine	615-05-4
5	NTO	1	0	1	NA	5-nitro-1,2-dihydro-3H-1,2,4-triazol-3-one	932-64-9
6	ATO	0	5	0	Reduction	5-amino-1,2-dihydro-3H-1,2,4-triazol-3-one	1003-35-6
7	DNT	1	0	0	NA	1-methyl-2,4-dinitrobenzene	121-14-2
8	DNBEZHY	0	7	0	Advanced Oxidation	2,4-dinitrobenzaldehyde	528-75-6
9	DNBAc	0	7	0	Advanced Oxidation	2,4-dinitrobenzoic acid	610-30-0
10	1,3-DNB	0	7	0	Advanced Oxidation	1,3-dinitrobenzene	99-65-0
11	3-NP	0	7	0	Advanced Oxidation	3-nitrophenol	554-84-7
12	2,4-DNP	0	7	0	Advanced Oxidation	2,4-dinitrophenol	51-28-5
13	NAN	1	0	0	NA	1-methoxy-4-nitrobenzene	100-17-4
14	AAN	0	13	0	Reduction	4-methoxyaniline	104-94-9
15	NB	1	0	0	NA	nitrobenzene	98-95-3
16	AN	0	15	0	Reduction	aniline	62-53-3
17	TNT	1	0	0	NA	2-methyl-1,3,5-trinitrobenzene	118-96-7
18	2,4-DANT	0	17	0	Reduction	4-methyl-5-nitro-1,3-benzenediamine	6629-29-4
19	2,6-DANT	0	17	0	Reduction	2-methyl-5-nitro-1,3-benzenediamine	59229-75-3
20	4ADNT	0	17	0	Reduction	4-methyl-3,5-dinitroaniline	19406-51-0
21	TAT	0	17	0	Reduction	2-methyl-1,3,5-benzenetriamine	88-02-8
22	4-NT	1	0	0	NA	1-methyl-4-nitrobenzene	99-99-0
23	4-AT	0	22	0	Reduction	p-toluidine	106-49-0
24	NQ	1	0	0	NA	1-nitroguanidine	556-88-7

25	AQ	0	24	0	Reduction	hydrazinecarboximidamide	79-17-4
26	2-M-3,5-DNP	0	17	0	Hydrolysis	2-methyl-3,5-dinitrophenol	497-56-3
27	4-M-3,5-DNP	0	17	0	Hydrolysis	4-methyl-3,5-dinitrophenol	63989-82-2
28	2-M-5-NP	0	1	0	Hydrolysis	2-methoxy-5-nitrophenol	636-93-1
29	4-M-3-NP	0	1	0	Hydrolysis	4-methoxy-3-nitrophenol	15174-02-4
30	5-oxo-1,2,4-TZ	0	5	0	Hydrolysis	5-Oxo-2,5-dihydro-1H-1,2,4-triazol-3-olate	133476-04-7
41	RDX	1	0	0	NA	1,3,5-trinitro-1,3,5-triazinane	121-82-4
45	MXN	0	41	0	Reduction	1,3-dinitro-5-nitroso-1,3,5-triazinane	5755-27-1
46	DNX	0	41	0	Reduction	1-nitro-3,5-dinitroso-1,3,5-triazinane	80251-29-2
47	TNX	0	41	0	Reduction	1,3,5-trinitroso-1,3,5-triazinane	13980-04-6
48	HMX	1	0	0	NA	1,3,5,7-tetranitro-1,3,5,7-tetrazocane	2691-41-0
49	MN-HMX	0	48	0	Reduction	1,3,5-trinitro-7-nitroso-1,3,5,7-tetrazocane	5755-28-2
50	2-ANT	1	7	0	(Proposed) Reduction	2-methyl-5-nitroaniline	99-55-8
51	4-ANT	1	7	0	(Proposed) Reduction	4-methyl-3-nitroaniline	119-32-4
52	DAT	1	7	0	(Proposed) Reduction	4-methyl-1,3-benzenediamine	95-80-7

Based on the experimental design outlined for the systematic testing of the transformation of (I)MCs in a common soil matrix (i.e., SSL) under different Redox conditions, all the microcosm units, electrode probes, and instrumentation were obtained and or custom-made. The experimental setup sketch is presented in **Figure 14**. In order to maintain anoxic conditions, each microcosm unit was capped with an air-tight-Teflon septum that was kept in place by a polypropylene threaded cap. The Teflon septum housed a gas sparging line and five ports (sampling, pH electrode, two ORP working electrodes, ORP reference electrode).

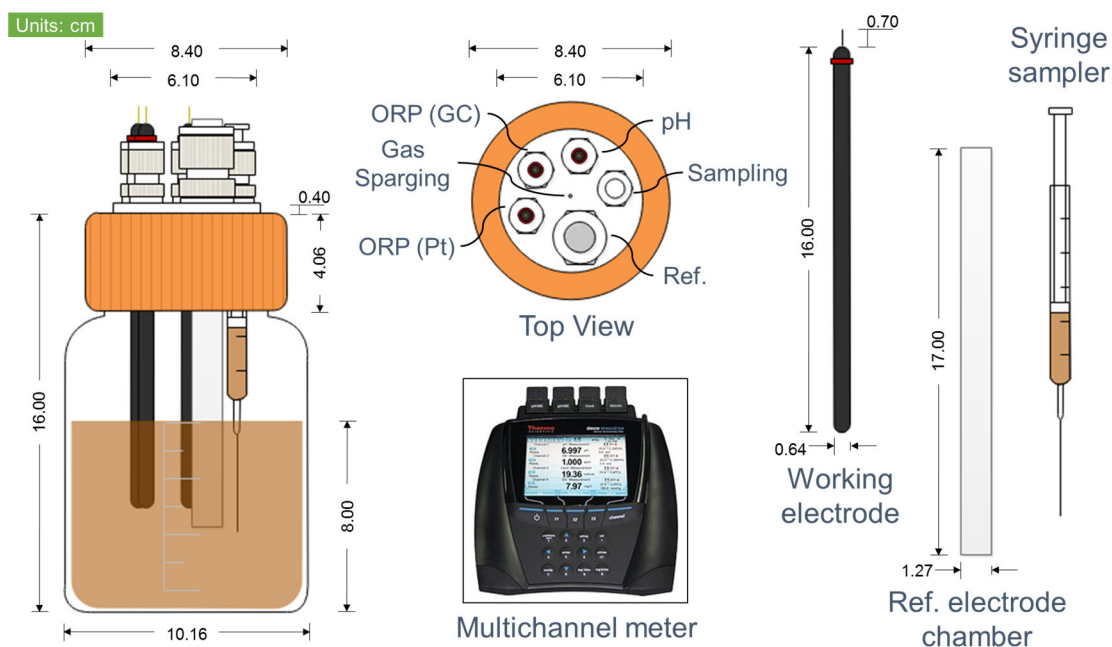


Figure 14. Microcosm experimental setup sketch, left to right: Microcosm unit capped with an air-tight-Teflon septum that is kept in place by a polypropylene threaded cap (side view), cap and electrode fittings (top view), multichannel meter for continuous and simultaneous measurement of pH and ORP using multiple electrodes, ORP working electrode, glass receptacle for ORP reference electrode (side view), and syringe sampler for withdrawal of slurry. The Teflon septum (white circle concentric with the orange screw cap) houses a gas sparging line (not pictured) and five ports: sampling, pH electrode, two ORP working electrodes (glassy carbon and platinum), ORP reference electrode (bigger fitting pictured).

Four reactors were set up with 10 g/ea of SSL. A volume of 600 mL of electrolyte solution composed of sodium bicarbonate (10 mM NaHCO_3) and lactic acid (10 mM $\text{C}_3\text{H}_6\text{O}_3$) was added to each reactor. A different Redox condition was promoted in each

reactor: (i) control, electrolyte solution + soil, (ii) treatment 1, electrolyte solution + soil + sodium sulfate (100 μM Na_2SO_4), (iii) treatment 2, electrolyte solution + soil + sodium nitrate (100 μM NaNO_3), and (iv) treatment 3, electrolyte solution + soil + 0.3 g iron oxide mineral (0.5 g/L Goethite). The four reactors rested on a magnetic multistirrer. Each reactor contained a caged, suspended magnetic stir bar that mixed the solution to keep the soil particles suspended. A photo of the experimental setup testing is presented in **Figure 15**.

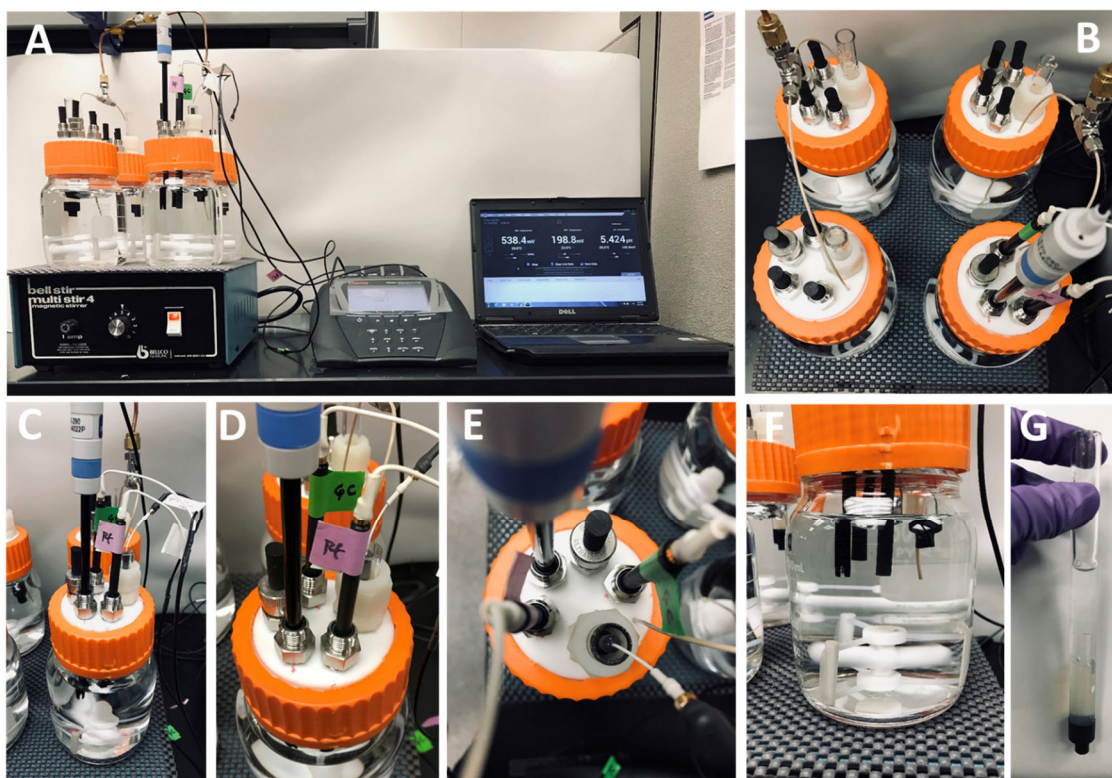


Figure 15. Microcosms experiments. A. Left to right: microcosms reactors on magnetic multistirrer, pH/ORP meter, and computer recording meter measurements on real-time. B. Top view of the four reactors. pH and ORP electrodes are placed in the bottom right reactor. Electrodes are removed and placed into the next reactor after a monitoring period of about 1h. C. and D. Side view of reactor being monitored for pH and ORP. E. Top view of electrode arrangement. Clockwise from bottom left: Pt ORP working electrode, pH electrode, sampling port with rubber stopper to prevent O_2 intrusion, glassy carbon (GC) ORP working electrode, N_2 sparging line, and Ag/AgCl reference electrode in reference electrode glass holder (G.). F. Side view of electrodes immersed in solution and caged, suspended magnetic stir bar. G. Reference electrode glass holder. Plugged at the bottom with 20% wt. Agarose made with the electrolyte/medium

solution, heat-shrink tubing at the bottom to prevent Agarose plug from sliding out, and electrolyte/medium solution overlaying the Agarose plug.

The measurement protocol consisted of placing the pH and ORP electrodes into a reactor unit at a time. Data was recorded every 5 min for a monitoring period of about 1h per reactor. At the end of this period, the electrodes were removed from the already measured reactor and placed into the next one. Rubber plugs were replaced into the ports of the measured reactor. Gas (N_2) was flushing each reactor continuously to keep a positive pressure inside the unit avoiding O_2 intrusion.

Tests were carried out for two months. Measurements of ORP and pH in each reactor were performed and recorded periodically. Initial ORP values were around 300 mV (v. SHE) for all reactors as shown in **Figure 16** along with the variation in pH. The largest drop in ORP for all reactors occurred towards the end of the first week. Both $+NO_3^-$ and Goethite reactors dropped down to approximately -300 mV while Ctrl and SO_4^{2-} reactors only reached down to about -100 mV. After the first week, ORP values only varied roughly ± 100 mV around 0 mV in all reactors. Respikes of fresh electrolyte solution into the reactors throughout the course of the experiment did not cause any significant effects on the ORP values, which was contrary to our expectations of reaching down to more reducing conditions (i.e., lower ORP values) if we promoted microbial activity.

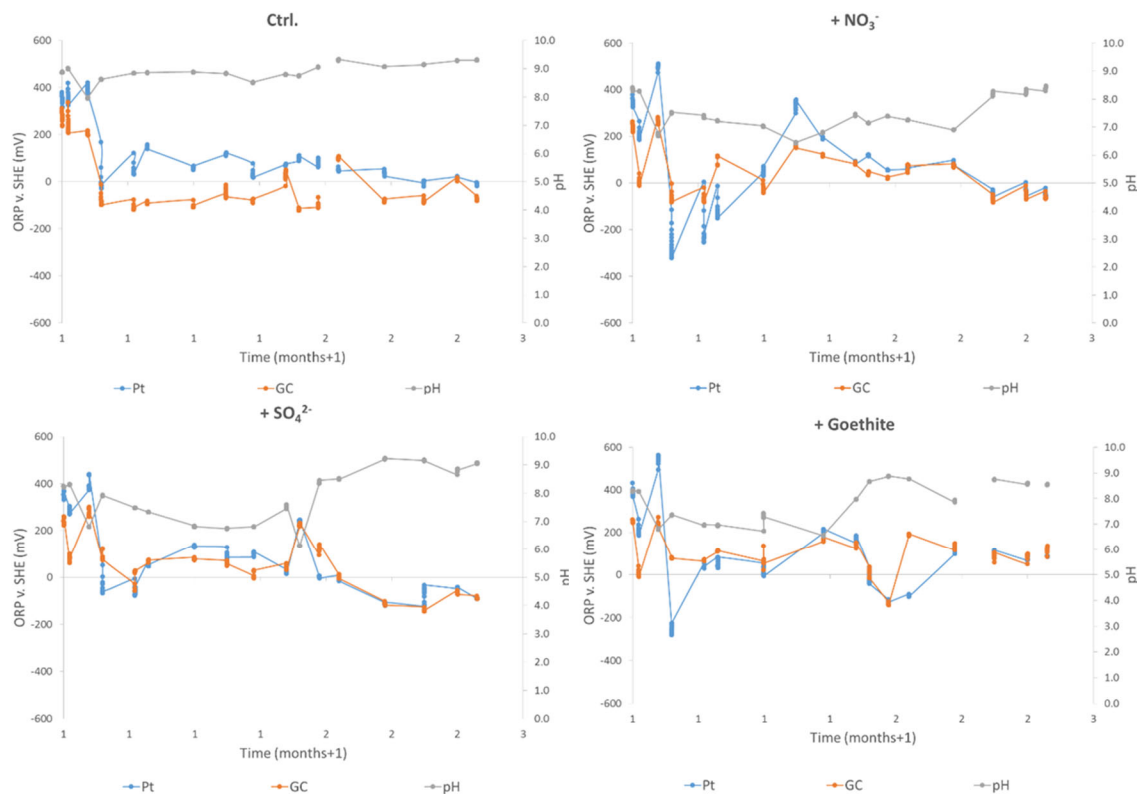


Figure 16. Monitoring data for testing of microcosms experiments. Four reactors with Sassafras Sandy Loam soil in an electrolyte solution composed of sodium bicarbonate (10 mM NaHCO₃) and lactic acid (10 mM C₃H₆O₃). A different Redox condition was promoted in one of four reactors: (i) “Ctrl.”, electrolyte solution + soil, (ii) “+NO₃⁻”, electrolyte solution + soil + sodium nitrate (100 μM NaNO₃), (iii) “+SO₄²⁻”, electrolyte solution + soil + sodium sulfate (100 μM Na₂SO₄), and (iv) “Goethite”, electrolyte solution + soil + 0.3 g iron oxide mineral (0.5 g/L Goethite). ORP: Oxidation Reduction Potential. SHE: Standard Hydrogen Electrode. GC: Glassy Carbon.

In summary, no increases in ORP beyond the initial value, or decreases beyond the initial drop, were observed in any of the reactors through the end of the testing period. We did observe, however, contrasting colors in the soil slurries among the reactors with the Ctrl and SO₄²⁻ reactors turning dark black and the +NO₃⁻ and Goethite reactors not showing much change with respect to their initial colors. Also, both glassy carbon and Pt electrodes exhibited the same ORP trends with only occasional discrepancies between them of around 200 mV. Photographs showing the progressive color change in soil slurries are displayed in **Figure 17**.

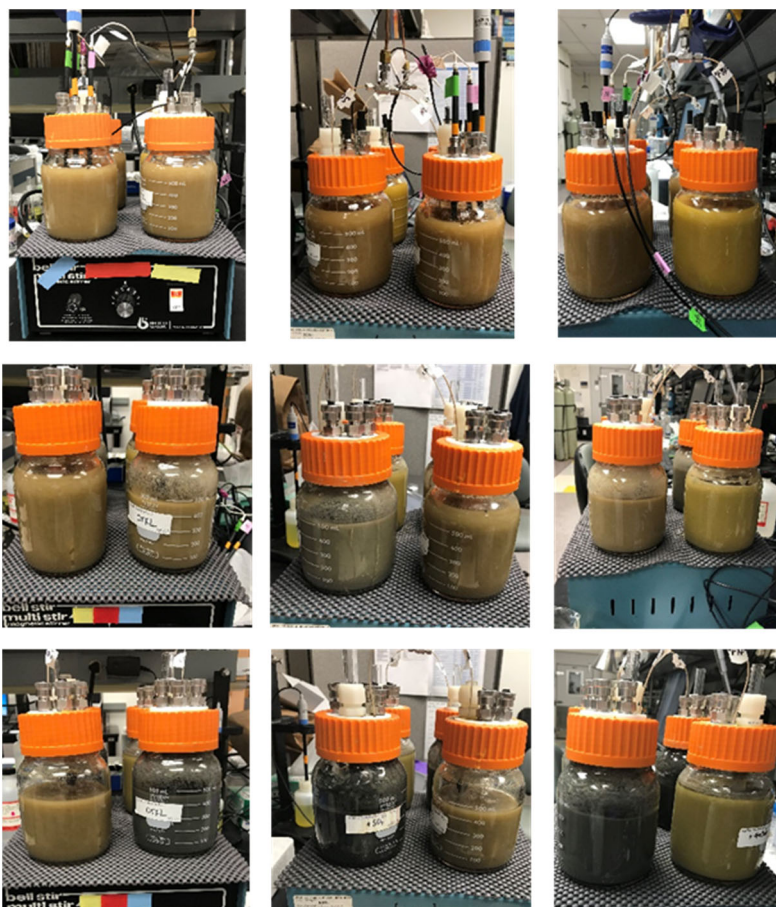


Figure 17. Photographs of reactors in microcosm experiments during tests. Rows, top to bottom: Initial (Day 1), middle (approx. 1 month), and end (approx. 2 months).

The addition of different terminal electrons (i.e., NO_3^- , SO_4^{2-} , and Goethite) was expected to promote markedly different Redox conditions in each reactor with the NO_3^- treatment showing ORPs in the 300 to 500 mV region and SO_4^{2-} and Goethite down between -200 to -400 mV, based on literature values. The fact that we observed similar ORP values (roughly ± 100 mV around 0 mV) for all reactors throughout most of the tested period but contrasting medium colors, however, suggests to us that the measurements we obtained with the electrodes are not representative of the actual Redox conditions in the reactors. We suspect that the reducing conditions were mostly present in the solid phase of the soil slurry (i.e., soil particles) and not in solution. Since the electrodes only detect species present in solution and the equilibration between the soil particles and the bulk phase is a slow process, the differences in Redox conditions among our reactors were imperceptible in our ORP measurements. This challenge has been

extensively studied and a solution proposed to circumvent the problem is to use dissolved mediators, i.e., Chemical Reactivity Probes (CRPs), that rapidly equilibrate with both the working electrode and the solid phase (Kocur et al., 2019; Sander et al., 2015).

With the purpose of exploring whether reducing conditions were actually present in the reactors, on the last week of the experiments, we spiked the reactors with 2,4-dinitroanisole (DNAN), an insensitive munition compound. We withdrew aliquots of the soil slurry from each reactor on three separate occasions over the course of 12 days after the spike and stored the filtered samples for consecutive HPLC (High Performance Liquid Chromatography) analysis. We completed those HPLC analysis to quantify the loss of DNAN in the reactors.

At the time of the DNAN spike, the ORP values were around 0 mV (v. SHE with Pt electrode) for the Ctrl and $+NO_3^-$ reactors and even more positive (approx. 100 mV) for the Goethite reactor, as shown in **Figure 18**. The SO_4^{2-} reactor was the only one registering negative ORP values (approx. -100 mV). Given that, theoretically, the reductive transformation of DNAN requires Redox conditions at lower ORP values (approx. -400 mV at pH 7), we were dubious about observing disappearance of this compound in the aqueous phase beyond that due to sorption. However, the concentrations measured in the soil slurry samples revealed almost complete disappearance of DNAN in all the reactors with the exception of the Goethite one by the first sampling day (4 days after the DNAN spike), as shown in **Figure 19**. The concentration of DNAN in the Goethite reactor, however, showed a descending trend over the sampling period to a total decrease of about 86% by the last sampling day.

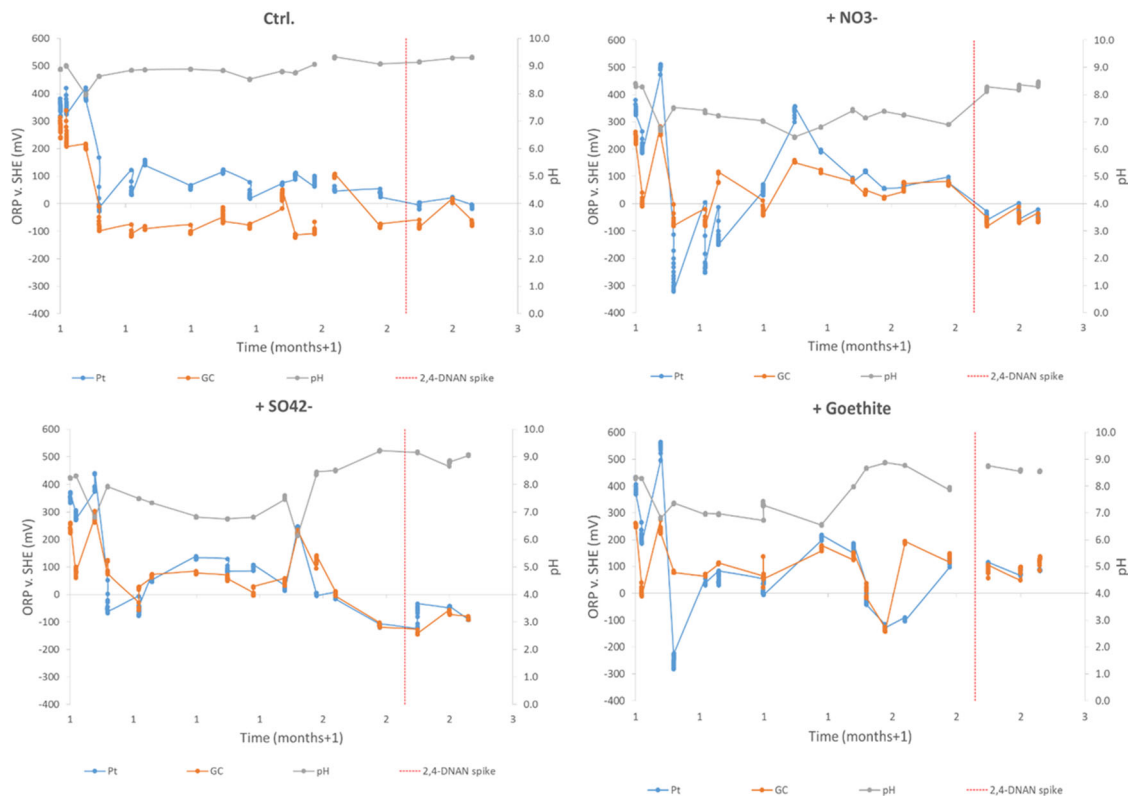


Figure 18. Monitoring data for tests of microcosms experiments and spike of insensitive munition compound, 2,4-dinitroanisole (2,4-DNAN). Four reactors with soil in an electrolyte solution. A different Redox condition was promoted in one of four reactors: (i) “Ctrl.”, electrolyte solution + soil, (ii) “+NO₃⁻”, electrolyte solution + soil + sodium nitrate (100 μM NaNO₃), (iii) “+SO₄²⁻”, electrolyte solution + soil + sodium sulfate (100 μM Na₂SO₄), and (iv) “Goethite”, electrolyte solution + soil + 0.3 g iron oxide mineral (0.5 g/L Goethite). ORP: Oxidation Reduction Potential. SHE: Standard Hydrogen Electrode. GC: Glassy Carbon.

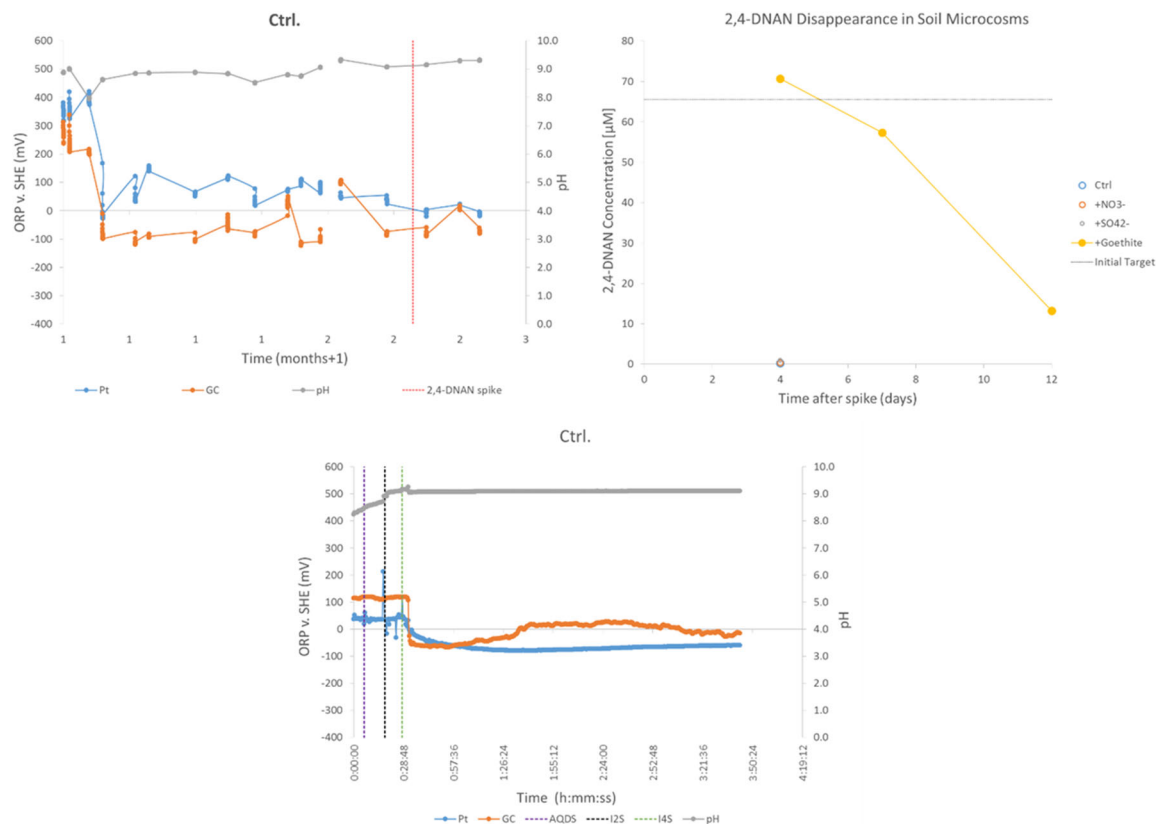


Figure 19. Clockwise from top left: (i) Monitoring data for Control reactor (electrolyte solution + soil) during tests for microcosms experiments. (ii) Concentration of 2,4-DNAN in filtered soil slurry aliquots sampled from microcosm reactors. (iii) Monitoring data for Control reactor upon addition of CRPs (Chemical Reactivity Probes) after tests for microcosms experiments. ORP: Oxidation Reduction Potential. SHE: Standard Hydrogen Electrode. Pt: Platinum. GC: Glassy Carbon.

The fact that we observed almost complete disappearance of DNAN in all of the reactors despite the positive ORP values measured with our electrodes, confirmed to us that the measurements we obtained with the electrodes were indeed not representative of the actual Redox conditions in the reactors. Therefore, we added the dissolved CRPs to use them as mediators that rapidly equilibrate with both the working electrode and the solid phase. We added three CRPs individually and subsequently to the Ctrl reactor. The CRPs were AQDS (9,10-Anthraquinone-2,6-disulfonate), I2S (5,5'-Indigo disulfonate), and I4S (Indigo tetrasulfonate) with formal one electron reduction potentials at pH 7 of -184 , -125 , and -46 mV (v. SHE), respectively. The first two CRPs had no significant effect on the ORP measurements compared to those measured before the addition of the mediators. The last CRP, I4S, however, did have a marked effect on the ORP values, as

shown on **Figure 19**. The ORP values dropped about 100 mV (with the Pt electrode. 200 mV with the GC electrode) from 0 mV almost immediately after the addition of I4S and stayed virtually constant at that –100 mV region for the entire period that we monitored the reactor (approx. 3 h).

In conclusion, the disappearance of DNAN and the drop in ORP values upon the addition of the appropriate CRP, are two key findings obtained from the testing of this methodology. Future methodologies should include the appropriate CRPs in the background solution from the beginning of the tests. This will allow to monitor redox conditions more accurately and, in turn, signal the appropriate period for the addition of the (I)MC so that the transformation can be monitored before the complete disappearance of the parent compound. These results can be used as guidance for the identification of (I)MCs transformation products/pathways via experimental configurations that promote different redox conditions while keeping other “static” physicochemical soil properties (mineral composition, organic matter content, etc.).

11.1.3. Improve Models (Task 1.3)

The five models that we surveyed in **Task 1.1 (Section 11.1.1)** were evaluated comparatively in this task to identify needed improvements for the accurate and efficient prediction of transformation products. Given the contrasting differences in output content and format among the models, we developed a semiquantitative metric to compare them. The metric renders a score that is calculated simply as a weighted summation considering five evaluation criteria: (i) Accuracy of predictions (*a*), (ii) Diversity of pathways (*p*), (iii) Mechanistic detail (*m*), (iv) Extent of degradation/transformation (*d*), and (v) Accessibility and ease of use (*e*):

$$Score = 0.3a + 0.3p + 0.2m + 0.1d + 0.1e \quad (1)$$

Accuracy of predictions rates the frequency of agreement between predicted products and those experimentally observed. *Diversity of pathways* evaluates the assortment of reaction types considered in the transformation route, especially whether those of environmental relevance are included. *Mechanistic detail* assesses the explicitness with which a reaction is described/shown in the model output, i.e., transference of electrons graphically represented with Arrows from and to atoms involved in the reaction. *Extent of degradation* refers to the depth of sequential levels to which a prediction is made down a transformation pathway, i.e., degradation predicted from parent compound down to complete mineralization. *Accessibility & ease of use* judges the convenience and

availability of the model either as a software or a web-based tool, the practicality of input formats and manageability of result outputs, and the intuitiveness of the user interface.

Figure 20 shows a graphical representation of the resulting scores. The model with the highest score is PPS (Score = 0.56). Given the abundant variety of biotic transformation routes harbored in this model's extensive database, its diversity of pathways is significantly wide. Also, PPS and its ML extension, enviPath (Score = 0.22), score well on *e* as they are both openly accessible online, have simple submission formats (SMILES and molecular structure sketch drawing), and a MS Excel spreadsheet output can be generated using a test user mode. As mentioned before, enviPath is still in the early stages of development, so the migration of rules and reactions from PPS is still underway and, hence, features such as the extent of degradation and diversity of pathways have a very intermittent performance.

CTS, RP, and Arrows have similar scores (**Figure 20**). CTS has a good level of accuracy of predictions as most of its rules have been encoded by experts based on their experimental observation of reaction products, which also provides a good score for its diversity of environmentally-relevant pathways. CTS falls short in its mechanistic detail and extent of transformation given that the output format shows the transformation products without insight on the reactants involved or the types of bonds broken/formed, and only a single type of transformation is triggered per degradation branch so there is a very limited number of depth levels predicted.

RP, in contrast, generates very mechanistically detailed outputs, which is expected given its chemical synthesis framework origin (**Figure 9** Error! Reference source not found.). This model, however, does not frequently match experimentally observed end products of reactions for I(MCs) because it mainly predicts elementary reactions with products that are likely unstable at environmentally relevant conditions.

Arrows has also a good score for mechanistic detail as all the reactants and products are explicitly shown in its output files as well as the corresponding reaction energetics (**Figure 11** Error! Reference source not found.). This model generates accurate predictions given that its estimates are rooted in patterns identified from a database of curated reactions. On the downside, however, Arrows has a very shallow level for extend of degradation at the moment because its pathway prediction capability is not built yet.

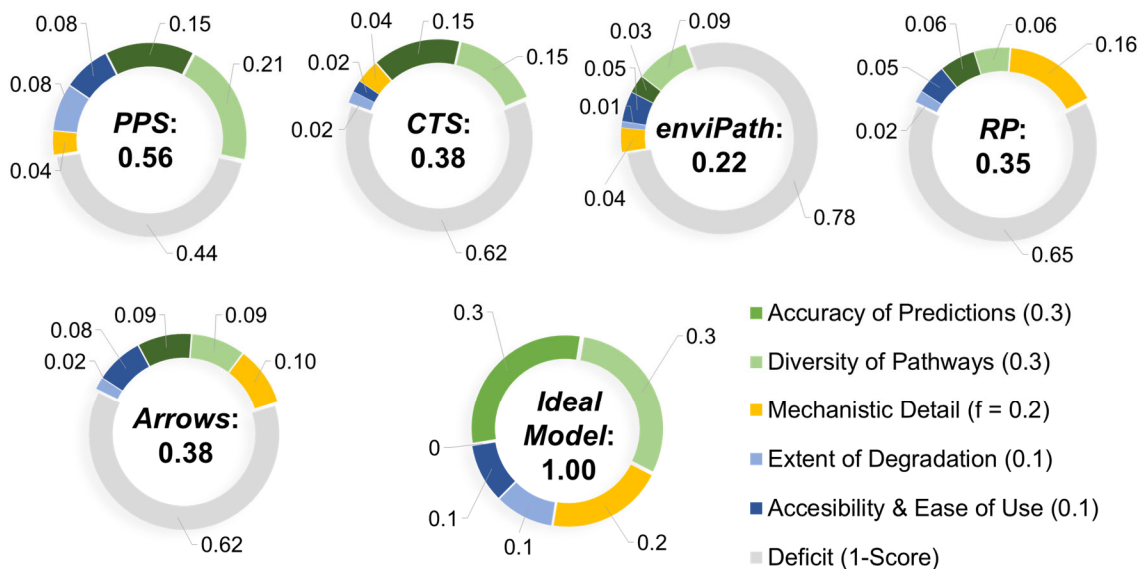


Figure 20. Comparison of existing pathway/product prediction models using a semiquantitative metric that considers five criteria (**Figure** legend). Model scores are calculated using Eq. (1). Models: EAWAG’s Pathway Prediction System (**PPS**) and **enviPath**, U.S. EPA’s Chemical Transformation Simulator (**CTS**), UCI’s Reaction Predictor (**RP**), and PNNL’s **Arrows**. The score distribution for an ideal model is included as a reference.

The results from our comparative assessment of five existing models that predict reaction products and transformation pathways of (I)MCs from molecular structure, helped us identifying aspects in which our prediction modeling tool, Arrows, could improve. Mainly, Arrows was lacking a graphical method to display the resulting network of transformation products and a clear user manual. Progress has now been made on both of those needs. A new feature that graphically displays the cascade of products per transformation level has been developed. The user can now access a “Reaction Prediction” feature. The tool requires a parent compound + reactant format input. Once this information is entered and the “run” request is executed, Arrows performs the reaction energy calculations. After the calculations are completed, the graphical output is displayed showing the parent compound, reactants, and primary and secondary products, in a SMILES format, for transformation level 1 along with the corresponding computed reaction energies, as shown in **Figure 21**. The output also includes the option to run a second transformation level, level 2, which once it is executed its transformation products

are appended to those of level 1 as shown in **Figure 22**. So far up to 3 transformation levels are available.

The Arrows user manual has also undergone improvements. The previous site has now a new and improved look and can be accessed at: https://nwchemgit.github.io/EMSL_Arrows.html. The manual can be easily navigated across ten different sections including how to use the Arrows API, how to generate a table of reactions, and how to fetch a full NWChem (the quantum chemical computational engine behind Arrows).

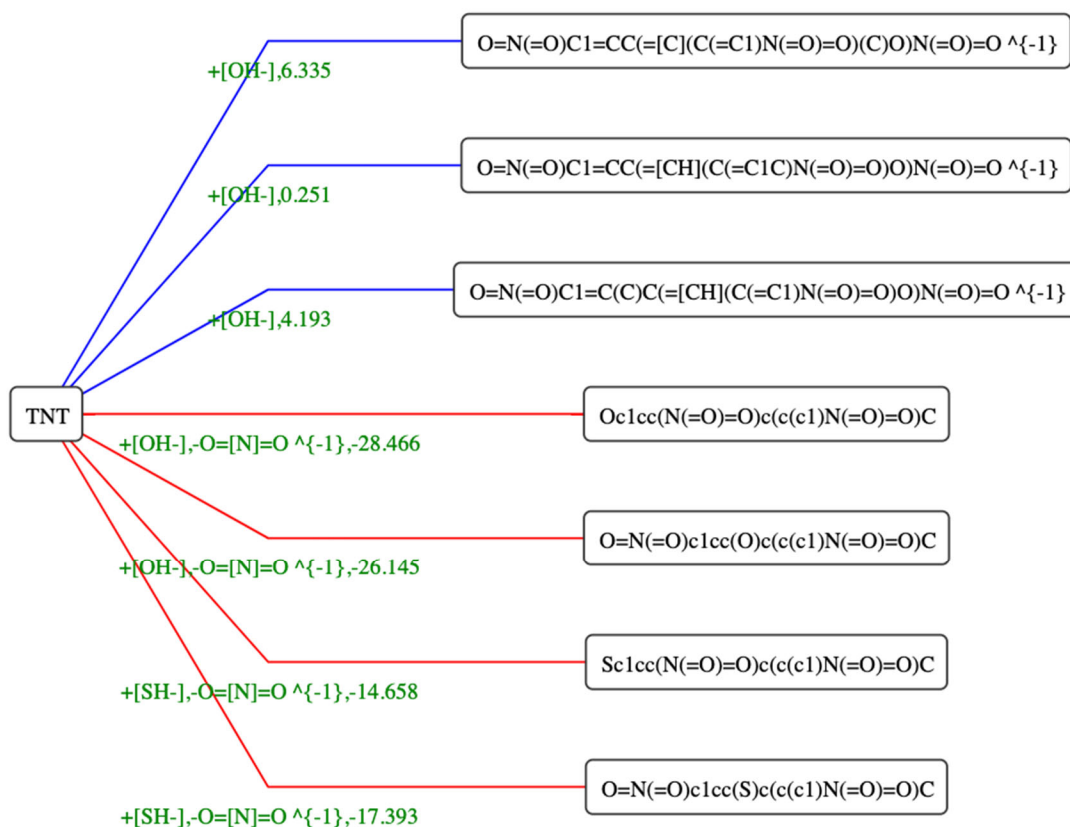


Figure 21. Arrows output for TNT transformation level 1. In green: reactants, secondary products, and quantum chemically computed reaction Gibbs free energy ($\Delta G_{rxn}(aq)$, kcal/mol).

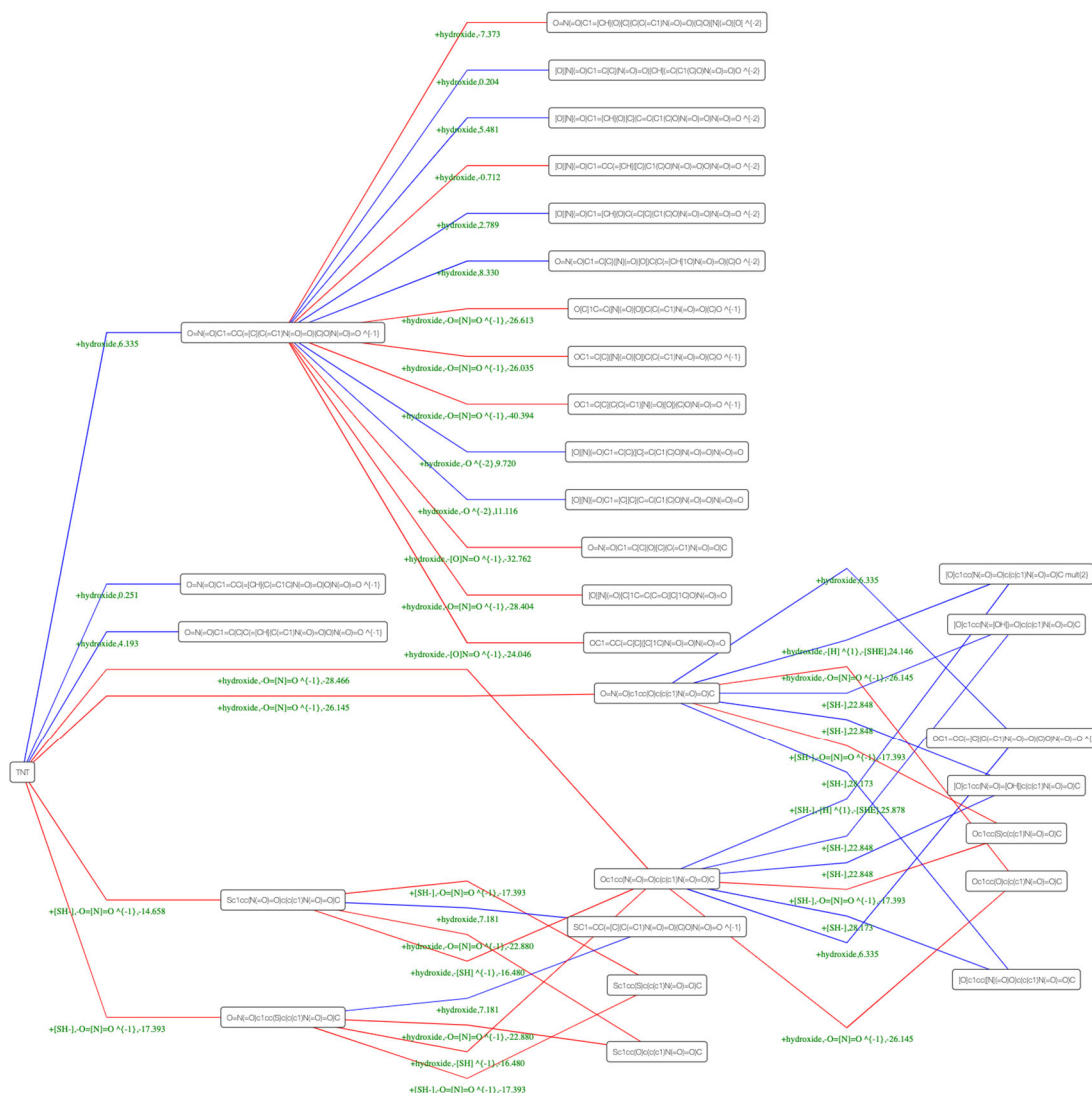


Figure 22. Arrows output for TNT multiple transformation levels. In green: reactants, secondary products, and quantum chemically computed reaction Gibbs free energy ($\Delta G_{rxn}(aq)$, kcal/mol).

11.2. Objective 2—Determination of Product Properties

Many of the degradation products identified in **Objective 1, Section 11.1**, had not been characterized in terms of environmental fate or potential effects. This objective focused on gathering data and rendering estimates on the fate/effects determining properties of the compounds. As in **Objective 1, Section 11.1**, the overall approach was to integrate and reconcile predictive model results with experimentally measured data, in

order to provide a greater level of confidence in both. **Objective 2** included three specific tasks.

11.2.1. Measured Properties (Task 2.1)

11.2.1.1. Environmental Mobility

In unison with data mining for **Task 1.2, Section 11.1.2.1**, collection of reported measured values for relevant environmental fate/effect determining physico-chemical properties (PChPs) was conducted. The PChPs include water solubility (S) and octanol-water partition coefficient (K_{ow}). While measured values for all the compounds in the data set were not found, values of $\log S$ range from -3.30 to -0.88 M for the parent compounds and from -4.18 to -0.41 M for the products, and values of $\log K_{ow}$ range from -1.70 to 2.37 L/kg and from -1.00 to 2.00 L/kg, respectively.

Figure 23 and **Figure 24** are graphical summaries of published PChPs data for parent compounds. Overall, limited and preliminary data were found for transformation products relative to parent compounds. **Figure 23** and **Figure 24** show that aliphatic IMCs (e.g., NTO and NQ) tend to be significantly more water soluble than legacy nitroaromatic MCs (e.g., TNT). In particular, NTO has higher solubility and lower $\log K_{ow}$ than both nitramine and nitroaromatic (I)MCs. Similarly, reduction products of tend to be more soluble and less lipophilic than their parent compound (**Figure 25** and **Figure 26**). These characteristics are indicative of poor sorption to soil, which raises concerns for increased environmental mobility.

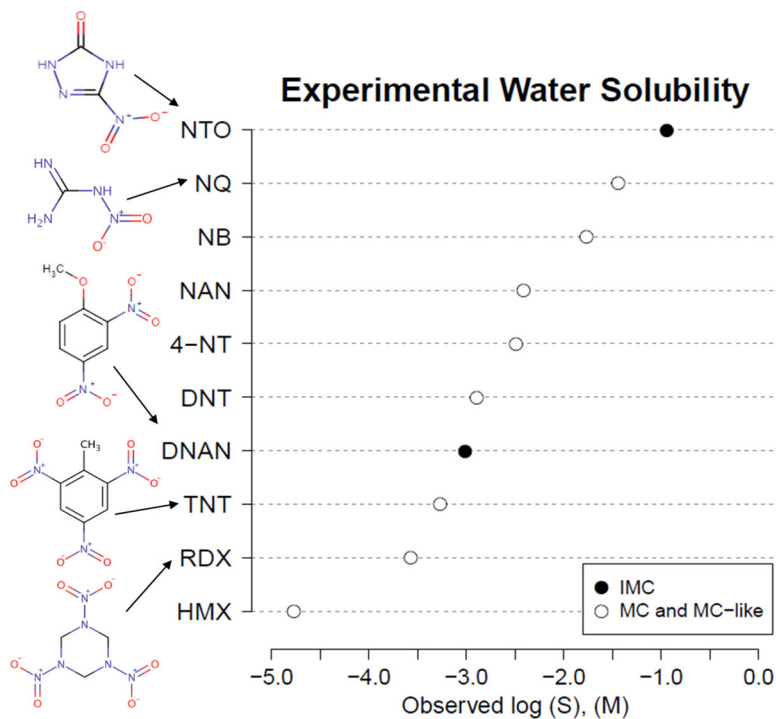


Figure 23. Literature data mined for water solubility (S) of (I)MCs and MC-like compounds.

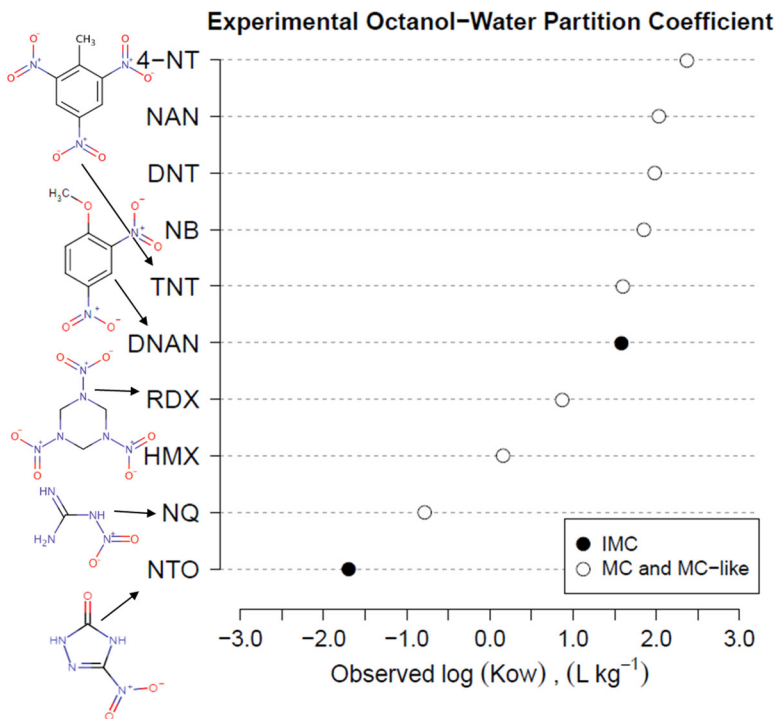


Figure 24. Literature data mined for octanol-water partition coefficient (K_{ow}) of parent (I)MCs and MC-like compounds.

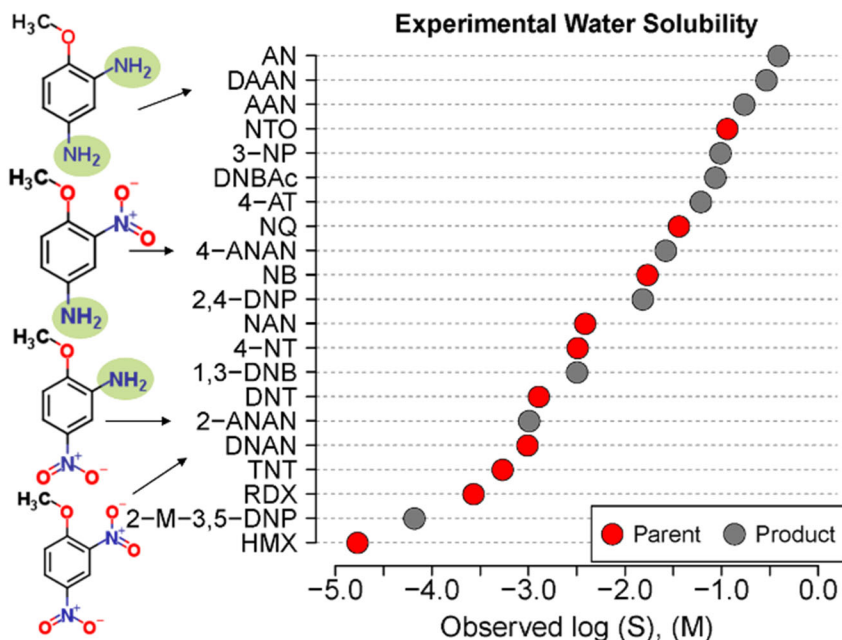


Figure 25. Literature data mined for water solubility (S) of parent and transformation products of (I)MCs and MC-like compounds. Molecular structures correspond to DNAN and its reduction transformation products.

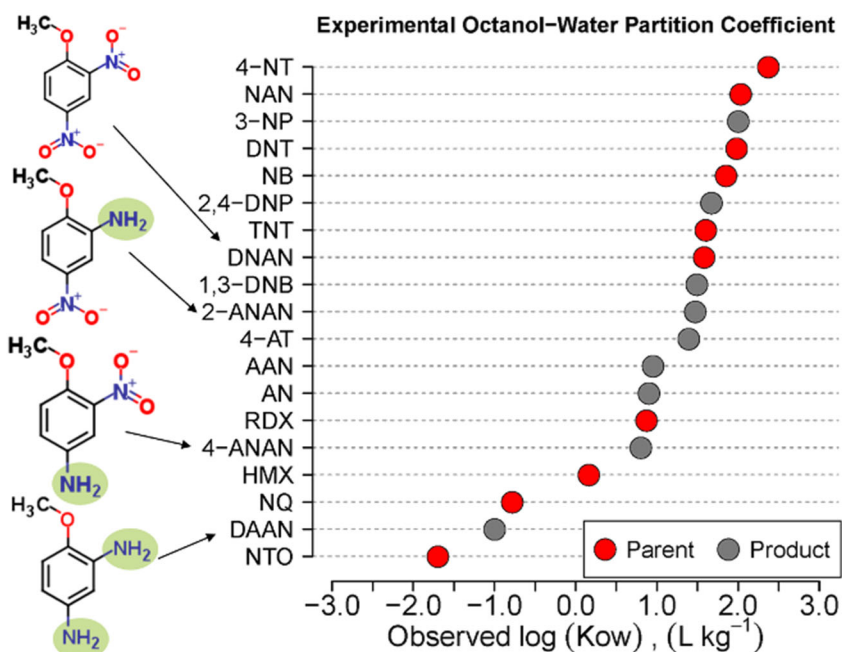


Figure 26. Literature data mined for octanol-water partition coefficient (K_{OW}) of parent and transformation products of (I)MCs and MC-like compounds. Molecular structures correspond to DNAN and its reduction transformation products.

11.2.2. Predicted Properties (Task 2.2)

11.2.2.1. Environmental Mobility

Giving the scarcity of data, one of the pressing needs for the environmental assessment of existing and candidate (I)MCs is to be able to predict the PChPs of parent compounds and transformation products from molecular structure. As a response to this need, three predictive models were evaluated in this task for the prediction of water solubility and K_{ow} : (i) U.S. EPA's EPI Suite, (ii) SPARC, and (iii) Abraham pp-LFERs (polyparameter linear free energy relationships).

The WSKOW module of EPI Suite and SPARC, both well-known and extensively used models, are mainly based on an atom/fragment contribution method while the pp-LFERs are based on additive energies of molecular interactions. In that regard, pp-LFERs are a more advanced tool that provides an alternative to fragment-based models when the compounds of interest contain less common or new fragments, as is the case for most of the aliphatic IMCs, namely, RDX, NQ, and NTO.

The experimentally measured values collected in **Task 2.1, Section 11.1.2.1**, were compared against estimates by these three molecular-structure-based models, as shown in **Figure 27**. As seen in the graphical results, SPARC performs far superiorly than pp-LFERs (with unoptimized molecular descriptors obtained from the UFZ database (Ulrich et al., 2017) and equally or better than EPI Suite in the prediction of water solubility and K_{ow} . SPARC yields the smallest prediction errors (root mean square error, RMSE = 0.777 and 0.571 for $\log(S)$ and $\log(K_{ow})$, respectively) among the tested models as it is highly calibrated, well tested, and actively maintained. EPI Suite renders smaller prediction errors (RMSE = 1.209 and 0.475 for $\log(S)$ and $\log(K_{ow})$, respectively) than the pp-LFERs (RMSE = 3.112 and 1.841 for $\log(S)$ and $\log(K_{ow})$, respectively) largely because most of the observed data collected belong to the EPI Suite calibration database. All three models predict nitroaromatic compounds well but yield large errors for nitramines (I)MCs, i.e., the outlier data points in **Figure 27**: RDX, HMX, NQ, and NTO.

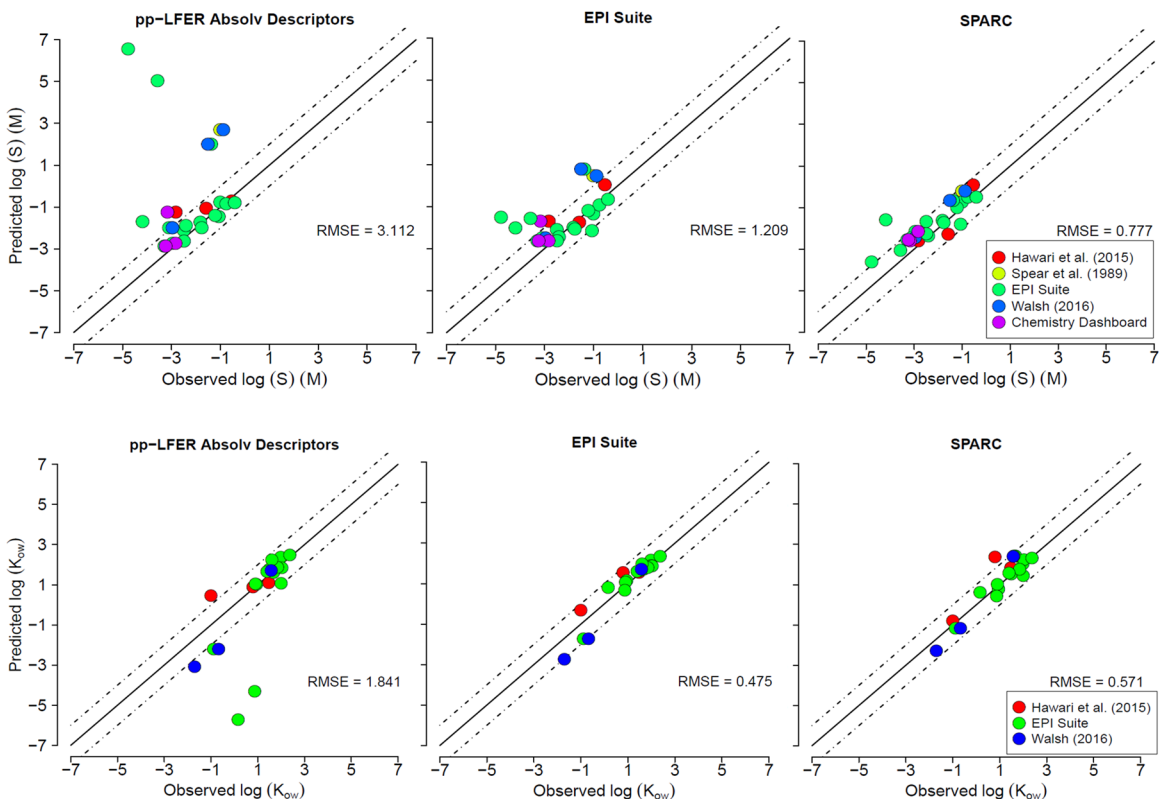


Figure 27. Predicted vs. observed water solubility (S , top row) and octanol-water partition coefficient (K_{ow} , bottom row) values. Three estimation models: pp-LFERs, EPI Suite, and SPARC. RMSE: Root Mean Square Error.

11.2.2.2. Potential for Treatability/Transformation

In addition to estimating molecular descriptors for the prediction of physicochemical properties, we estimated molecular descriptors that assess the reactivity to redox-type transformations, which can then be used to estimate reaction rate constants, for the set of parent and product compounds collected in the previous task (**Task 2.1, Section 11.2.1**). These descriptors are the energies for both the highest occupied molecular orbital (E_{HOMO}) and lowest unoccupied molecular orbital (E_{LUMO}). E_{HOMO} and E_{LUMO} are frequently and strongly correlated with redox potentials and, by extension, with reduction and oxidation rate constants as these types of reactions generally involve the gain or loss of electrons to or from the LUMO and the HOMO, respectively (Méndez-Hernández et al., 2013; Miehr et al., 2004). We performed the computations using Density Functional Theory (DFT) for 39 unique molecules.

Details of the computational methods are as follows. DFT computations were performed using four different exchange-correlation functionals (xcFncs): viz. B3LYP,

PBE0, PBE, and M06-2X. These are well-known and commonly used xcFncs that differ on the fashion in which they model the exchange and correlation interactions between electrons. All four are hybrid xcFncs, which means that they combine a portion of exchange energy (released when two or more electrons exchange positions in orbitals) from Hartree-Fock theory (in which the motion of an electron is modeled with a single-particle function independent of the instantaneous motion of other individual electrons, but rather assumed as an average electron charge cloud/distribution) with the rest of the exchange-correlation energy from other sources (e.g., local density approximation, generalized gradient approximation). The distribution and sources of said portions mark the differences among the four xcFncs.

We have summarized the results of the computations in **Figure 28**, **Figure 29**, and **Figure 30**, which include both scatter and bar figures of E_{HOMO} , E_{LUMO} , or E_{Gap} ($= E_{\text{LUMO}} - E_{\text{HOMO}}$) vs. compound ID/name in ascending order based on B3LYP values. For most of the molecules the trends in E_{HOMO} and E_{LUMO} values were similar among the different xcFncs. A few discrepancies were observed in the E_{LUMO} values for some of the product molecules.

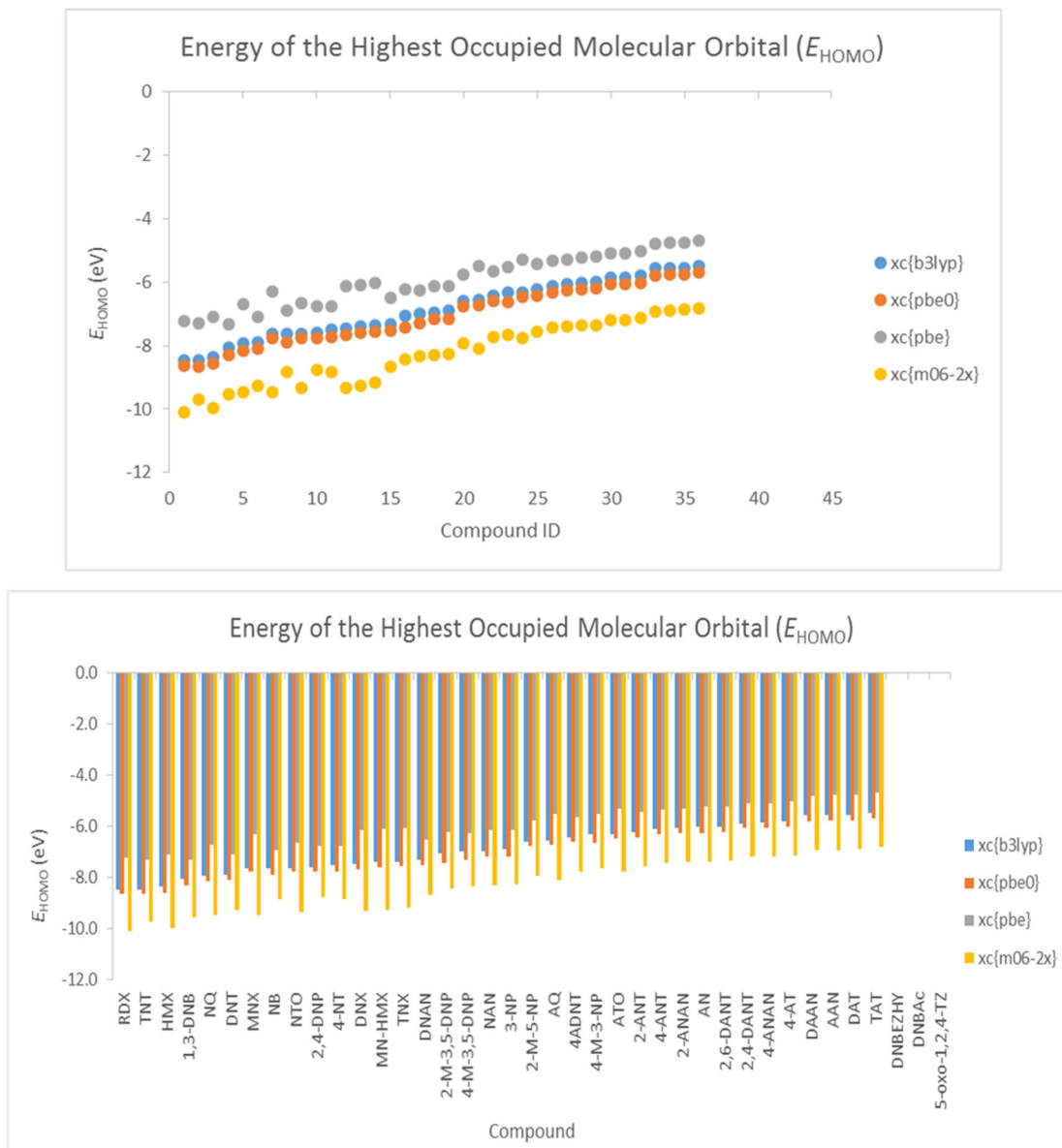


Figure 28. Computationally estimated energies of the highest occupied molecular orbital (E_{HOMO}) for parent (I)MCs and their transformation products. Theory level: Density Functional Theory (DFT). Exchange-correlation functionals (xcFncs): B3LYP, PBE0, PBE96, and M06-2X. Basis set: 6-311++G(2d,2p).

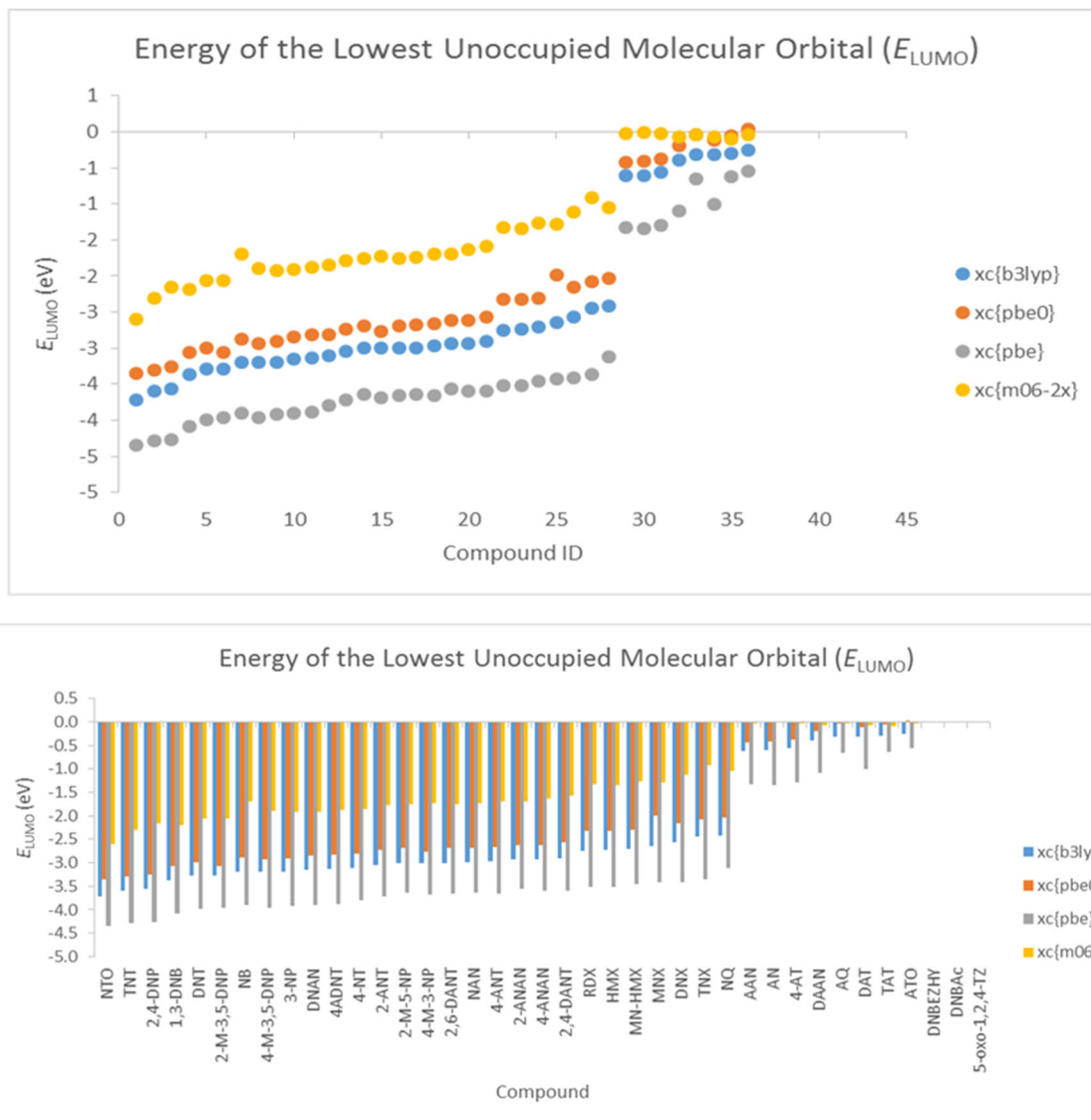


Figure 29. Computationally estimated energies of the lowest unoccupied molecular orbital (E_{LUMO}) for parent (I)MCs and their transformation products. Theory level: Density Functional Theory (DFT). Exchange-correlation functionals (xcFncs): B3LYP, PBE0, PBE96, and M06-2X. Basis set: 6-311++G(2d,2p).

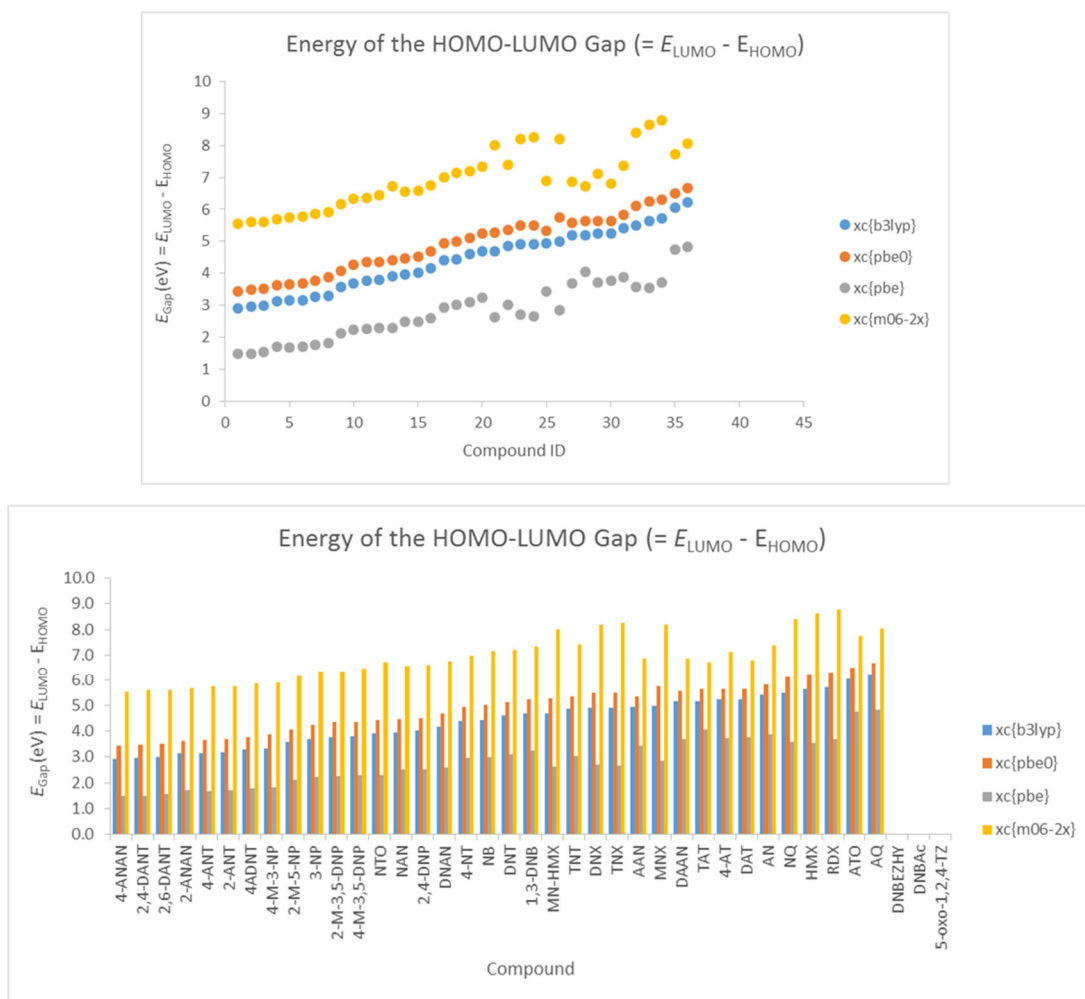


Figure 30. Computationally derived HOMO-LUMO Gap ($= E_{\text{LUMO}} - E_{\text{HOMO}}$) for parent (I)MCs and their transformation products.

E_{LUMO} of parent (I)MCs tend to be more negative than those of their reductively transformed products (e.g., trinitrotoluene vs. triaminotoluene). Larger negative E_{LUMO} values are indicative of easiness for reductive transformation of a molecule as they are closely related with the amount of energy released when an electron is added to the LUMO. Hence, it is not surprising that the parent compounds which contain electron withdrawing moieties ($-\text{NO}_2$ functional groups), have more negative E_{LUMO} values than their reduced products ($-\text{NH}_2$ -containing molecules).

Equivalently, E_{HOMO} of parent (I)MCs tend to be more negative than those of their reductively transformed products. Less negative E_{HOMO} values are indicative of easiness for oxidative transformation of a molecule as they are closely related with the amount of energy required to remove an electron from the HOMO. Therefore, as expected, the

reductively transformed products which contain electron deprived moieties ($-\text{NH}_2$ functional groups), have less negative E_{HOMO} values.

This computational assessment of reactivity indicates that the transformation products second stage of treatment technologies targeted to parent (I)MCs, which generally consists mainly based on reductive reactions, that are coupled might not be as effective for their transformation products. This in addition to the increased environmental mobility of the products, as described above from their higher water solubility and lower sorption tendencies, presents a challenge for the

11.2.2.3. Potential for Toxicity

We obtained results for this component with the U.S. EPA EcoSAR model. Using only the molecular structure of the compounds and EcoSAR's K_{OW} -based structure activity relationships, we estimated toxicity values for a few organisms (e.g., fish, daphnid, earthworm) at different endpoints (e.g., 48 h LC50, 14 d LC50, Chronic Value) and compared them graphically in **Figure 31**. The results of this hierarchical comparison, i.e., compounds ordered in decreasing toxicity from left-to-right and top-to-bottom in **Figure 31**, suggest that nitroaromatic (I)MCs (i.e., DNAN, TNT) tend to be more toxic than nitramines (I)MCs (i.e., RDX, NQ). Also, observed reduction transformation products (gray boxes in **Figure 31**) of both NACs and nitramines tend to be less toxic than their corresponding parent compounds (red boxes in **Figure 31**).

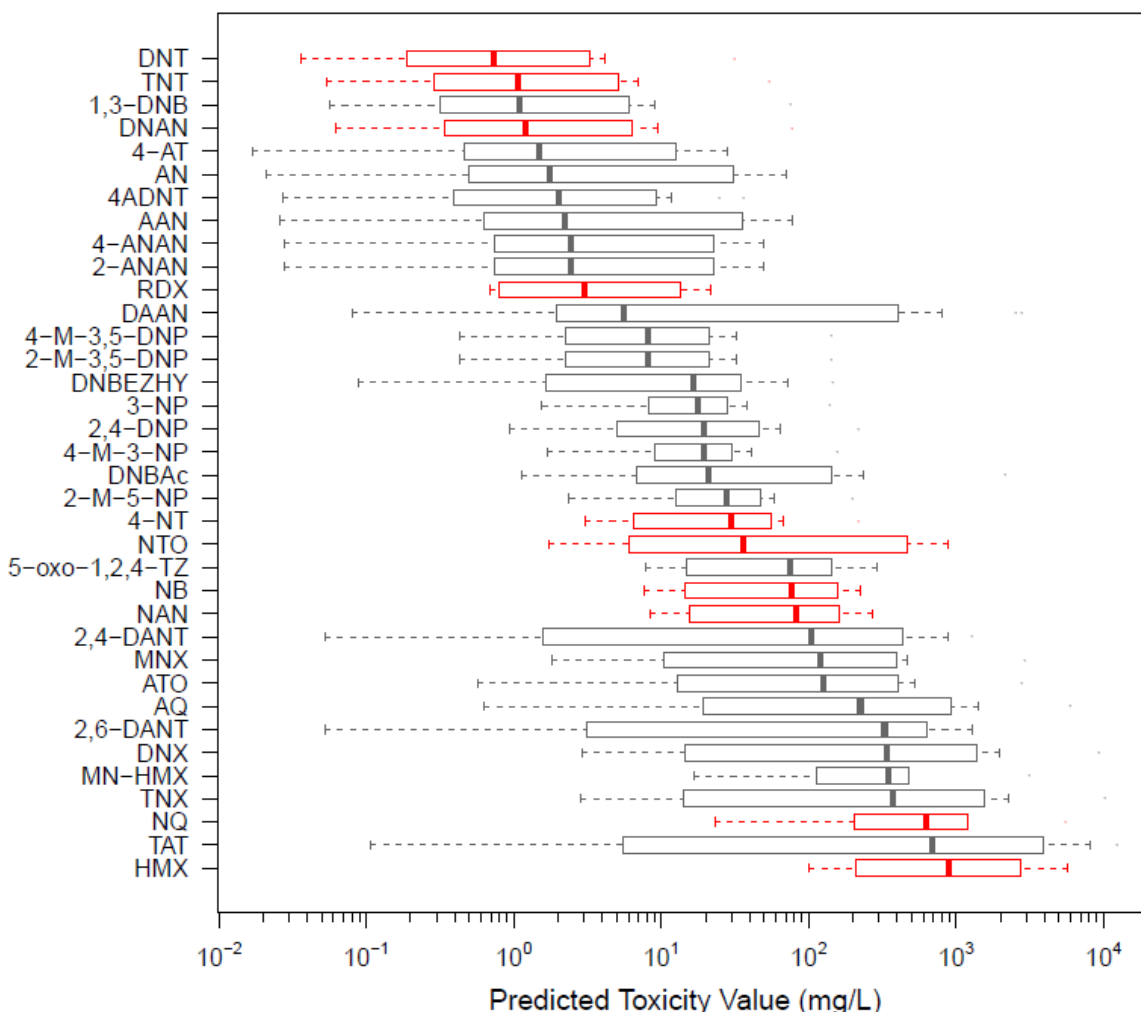


Figure 31. U.S. EPA EcoSAR estimated toxicity values for (I)MCs (red) and their observed transformation products (gray). Boxes contain data for different organisms and different endpoints.

11.2.3. New Prediction Methods (Task 2.3)

In this task, we began to explore the even greater challenge of predicting transformation reaction *rates* (i.e., kinetic rate constants), which is not addressed in any of the existing models discussed above. We set out to build on existing modeling frameworks, with re-calibration and validation of the model, as necessary. For example, the QSARs we have just developed for oxidation of aromatic amines by manganese oxides (Objective 4 of ER-1734, and **Figure 3** above) will cover the main transformation products of DNAN, and might be applicable to the products of NTO transformation with further calibration. QSARs for other transformation pathways (e.g., nitro reduction) could

be identified, validated (by comparison to data from Task 2.1), and adapted, when feasible.

Estimating relative rates of all the steps in a (predicted) IMC transformation network would require determining the reaction barriers (activation energies) for each reaction step. This is not feasible experimentally, but they can be calculated *in silico* using Arrows to automate determination of the reaction pathways and transition-states. For example, **Figure 32** shows the relative reaction energies and barriers for the first three steps in the hydrolysis reactions of TNT obtained using Arrows. These types of calculations are fairly well automated with Arrows, but the amount of computer time needed to completely evaluate the transformation pathways for most IMCs makes this impractical for most users. One approach to overcoming this problem is to estimate the activation barriers using a single parameter linear Gibbs energy relationship, commonly known as a linear free energy relationship (LFER). Using a LFER, such as the Bell-Evans-Polanyi principle, where the activation energy is linearly related to the reaction energy or reaction enthalpy is a well-known way around this problem that is used in environmental chemistry, catalytic chemistry, and other chemistry fields. The basic justification for using this relationship is that the difference between the activation energy and a reaction energy is approximately a constant, for specific type of chemical reaction.

For the example shown in **Figure 32**, the activation barriers were estimated from LFERs based on the differences between activation and reaction energies from PBE0 AIMD/MM reaction pathway simulations calculated using Arrows. The LFER parameters for the different reaction types, denoted by $\Delta\gamma^\ddagger = \Delta G^\ddagger - \Delta G_{rxn}$, were estimated to be 48.6 kcal/mol ($\sigma = 2.4$ kcal/mol), 19.4 kcal/mol ($\sigma = 2.4$ kcal/mol), 27 kcal/mol, and 31 kcal/mol for the Meisenheimer complex (4 reactions available), nitroaromatic substitution (5 reactions), proton abstraction (1 reaction) and Murto-Tomilla (1 reaction) reaction types. Inverting this relation, the reaction barrier for an unknown reaction with a specific reaction type is estimated using $\Delta G^\ddagger = \Delta\gamma^\ddagger - \Delta G_{rxn}$. This type of LFER relation is obviously approximate and estimating the exact kinetics will have large errors. Furthermore, the LFER parameters will also depend on the exact reaction conditions (e.g., temperature, pressure, catalyst & solvent). However, this approximation seems appropriate for use with RCDs that are meant to provide graphical insight for interpreting reaction networks and narrowing the list of reactions for further calculations and experimental study. We note there are several ongoing research and software development efforts that are combining the automation of electronic structure

calculations, such as Arrows, with direct kinetic modeling and machine learning to improve reaction kinetic estimates of complex chemical networks.

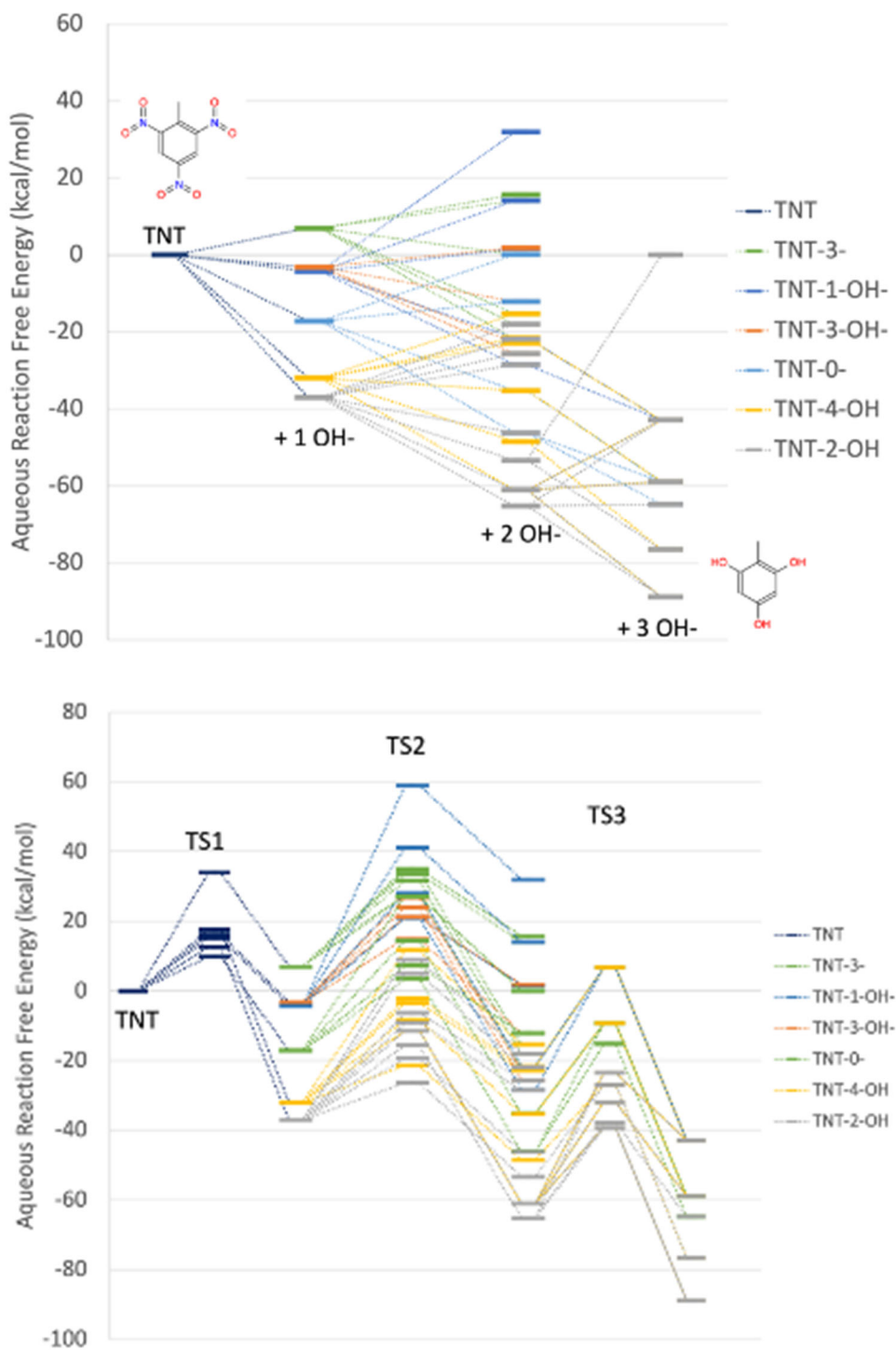


Figure 32. Reaction coordinate diagrams (RCDs) for the first three hydrolysis reactions of TNT in aqueous solution. The left figure shows the RCDs without transition states,

and the right figure includes transition states. The lowest energy metabolite is shown on the bottom right (2,4,6-Trihydroxytoluene, SMILES Cc1c(O)cc(O)cc1O). Results are for calculations using the B3LYP DFT exchange-correlation functional, 6-311++G(2d,2p) basis set and COSMO solvation model for water. The RCDs are selectively pruned to emphasize the lower energy levels. The spreadsheet and database capabilities in Arrows were used for these calculations.

11.3. Objective 3—Development of Transformation Models

The transformation pathways and products determined in **Objective 1, Section 11.1**, and fate-determining properties of transformation products determined in **Objective 2, Section 11.2**, provided the background necessary to incorporate transformation into models of the fate, transport, and risk of IMCs. In this objective, the approach emphasized collaboration with existing efforts to develop models of IMC fate and transport for risk assessment and site management (i.e., TREECS). The overall approach was to provide a validated framework for modeling IMC transformation that could be integrated into TREECS or other site management tools. **Objective 3** required three specific tasks.

11.3.1. Basic Transformation Model (Task 3.1)

We built a simplified, flexible, and general kinetic model for IMCs transformation using Matlab Simulink. The model was meant to utilize the pathways determined by **Objective 1, Section 11.1**, and parameters assembled under **Objective 2, Section 11.2**. We built the first order decay component which consists of an integrator (to solve the Ordinary Differential Equation, ODE) with two inputs that are the initial concentration of the contaminant and the predicted decay rate. The output loops back around to the integrator at each predicted time point. A figure showing the results of the calculation is generated in a pop-up window when the model is run as shown in **Figure 33**. The next step was to generate the component for the appearance of the products and nest it within the parent compound decay module.

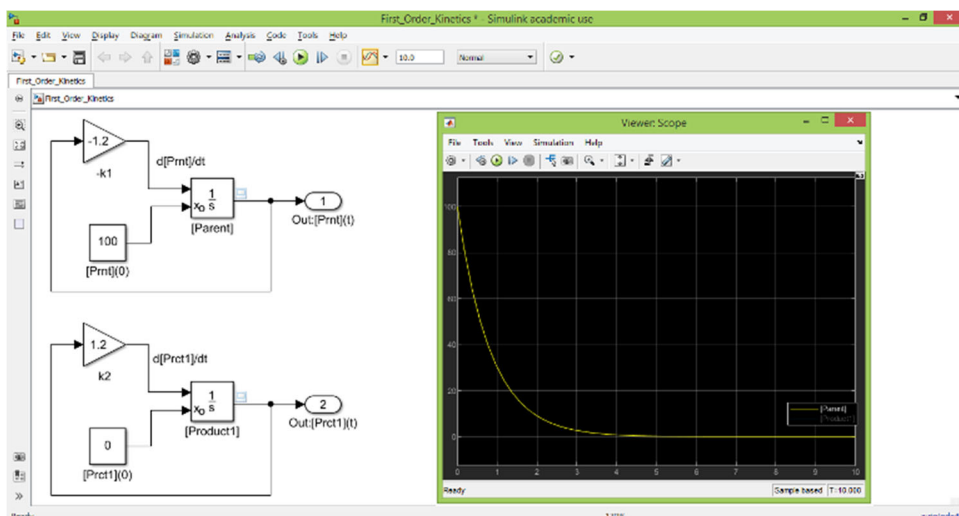


Figure 33. Screenshot of graphical first order kinetic model built using Matlab Simulink. Top left schematics represents the decay of the parent compound. Numbers for the decay rate (k) and the initial contaminant concentration ($[Prnt](0)$) were taken from results of in-house reduction kinetic experiments with a nitroaromatic model compound, nitrobenzene. Bottom left is a template for the appearance of the first transformation product, will be edited next. Scope window on the right is a graphical output of the models on the left showing contaminant concentration vs. time.

We extended the simplified “sandbox” Matlab Simulink transformation model to include both the disappearance of the (I)MCs parent compounds and the appearance of the transformation products for reductive transformation including intermediates. **Figure 34**, **Figure 35**, and **Figure 36** show the results of the calculations, a pop-up window is generated when the model is run. We performed demonstration runs using provisional numbers for reduction rate constants for nitrobenzene (NB), nitrotriazolone (NTO), DNAN, and TNT.

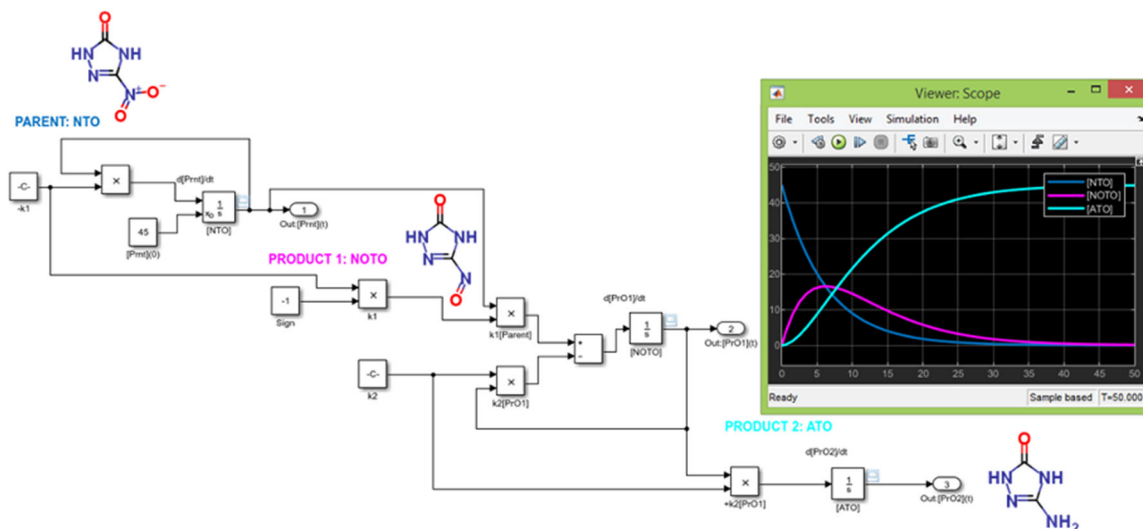


Figure 34. Screenshot of results from demonstration run of our “sandbox” transformation model built using Matlab Simulink: First order kinetics for reduction of NTO. Inset figure shows concentration vs. time.

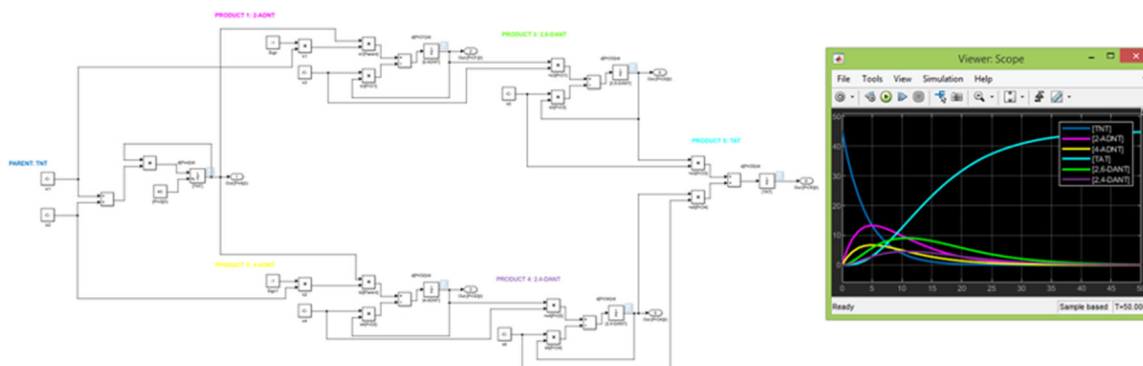


Figure 35. Screenshot of results from demonstration run of our “sandbox” transformation model built using Matlab Simulink: First order kinetics for reduction of TNT. Inset figure shows concentration vs. time.

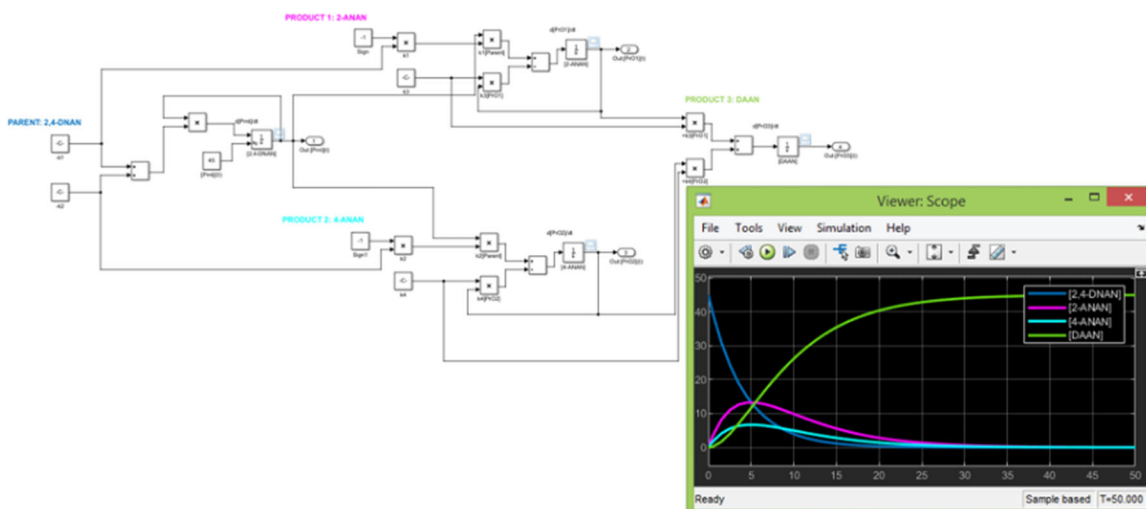


Figure 36. Screenshot of results from demonstration run of our “sandbox” transformation model built using Matlab Simulink: First order kinetics for reduction of DNAN. Inset figure shows concentration vs. time.

11.3.2. Model Validation (Task 3.2)

With the purpose of evaluating the performance of the Matlab Simulink model described in the previous Task and given the lack of experimental data for IMC reduction kinetics in a simplified system, we simulated the reduction of NB to AN (aniline) and compared the results to the experimental data reported in (Gorski et al., 2016), as shown in **Figure 37**. The Matlab Simulink model was able to predict both the decay of the parent compound, NB, and the exponential increase in the concentration of the reduced transformation product, AN, as seen in **Figure 37**, where the model estimated trends resemble those exhibited by the measured data.

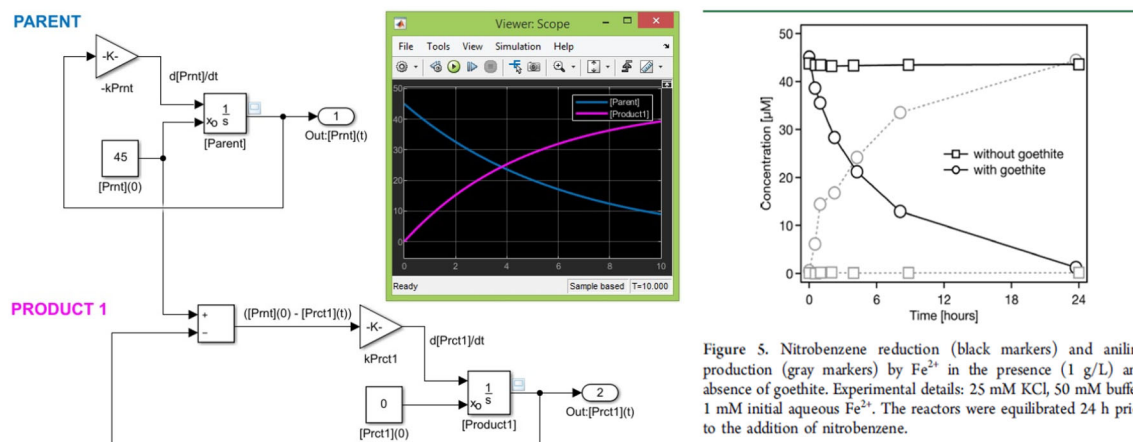


Figure 37. Screenshot of results from demonstration run of our “sandbox” transformation model built using Matlab Simulink. Left panel: First order kinetics for reduction of NB (to AN, aniline) with inset figure showing concentration vs. time. Right panel: Figure showing experimental data on NB reduction to AN, taken from (Gorski et al., 2016) for illustrative comparison purposes.

To assess the sensitivity of the model simulation to the input AN decay rate, we tested decay rate values that were about a factor of two above (0.42 h^{-1}) and below (0.10 h^{-1}) the rate obtained from fitting the experimental data (0.21 h^{-1}). The results are shown in **Figure 38** where the predicted simulations using either of the alternative decay values miss the trend displayed by the AN experimental data increasing the prediction RMSE (root mean square errors) by about 30%.

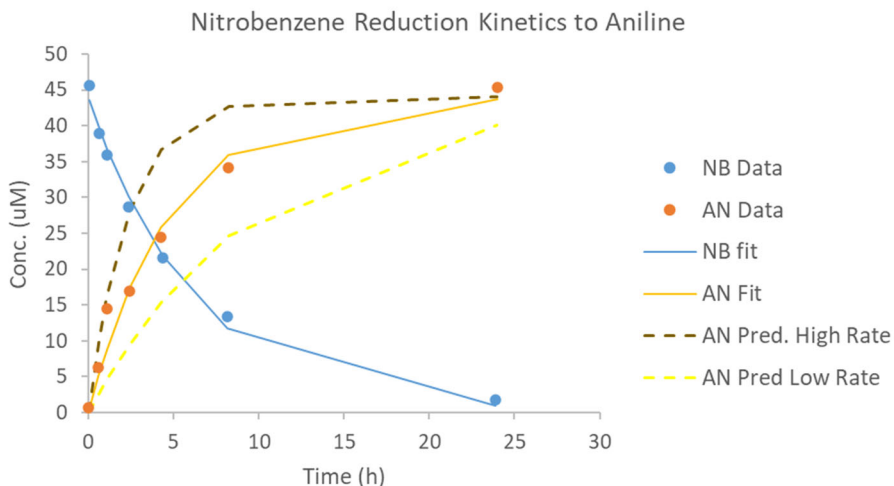


Figure 38. Modeled first order kinetics for reduction of NB to AN (aniline) showing sensitivity of predicted AN concentration to decay rates. Data from (Gorski et al., 2016).

These results indicate that the Matlab Simulink “sandbox” model can be used to generate simulations of decay of IMCs parent compounds as well as exponential increase in their corresponding reduction products, which are expected to be those of major importance. These simulations can also be used to forecast the time needed for complete transformation of the parent compounds across a series of decay rates that may vary as a function of environmental conditions at different training sites.

11.3.3. Adapt for TREECS (Task 3.3)

11.3.3.1. Data for TREECS calibration and testing

We have compiled a set of 35 experimental literature reaction rate constants for 6 compounds including (I)MCs, their transformation products, and munitions-like compounds (e.g., Nitrobenzene). The data are presented in **Table 3**. The reaction processes include chemical reduction and biodegradation. Since it has been very challenging to find uniform datasets across different sources, we have adopted a normalization strategy that consists of calculating relative reaction rates using Nitrobenzene as a reference compound. This normalization allows us to use data that otherwise would be of no use due to mismatching experimental conditions. This dataset can now be incorporated as a validation set in the development of TREECS to test the performance of the fate and transport model.

Table 3. Literature reaction rate constants (k) for (I)MCs, their transformation products, and munitions-like compounds. These data are for a subset of compounds from our larger physicochemical properties experimental dataset from where the entry ID numbers originate.

ID	Chem. Abbrev.	CAS	Transformation Type	k (M ⁻¹) d ⁻¹	Reaction Order	Reactant	$k_{\text{Rel. NB}}$	Source
35	DNAN	119-27-7	Photodegradation	2.62E-01	1			(Halasz et al., 2018)
36	NTO	932-64-9	Photodegradation	3.49E-01	1			(Halasz et al., 2018)
37	NQ	556-88-7	Photodegradation	1.18E+00	1			(Halasz et al., 2018)
53	DNAN	119-27-7	Abiotic Reduction	-1.90E+00	1	Fe(II)		(Niedźwiecka et al., 2017)
54	DNAN	119-27-7	Abiotic Reduction	-1.34E+02	1	Fe(II)		(Niedźwiecka et al., 2017)
55	2-ANAN	99-59-2	Abiotic Reduction	1.03E+04	2	Lawson Singly Deprotonated (quinone; HLAW ⁻)	0.10	(Murillo-Gelvez et al., 2019)
56	2-ANAN	99-59-2	Abiotic Reduction	1.86E+07	2	Lawson Doubly Deprotonated (quinone; LAW2 ⁻)	0.14	(Murillo-Gelvez et al., 2019)
57	2-ANAN	99-59-2	Abiotic Reduction	2.68E+03	2	AQDS Fully Protonated (quinone, AH2QDS)	0.31	(Murillo-Gelvez et al., 2019)
58	2-ANAN	99-59-2	Abiotic Reduction	1.24E+06	2	AQDS Single Deprotonated (quinone, AHQDS ⁻)	0.13	(Murillo-Gelvez et al., 2019)
59	2-ANAN	99-59-2	Abiotic Reduction	2.32E+04	2	AQS Fully Protonated (quinone, AH2QS)	1.16	(Murillo-Gelvez et al., 2019)
60	2-ANAN	99-59-2	Abiotic Reduction	1.35E+07	2	AQS Singly Deprotonated (quinone, AHQS ⁻)	0.13	(Murillo-Gelvez et al., 2019)
61	4-ANAN	577-72-0	Abiotic Reduction	1.56E+07	2	Lawson Doubly Deprotonated (quinone; LAW2 ⁻)	0.12	(Murillo-Gelvez et al., 2019)
62	4-ANAN	577-72-0	Abiotic Reduction	3.70E+04	2	AQDS Fully Protonated (quinone; AH2QDS)	4.28	(Murillo-Gelvez et al., 2019)
63	4-ANAN	577-72-0	Abiotic Reduction	3.52E+06	2	AQDS Singly Deprotonated (quinone; AHQDS ⁻)	0.36	(Murillo-Gelvez et al., 2019)
64	4-ANAN	577-72-0	Abiotic Reduction	1.34E+05	2	AQS Fully Protonated (quinone; AH2QS)	6.68	(Murillo-Gelvez et al., 2019)
65	4-ANAN	577-72-0	Abiotic Reduction	1.99E+07	2	AQS Singly Deprotonated (quinone; AHQS ⁻)	0.20	(Murillo-Gelvez et al., 2019)
66	NB	98-95-3	Abiotic Reduction	1.05E+05	2	Lawson Singly Deprotonated (quinone; HLAW ⁻)	1.00	(Murillo-Gelvez et al., 2019)

67	NB	98-95-3	Abiotic Reduction	1.35E+08	2	Lawson Doubly Deprotonated (quinone; LAW2 ⁻)	1.00	(Murillo-Gelvez et al., 2019)
68	NB	98-95-3	Abiotic Reduction	8.64E+03	2	AQDS Fully Protonated (quinone, AH2QDS)	1.00	(Murillo-Gelvez et al., 2019)
69	NB	98-95-3	Abiotic Reduction	9.76E+06	2	AQDS Single Deprotonated (quinone, AHQDS ⁻)	1.00	(Murillo-Gelvez et al., 2019)
70	NB	98-95-3	Abiotic Reduction	2.00E+04	2	AQS Fully Protonated (quinone, AH2QS)	1.00	(Murillo-Gelvez et al., 2019)
71	NB	98-95-3	Abiotic Reduction	1.00E+08	2	AQS Singly Deprotonated (quinone, AHQS ⁻)	1.00	(Murillo-Gelvez et al., 2019)
72	NTO	932-64-9	Anaerobic Reduction	5.10E+02	2	H2 added to Catlin Soil (IL)		(Krzmarzick et al., 2015)
73	NTO	932-64-9	Anaerobic Reduction	2.50E+02	2	H2 added to Maricopa (AZ)		(Krzmarzick et al., 2015)
74	NTO	932-64-9	Anaerobic Reduction	2.30E+02	2	H2 added to Roger Road (AZ)		(Krzmarzick et al., 2015)
75	NTO	932-64-9	Anaerobic Reduction	7.50E+02	2	H2 added to Camp Ripley (MN)		(Krzmarzick et al., 2015)
76	NTO	932-64-9	Anaerobic Reduction	8.00E+00	2	NO H2 added to Camp Ripley (MN)		(Krzmarzick et al., 2015)
77	NTO	932-64-9	Anaerobic Reduction	1.25E+03	2	H2 added to Camp Navajo (AZ)		(Krzmarzick et al., 2015)
78	NTO	932-64-9	Anaerobic Reduction	4.80E+01	2	NO H2 added to Camp Navajo (AZ)		(Krzmarzick et al., 2015)
79	NTO	932-64-9	Anaerobic Reduction	7.40E+02	2	H2 added to Florence (AZ)		(Krzmarzick et al., 2015)
80	NTO	932-64-9	Anaerobic Reduction	1.20E+01	2	NO H2 added to Florence (AZ)		(Krzmarzick et al., 2015)
81	NTO	932-64-9	Biodegradation	3.29E+00	1	Non-sterile Catlin stop-flow soil		(Mark et al., 2017)
82	NTO	932-64-9	Abiotic Degradation	1.15E+00	1	Sterile Catlin Flow stop-flow soil		(Mark et al., 2017)
83	NTO	932-64-9	Biodegradation	7.20E-01	1	Non-sterile Sassafras stop-flow soil		(Mark et al., 2017)
84	NTO	932-64-9	Abiotic Degradation	1.20E-03	1	Sterile Sassafras stop-flow soil		(Mark et al., 2017)

In addition, a new version of TREECS (v. 6.1) has been recently released along with the upcoming version 0.1 of its successor, the Fate and Risk Evaluation System for Contaminants (FRESCO). Ideally the literature set will serve to test the capabilities of both of these new releases.

11.3.3.2. Model and results from TREECS team

The Training Range Environmental Evaluation and Characterization System (TREECS™) was developed for the Army to forecast the fate of and risk from munitions constituents (MC) within and transported from firing/training ranges to surface water and groundwater. The objective of this project component is to modify the TREECS tool for assessing and managing the environmental risks of MC by extending the capability to model the products of MC transformation. The chemical transformation capability allows for the modeled removal of parent compounds and generation of daughter products in sequenced chemical reactions. The modifications to allow for chemical transformations will improve the accuracy of TREECS Tier 2 estimates of soil concentrations and allow for estimates of the mass flux of daughter products to and transport through downstream modules.

The TREECS Tier 2 methods within TREECS include time-varying analysis and MC degradation and are the most appropriate workflow to adapt for chemical transformations. TREECS Tier 2 consists of a mass loading module to determine the mass of MC applied to an area of interest (AOI), a soil module that translates MC mass inputs directly to surface water or to deeper groundwater pathways, a vadose zone module for vertical transport through the unsaturated zone to an aquifer, an aquifer module for lateral transport to a receptor well, and a surface water module with two models for the fate of MC in flowing or standing water. **Figure 39** describes summarizes the component models contained in TREECS and details about the TREECS modules, formulation, and validation are available in Dortch et al. (Dortch et al., 2011) and at the TREECS website (<https://treecs.el.erdc.dren.mil/>). Additional details regarding the development of the transformation capability as well as example model files are available in Ryder et al. (Ryder et al., 2023). All TREECS developments described herein have also been incorporated into TREECS successor, the Fate and Risk Evaluation System for Contaminants (FRESCO), and at the time of this report FRESCO v1.1 is available by email request to Dr. Ryder (jodi.l.ryder@usace.army.mil).

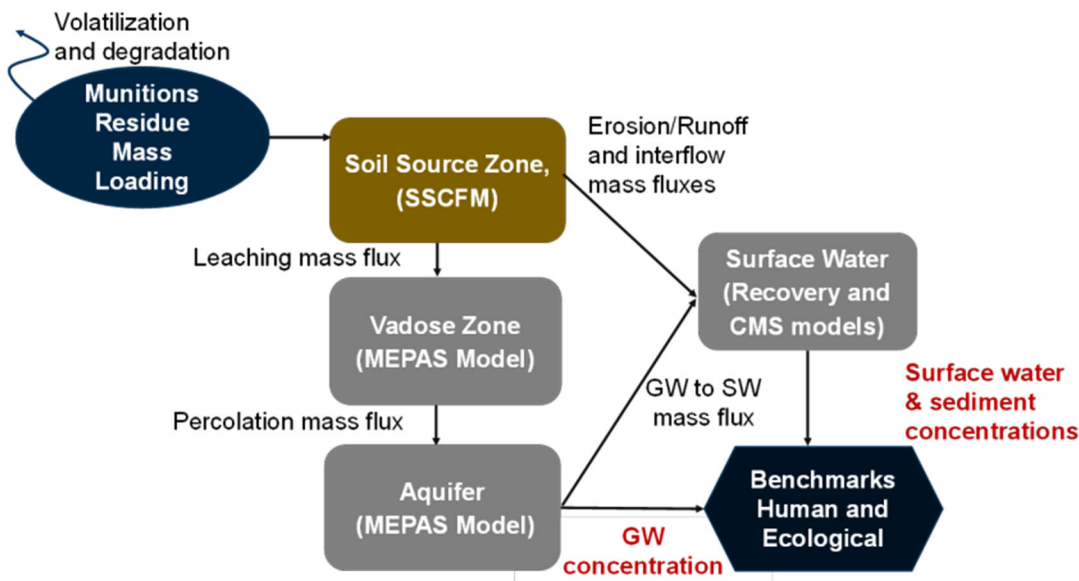


Figure 39. Component fate models and mass transport pathways available within the TREECS interface

The TREECS vadose and groundwater modules (based on the MEPAS model) already allows degradation to daughter products as well as the reporting of mass fluxes of both parent and daughter products. As transformation in the vadose and groundwater modules creates daughter products, those products are transferred to the surface water module. Both models within the surface water module (RECOVERY and CMS) also include the ability to degrade MC to un-tracked mass. However, the Tier 2 surface soil contaminant fate model (SSCFM) required modification to enable the generation of daughter products and tracking of daughter product mass as inputs to downstream modules. The surface soil contaminant fate model (SSCFM) included in TREECS simulates the fate and transport of MCs in the area of interest (AOI) within an installation. The SSCFM model solves mass balance equations for both solid and non-solid phase MC within the AOI. This model was modified and enhanced to include simulating interactions and transformation products of MC for assessing and managing the environmental risks of MC. The new SSCFM was fully tested and verified against its previous versions.

The approach here was to retain and use the existing TREECS framework and associated databases, while implementing upgrades in three components to enable the transformation capabilities, 1) the constituent chemical databases 2) the Surface Soil Contaminant Fate Model (SSCFM), and 3) the user interface.

11.3.3.2.1. TREECS Modifications Overview

Previously, TREECS Tier 2 contaminant fate and transport models included a zero dimensional, fully mixed surface soil model; a one-dimensional (1D) vadose zone model; an aquifer model with 1D longitudinal advection and three-dimensional (3D) dispersion; and a 1D longitudinal surface water model for streams (**Figure 39**). The Tier 2 soil module is a time-varying model that translates MC mass inputs to surface water via rainfall-extracted runoff and erosion and to the vadose zone via infiltration or leaching. The Tier 2 soil module allows MC to exist in solid and non-solid (dissolved) phases and mass losses can occur due to volatilization, degradation (previously only by transformation to an un-tracked species), and sorption.

11.3.3.2.2. TREECS Chemical Databases

TREECS utilizes four databases to reference constituent properties relevant for fate and transport such as mass partitioning parameters, diffusion coefficients, and degradation rates. These databases include the Army Range Constituent Database (Dortch et al., 2005; ZaZakikhani et al., 2002), Framework for Risk Analysis in Multimedia Environmental Systems database (FRAMES (Whelan et al., 1997)), and the Risk Assessment Information System (RAIS) constituent database, (<https://rais.ornl.gov/home/about.html>). During the setup of a new model the user selects parameter values from one of the included databases or inputs their own values. Because the previous version of TREECS allowed contaminants to degrade but did not track the mass flux of the degradation daughter products, there was no information stored in the databases to link one compound to another. A numbering schema and capability to track daughter products was developed to enable continuity from the SSCFM to the downstream vadose, aquifer, and surface water modules.

Chemical specific parameters for each constituent (parent or daughter) such as the aqueous solubility, Henry's Law constant, molecular weight, solid density and solid partitioning coefficient are required to run the TREECS modules. Degradation kinetics are expressed in terms of half-lives and also must be provided. A volatilization rate or MC diffusion coefficient in air is required. TREECS employs multiple databases of contaminant properties as a component of the Hydro-GeoChemical Toolkit. These databases include the ARCDDB, FRAMES, and RAIS. Users can also input values for any parameter manually. As part of the larger project a basic transformation model is being developed which will allow generation of kinetic rates for transformations in a degradation pathway. To allow the utilization of the basic transformation model a new

form in the Hydro-Geo-Chemical Toolkit allows the user to identify the chemical parent and daughter species and assign positions in the overall degradation pathway as well as select a database for the chemical properties or enter those generated by the basic transformation model.

The TREECS SSCFM allows for first order decay rates described as half-lives. Mathematically for growth this is equivalent to a doubling time. A numbering system was needed to allow the kinetic rates to be selected in order to determine the mass of each node at each timestep during the soil model solution. In order to identify which decay and growth reaction pathways (the Arrows on the degradation tree below) apply to a given chemical compound a numbering scheme was devised. This allows information from the basic transformation model to inform the soil model how many chemical compounds are in the degradation tree, how they are connected (i.e., which reactions apply to each node), and which decay or growth terms to include in the mass balance for each chemical compound. Each pathway in the tree is described in three parts, the parent node, the target node, and all daughter nodes.

A new form in the Hydro-Geo-Chemical-Toolkit called the Degradation Pathways table allows users to enter the decay rate constants for each transformation pathway as well as the description of the network using the numbering scheme described below.

A chemical degradation pathway can be thought of as a tree type network with chemical species as nodes and kinetic rates as branches connecting the nodes (**Figure 40**). In order to quantify the mass of each parent and daughter product at each timestep the kinetic relationship between each parent and daughter pairing must be known. In the case of DNAN (2,4-dinitroanisole) there are three levels to the degradation pathway resulting in six unique compound species: M1, M2, M3, M4, M5, and 4-ANAN (**Figure 40**) (Schroer, 2018). Restricting the reactions to the net reactions that generate the six compounds in the degradation tree there are nine unique first order reaction rates needed to describe the system. Since one munition may produce multiple daughter compounds, it is assumed that each constituent it has a unique decay pathway that is independent of any other starting constituent.

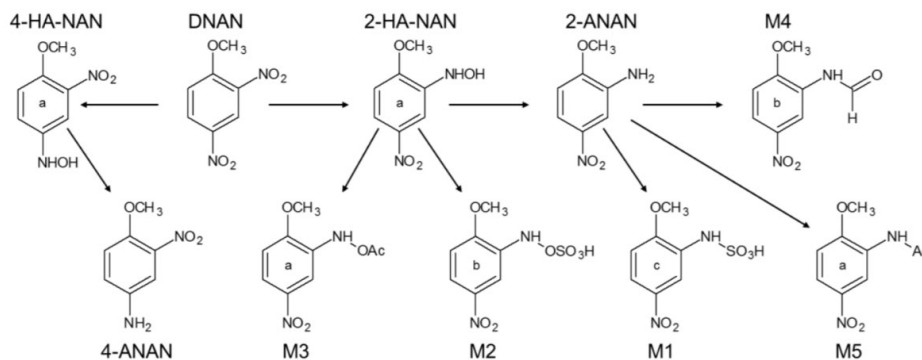


Figure 40. Sample transformation pathway scheme for DNAN (Schroer, 2018).

The degradation tree and the connections between chemical compounds are essential to develop equations relating the decay of each compound and transformation to new compounds to the total mass or concentration of each compound in the environmental media. Within the TREECS chemical database system degradation pathways will be designated with a constituent labeling system as described here. For example, there are two decay pathways that produce compound 4 and one pathway that consumes compound 4 in a simplified reaction tree shown in **Figure 41** which require four degradation rates and transformation yield rates to be defined.

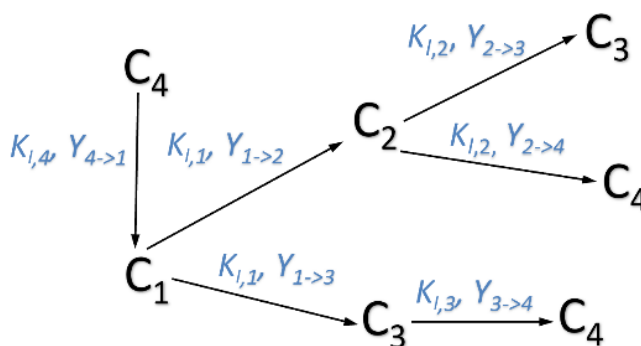


Figure 41. Schematic representation of a hypothetical transformation pathway for four constituents.

For four MC compounds C1 to C4, the reaction processes that directly produce daughter products are defined and shown in **Figure 40**. The degradation rates and yield coefficients are named to reflect the parent and daughter of each transformation pair. The transformation process is represented as a first order reaction occurring only in the liquid phase and is defined as follows:

$$\frac{dC_1}{dt} = Y_{4 \rightarrow 1} K_4 C_4 - K_1 C_1 \quad (2a)$$

$$\frac{dC_2}{dt} = Y_{1 \rightarrow 2} K_1 C_1 - K_2 C_2 \quad (2b)$$

$$\frac{dC_3}{dt} = Y_{1 \rightarrow 3} K_1 C_1 + Y_{2 \rightarrow 3} K_2 C_2 - K_3 C_3 \quad (2c)$$

$$\frac{dC_4}{dt} = Y_{2 \rightarrow 4} K_2 C_2 + Y_{3 \rightarrow 4} K_3 C_3 - K_4 C_4 \quad (2d)$$

Where: C_1, C_2, C_3, C_4 are the concentrations of compound 1 to 4, K_1, K_2, K_3, K_4 are the first order decay rates for compound 1 to 4, t is time, $Y_{1 \rightarrow 2}, Y_{1 \rightarrow 3}$ are $Y_{1 \rightarrow 2}$ are the transformation yield coefficients from compound 1 to 2 and 1 to 3. $Y_{2 \rightarrow 3}$ and $Y_{2 \rightarrow 4}$ are the transformation yield coefficients from compound 2 to 3 and 2 to 4, $Y_{3 \rightarrow 4}$ is the transformation yield coefficient from compound 3 to 4, $Y_{4 \rightarrow 1}$ is the transformation yield coefficient from compound 4 to 1.

The TREECS SSCFM allows for first order decay rates described as half-lives. Mathematically for growth this is equivalent to a doubling time. The numbering system allow the kinetic rates to be selected in order to determine the mass of each node at each timestep during the soil model solution. In order to identify which decay and growth reaction pathways (the Arrows on the degradation tree) apply to a given chemical compound a numbering scheme was devised. This allows information from the basic transformation model to inform the soil model how many chemical compounds are in the degradation tree, how they are connected (i.e., which reactions apply to each node), and which decay or growth terms to include in the mass balance for each chemical compound. Each pathway in the tree is described in three parts, the parent node, the target node, and all daughter nodes.

11.3.3.2.3. *TREECS Surface Soil Contaminant Fate Model*

The TREECS vadose and groundwater modules (based on the MEPAS model) do allow degradation to daughter products as well as the reporting of mass fluxes of both parent and daughter products. As transformation in the vadose and groundwater modules creates daughter products, those products are transferred to the surface water module. Both models within the surface water module (RECOVERY and CMS) include the ability to degrade MC to un-tracked mass. However, the proportion of time a contaminant

spends in the soil and groundwater compartments of the model are significantly longer than the amount of time spent in the surface water compartment. Thus, the ability to create new daughter products was not incorporated into the surface water module and the primary modification to TREECS to handle MC transformations was the addition of a capability to track the production of daughter products in the Surface Soil Contaminant Fate Model (SSCFM) and pass those daughter products as mass fluxes to the surface and groundwater compartments. Incorporating the MC transformation processes and tracking the flux of parent and daughter products from mass loading at the surface to eventual reception at surface and groundwater targets improves the ability to comprehensively assess and manage the impacts of MC at DOD sites.

As described in Dortch et al. (Dortch et al., 2011), there are two functions of the SSCFM: 1) hydrologic routing by division of the incoming precipitation into runoff, soil interflow, and infiltration and 2) estimation of MC mass flux time series delivered to the vadose and/or stream models via each of those flows. No changes are needed to the hydrology aspects of the SSCFM but the quantification of daughter products necessitates a new solution for the constituent mass balance. As shown in **Figure 42**, each MC is considered independently from all others in a homogenous continuously stirred tank reactor formulation that allows for solid and non-solid MC phases. Two alterations were made to the SSCFM, the addition of a source removal term to the solid mass balance and the addition of a transformation flux to the non-solid mass balance.

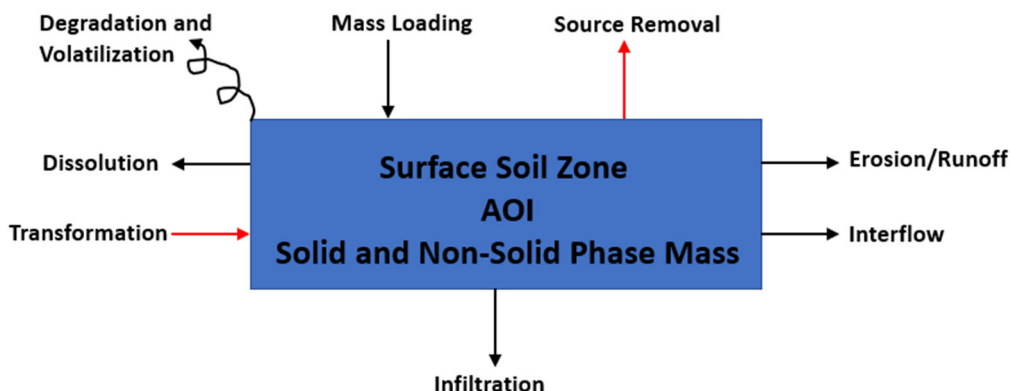


Figure 42. Schematic representation of the fluxes in the SSCFM.

The solid phase mass balance with source removal is given by

$$\frac{dM_s}{dt} = L(t) - F_{dis} - F_{es} + F_{precip} - R_s M_s - SR \quad (3)$$

where M_s is the solid phase mass (g), t is the time, $L(t)$ is time varying solid phase MC loading, F_{dis} is dissolution flux, F_{es} is the erosion flux of solid phase particles, F_{precip} is the precipitation flux due to dissolved pore water concentration exceeding the water solubility limit, R_s is the first-order MC source removal rate (yr^{-1}), and SR (g/yr) is a zero-order MC removal rate. It is noted that M_s is the mass of MC prior to dissolution into water where it can partition among water, soil, and air within the soil matrix.

The last two terms on the right-hand-side of **Eq. (3)** are new compared to the original solid phase mass balance of the MC however the remainder of the constituent formulations for the fluxes are detailed in Dortch et al. (Dortch et al., 2011). The source removal rate R_s is related to the source removal process. For soil removal, the rate is estimated by

$$R_s(\text{soil removal}) = \frac{S_r}{(\rho_b + \theta_w \rho_w) A Z_b} \quad (4)$$

where S_r is the soil removal rate (metric tons/yr), and ρ_b is the soil dry bulk density (g/cm^3), θ_w is the volumetric soil moisture content, A is the AOI surface area (m^2), and Z_b is the AOI active (contaminated) soil layer thickness (m) where most of the MC mass residue is believed to reside.

The non-solid mass balance is given by

$$\frac{dM_{ns}}{dt} = F_{dis} - F_r - F_e - F_l - F_{decay} - F_{vol} - F_{precip} + F_{tran} \quad (5)$$

where M_{ns} is the non-solid phase mass (g), F_r is the rain-induced pore water ejection and runoff flux, F_e is MC flux due to soil erosion, F_l is leaching flux, F_{decay} is degradation flux, F_{vol} is volatilization flux, and F_{tran} is the transformation flux to account for mass gained due to transformations. The mass unit is grams and the time unit is years so all the flux and load terms have units of grams per year. The complete formulation of the individual fluxes can be found in Dortch et al. (Dortch et al., 2011).

The transformation process is represented as a lumped first-order degradation kinetics operating on the compartment of the reactants with user-specified yield rate constants. When two or more constituents are simulated as shown in **Figure 42**, the total mass flux

for the transformation processes that convert multi- constituents into one daughter product is computed from

$$F_{tran} = \sum_j^{nC} Y_{tran} A Z_b \lambda_l \theta_w C_l \quad (6)$$

where F_{tran} is the total transformation flux (g/yr), j is a chemical index ($j = 1$ to nC) that can be transformed into daughter products (unitless), Y_{tran} is the transformation yield coefficient from chemical j to its daughter product. λ_l is the degradation rate (yr^{-1}), and C_l is the constituent concentration.

Any combinations of transformation processes between simulated constituents can be applied. Yield rate coefficients can be applied at varying strengths. The mass of non-solid MC must be reduced to account for transformation gains for a daughter product. Additional source and sink terms are required to be incorporated into the mass balance equations (**Eq. (5)**) for non-solid MC, the complete formulation of which for a case allowing for source removal as an example best management practice (BMP) can be found in Dortch et al. (Dortch, 2013).

The mass balance for non-solid phase MC mass, including source removal, is stated as

$$\begin{aligned} \frac{dC_{tt}}{dt} = & \frac{F_{dis}}{AZ_b} - \left[\frac{d_e}{Z_b} (1 - e^{-\kappa}) N + \frac{E}{Z_b} + \frac{q_w}{\theta_w Z_b} F_{dp} + (\lambda_l F_{dp} + \lambda_a F_{pp}) \right. \\ & \left. + \frac{K_v}{Z_b} F_{ap} + R_{ns} \right] C_{tt} - \frac{F_{precip}}{AZ_b} + \frac{F_{tran}}{AZ_b} \end{aligned} \quad (7)$$

Where C_{tt} is the total concentration of MC in the soil domain, q_w is the average annual Darcy water infiltration rate (m/yr), λ_a is the degradation rate (yr^{-1}) for the adsorbed concentration, K_v is the volatilization rate (m/yr), or vapor escape rate from soil to overlying air, E is the average annual soil erosion rate (m/yr), d_e is the soil exchange layer thickness (m), N is the average number of rainfall events per year (yr^{-1}), and F_{dp} , F_{ap} , and F_{pp} are used to convert from total concentration on a total volume basis to dissolved, particulate, and air concentrations respectively in the porous media on a total volume basis (see Dortch et al., 2011).

The Runge-Kutta (RK) method was used for solving the two coupled 1st order ordinary differential **Eq. (5)** and **Eq. (7)** under the initial conditions. Two numerical schemes of the RK method were implemented in the SSCFM: RK fourth-order accurate

(RK4) with a constant time step and RK fifth-order accurate (RK45) with adaptive time step. The methods are denoted by RK4 and RK45, respectively. The difference between the two schemes is that RK4 uses the classic fourth-order formulae while RK45 uses the fourth and fifth-order formulae. The model time step is automatically computed by RK45 to maintain model stability.

11.3.3.2.4. *TREECS/FRESCO Interface*

The SSCFM code was modified and upgraded for simulating transformations between constituents. The updated SSCFM has an option for simulating transformations between constituents as well as all capabilities previously implemented in the model. The updated model has the same input requirements as previous version under the different hydrologic input conditions except for the transformation option and associated parameters in the input control file.

Each pathway in the contaminant degradation tree is described in three parts, the parent node, the target node, and all daughter nodes. A new form in the Hydro-Geo-Chemical-Toolkit (available in the latest FRESCO v1.1) called the Degradation Pathways table allows users to enter the decay rate constants for each transformation pathway as well as the description of the network using the numbering scheme described above. If an option for simulating transformations (flag = 1) is included, the additional transformation parameters (constituent number, transformation yield coefficient) for each constituent must be provided in the input control file. For example, four constituents are simulated as shown in **Figure 41**, transformation processes and yield coefficients from one constituent to another one need to be defined.

The SSCFM with transformations can be applied in the average annual (AA) mode or the time varying (TV) mode. The hydrologic inputs used under the average annual condition are single, constant, average annual values for rainfall, precipitation, runoff, soil erosion, net infiltration, etc. The model simulation unit for time is a year and the model time step is in units of years. The SSCFM for the time varying condition requires time-varying hydrologic inputs including rainfall, precipitation, runoff, soil erosion, infiltration (interflow and groundwater percolation), and volumetric soil moisture content values. Note that the model unit for time under the TV condition is a day and the model time-step is expressed in units of days. All mass fluxes and rates used to compute mass fluxes are per day units rather than per year units.

11.3.3.2.5. *TREECS Transformation Proof-of-Concept*

A Proof-Of-Concept (POC) application of the enhanced SSCFM to a small arms firing range located at Fort Leonard Wood (FLW), MO was conducted to demonstrate the new capability to simulate transformations. The new model was applied to simulate the fate and transformation of two insensitive munitions (IM) compounds: NTO (3-nitro-1,2,4-triazol-5-one) and DNAN (2,4-dinitroanisole). These MCs exhibit high aqueous solubility and, hence, potential mobilization to groundwater (Dontsova et al., 2014; Mark et al., 2016).

To test and demonstrate transformation capabilities implemented in SSCFM, re-application of the SSCFM to a small arms firing range located at Fort Leonard Wood (FLW), MO was chosen. Site characterization information for this site had already been collected and previous studies including the SSCFM of this firing range are described in detail by Dortch (Dortch, 2013, 2014). The FLW installation is approximately 61,410 acres. The area of the AOI polygon is approximately 294,000 m². The AOI for this site consisted of Ranges 20–22, which drain to an unnamed tributary to Falls Hollow eventually converging with Little Bald Creek and Bald Creek, which flow east into the Big Piney River (**Figure 43**). The soil properties, hydrologic inputs, and mass loadings of lead were transferred from the previous application. Hypothetical loading of NTO and DNAN and their main transformation products were selected for this POC application of the enhanced SSCFM. First POC application simulates Pb, NTO and its transformation constituents, the second one simulates DNAN and its transformation constituents. Pb was used to verify the enhanced model, and the remaining IM constituents were used to demonstrate how to apply the SSCFM for tracking daughter products transformed from their parent compounds.

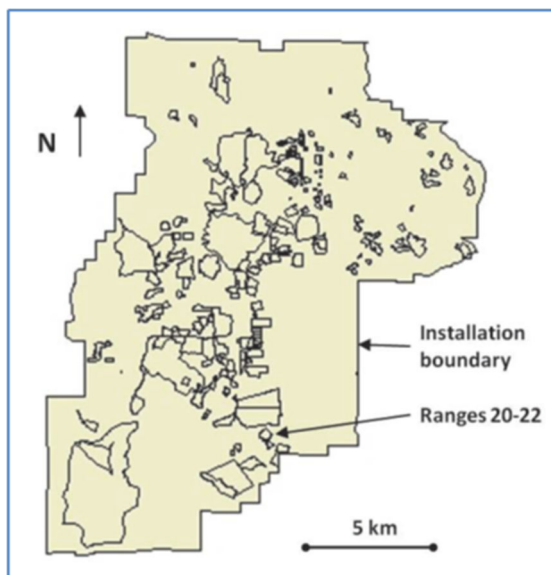


Figure 43. Study site map of Fort Leonard Wood, MO.

The first POC application focused on NTO and its transformation products. Microbial transformation studies have shown that NTO can undergo nitroreduction under anaerobic conditions (Krzmarzick et al., 2015) and form ATO (5-amino-1,2,4-triazol-3-one). As shown in **Figure 44**, NTO reduction, a first step in its transformation, is reduced to 5-amino-1,2,4-triazol-3-one (ATO) with small amounts of the intermediate HTO (3-hydroxylamino-1,2,4-triazol-5-one). HTO is an intermediate of NTO transformation. HTO does not accumulate, it is quickly reduced to ATO. The yield of ATO production as a fraction of NTO removed was stoichiometric, averaging $95.3 \pm 9.4\%$. The ATO formed, however, was not further metabolized under anaerobic conditions. Under aerobic conditions, ATO can further transform to CO₂, urea, and inorganic nitrogen species (Krzmarzick et al., 2015). Photodegradation or biotransformations of NTO were also recognized by some researchers (Dontsova et al., 2014).

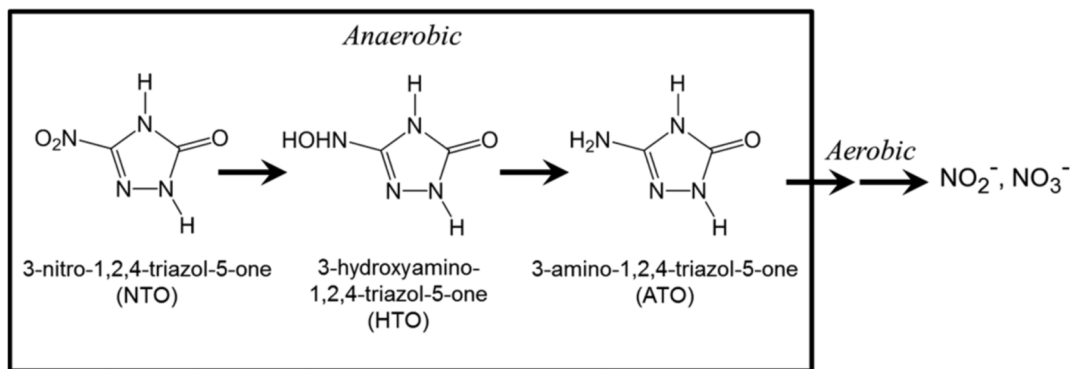


Figure 44. Transformation pathways of NTO and its daughter products (Krzmarzick et al., 2015).

Many of the inputs are the same as those used in the original Falls Hollow application described by Dortch (Dortch, 2013) including the same hydrology inputs. Differences in the original inputs and the present application include the chemical properties required for NTO, ATO, and NOx. There are six chemical-specific physical/chemical properties required by the model. They are the soilwater distribution (or partition) coefficient for soil (L/kg); the decay/degradation rate of liquid (water) phase constituent, (yr^{-1}); the decay/degradation rate of adsorbed (particulate) phase constituent (yr^{-1}); molecular weight (g-mole); diffusivity in water (cm^2/sec); Henry's constant ($\text{atm}\cdot\text{m}^3/\text{g}\cdot\text{mole}$), octanol-water partition coefficient (ml/ml), water solubility limit (mg/L); and solid phase particle mass density (g/cm^3). See Dortch et al (Dortch et al., 2011) for a complete description of the necessary parameters to run the SSCFM.

NTO is very mobile and adsorption in soils is low (Arthur et al., 2018). NTO is highly soluble and negatively charged under conditions usually encountered in soils, and therefore weakly adsorbed, with K_d ranging between 0.02 and $0.51 \text{ cm}^3 \text{ g}^{-1}$ (Mark et al., 2016). NTO is not expected to volatilize from dry soil surfaces based upon its vapor pressure. ATO exhibits high aqueous solubility (Smith & Cliff, 1999) and toxicity (Le Champion et al., 1999; Mullins et al., 2016). Key transport and chemical specific parameters of Pb, NTO, ATO and NOx used in the SSCFM for this POC application are summarized in **Table 4**.

Table 4. Chemical specific parameters for Pb and NTO constituents.

Parameter	Units	Munition Constituents			Description
		Pb	NTO	ATO	
MW	g/mol	207	130	100	Molecular weight

ρ_{sm}	g/cm ³	6.6	1.93	-	Solid phase constituent mass density
K _d	L kg ⁻¹	597	0.02 - 0.51	-	Sorption partitioning constant
Log K _{OW}	-	-	0.37 - 1.03	-	Octanol-water partitioning constant
λ_l	yr ⁻¹	1.9*10 ⁻²³	3.5 - 193.6	-	Decay/degradation rate of liquid (water) phase constituent
λ_a	yr ⁻¹	1.9*10 ⁻²³	3.5 - 193.6	-	Decay/degradation rate of adsorbed (particulate) phase constituent
C _s	mg/L	3.85	16642	11000	Aqueous solubility
Transformation yield coefficient					
Y _{1->2}		-	0	-	Pb → NTO
Y _{1->3}		-	-	0	Pb → ATO
Y _{2->1}		0	-	-	NTO → Pb
Y _{2->3}		-	-	95.3±9.4%	NTO → ATO

Operational inputs for this POC application are the same as those used in the original FLW application developed from the training ammunition usage reports. MC mass delivered depends on the amount of MC mass per item fired (identified by a Department of Defense Identification Code or DODIC) and the number of items fired per DODIC on Ranges 20-22 during the period 1999 through 2012. The total number fired per year was 2,432,568. We applied the same MC mass residue loading rates used in the previous application for metals for this hypothetical POC application.

The second POC application focused on DNAN and its transformation products. This application simulated four constituents and their multi-transformation pathways and daughter products for one-year period. MC mass residue loading was not included for DNAN and its transformation products. Instead, an initial MC concentration in soil was specified as 100 mg/kg for this application.

According to the transformation study of DNAN investigated by Hawari et al. (Hawari et al., 2015), DNAN was subsequently reduced to 2-ANAN (2-amino-4-nitroanisole) and 4-ANAN (4-amino-2-nitroanisole). 2-ANAN further transformed to DAAN (2,4-diaminoanisole) (**Figure 45**). Several laboratory studies also identify the reduced products 2-ANAN and DAAN as key final products (Ahn et al., 2011; Olivares

et al., 2013). In general, 2-ANAN was observed in quantities at least two orders of magnitude greater than those of 4-ANAN, indicating that ortho reduction of DNAN was the most dominant transformation pathway.

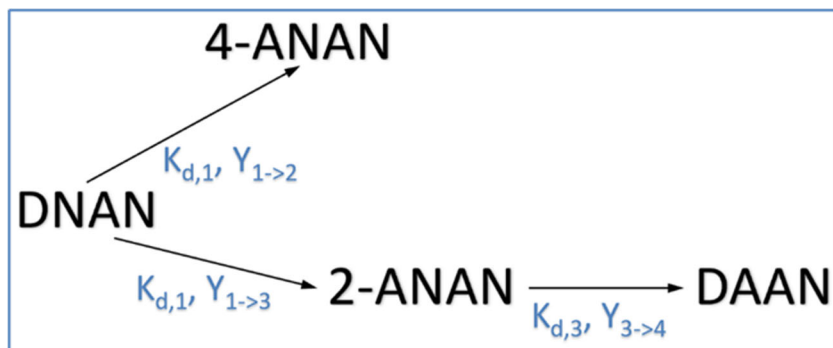


Figure 45. DNAN transformation pathways and its amino products.

With only limited knowledge available on the transport, transformation, and toxicity of DNAN, the degradation of DNAN in soils is assumed to follow a pseudo-first-order kinetics with a rate constant of $0.47 \pm 0.03 \text{ d}^{-1}$ (Hawari et al., 2015). Key transport and chemical specific parameters of DNAN and its transformation constituents used in SSCFM for this POC application are summarized in **Table 5**.

Table 5. Chemical specific parameters for DNAN and its transformation constituents.

Parameter	Units	Munition Constituents				Description
		DNAN	4-ANAN	2-ANAN	DAAN	
MW	g/mol	198	182	169	138	Molecular weight
ρ_{sm}	g/cm ³	-	-	-	-	Solid phase constituent mass density
Kd	L kg ⁻¹	73	29	46	0.01	Sorption partitioning constant
Log K _{OW}	-	1.58	0.8	1.47	-	Octanol-water partitioning constant
λ_l	yr ⁻¹	80.3	40.3	40.3	40.3	Decay/degradation rate of liquid (water) phase constituent
C _s	mg/L	213	4430	252	40000	Aqueous solubility
Y _{1->2}		-	0.3	-	-	DNAN → 4-ANAN
Y _{1->3}		-	-	0.6	-	DNAN → 2-ANAN
Y _{3->4}		-	-	-	0.9	2-ANAN → DAAN

The SSCFM can generate the mass transfer and biogeochemical fluxes in the AOI soil, the mass export flux from the AOI soil to surface water, and AOI soil concentrations. The option for solid phase MC erosion was turned off. The fluxes of five processes in AOI soil include: leaching, runoff, volatilization, degradation, and transformation. AOI soil concentrations are computed from AOI MC mass divided by AOI soil mass, which is the AOI soil volume times the dry bulk density of the soil. There are no measured soil concentrations of lead for FLW.

11.3.3.2.6. TREECS Transformation Results and Discussion

To verify the numerical solution of the enhanced SSCFM, output for lead concentrations and fluxes from the enhanced model were compared to output produced with the pre-transformation enabled code (labeled SSCFM on **Figure 46**). **Figure 46A** and **B** verify that the enhanced code computes identical total soil concentration and dissolved phase soil concentration of Pb over time as the original SSCFM. **Figure 46C** and **D** verify that the enhanced code computes identical degradation flux and leaching to groundwater flux of Pb versus time as the original. There was no observed Pb data for model validation. This result confirms the model and its numerical solutions were correctly implemented in the enhanced SSCFM.

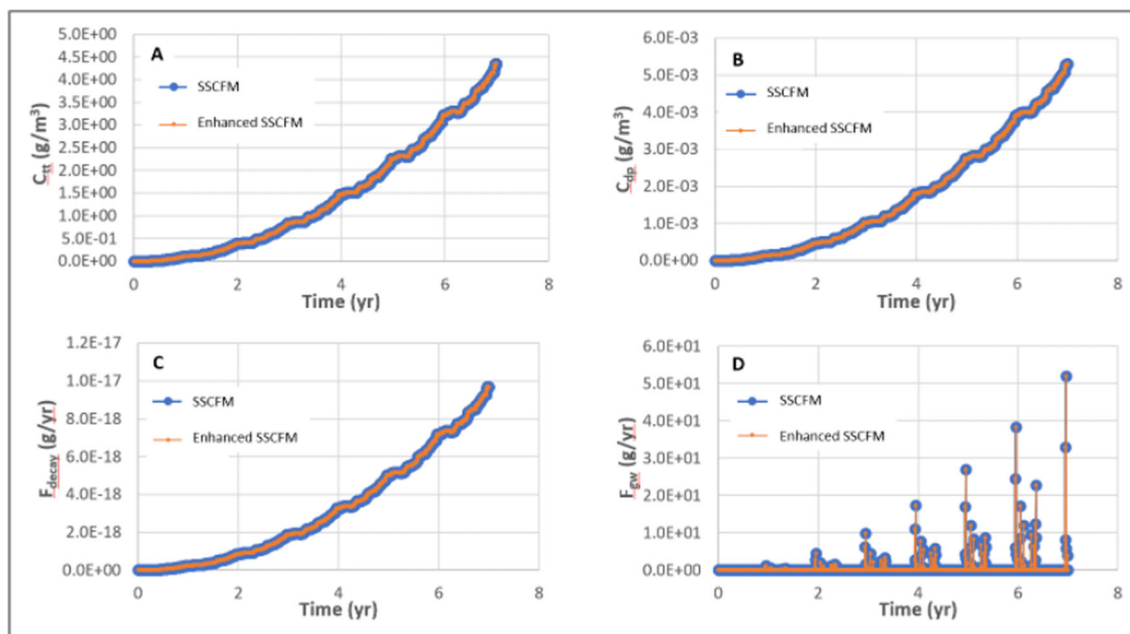


Figure 46. Comparison of lead fluxes and concentrations computed with the original SSCFM and the enhanced SSCFM that includes transformation: A) total soil concentration of P_b versus time, B) dissolved water concentration of P_b versus time, C) degradation flux of P_b versus time D) P_b flux to groundwater versus time.

Model results of NTO and ATO for this POC application are presented below. **Figure 47** presents model computed dissolved water concentrations of NTO (C_{dp}) versus time for this application. **Figure 48** presents computed degradation process fluxes of NTO versus time (F_{decay}). The time series pattern is dominated by the timing for rainfall events, as expected for a highly soluble MC as NTO. It appears that the first order degradation rate of NTO provided in the literature and used in HYDRUS-1D model (Mark et al., 2017) is much higher than conventional explosive compounds. SSCFM computed degradation flux reflects that trend.

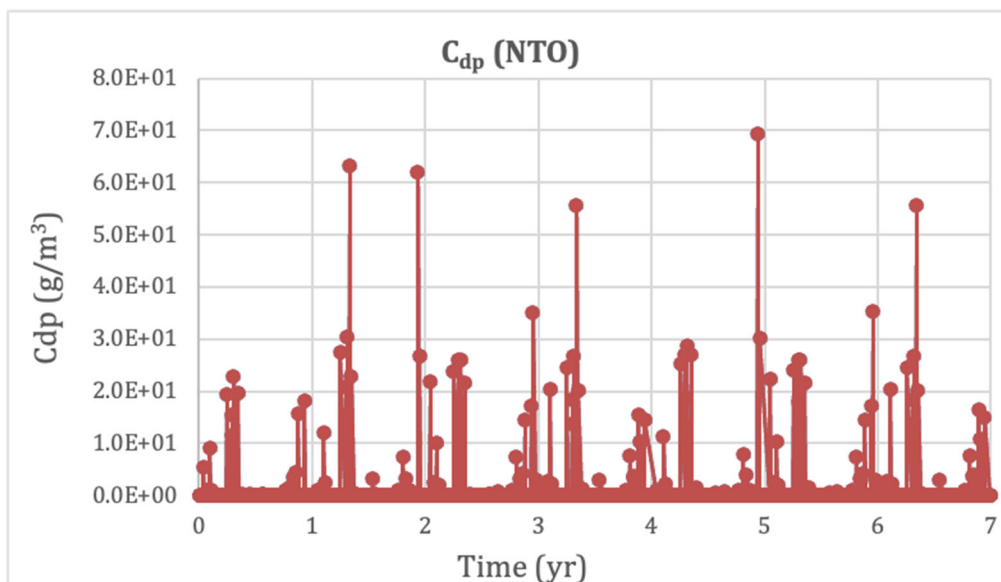


Figure 47. Computed dissolved water concentration of NTO versus time.

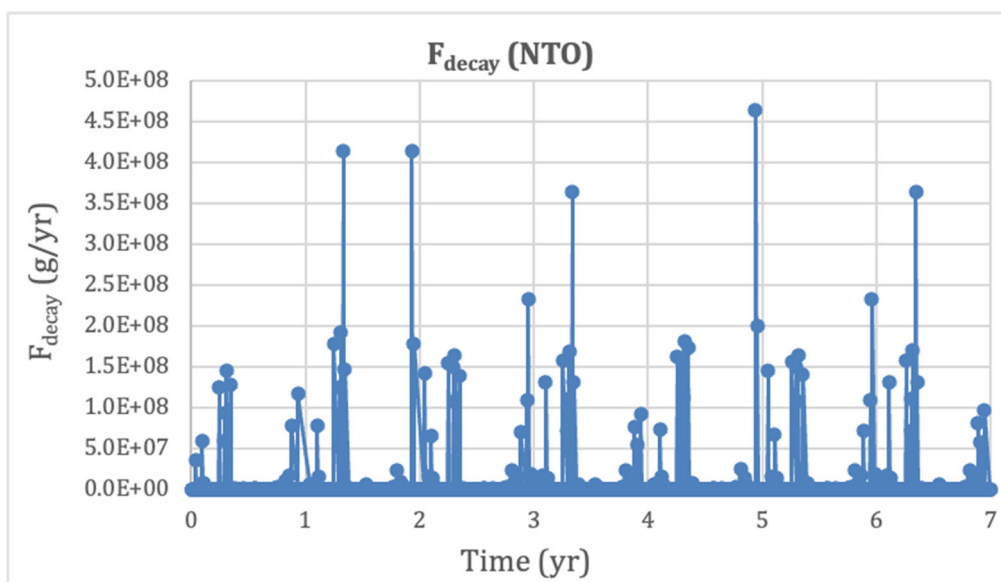


Figure 48. Computed degradation flux of NTO versus time.

As given by Krzmarzick (Krzmarzick et al., 2015; Mark et al., 2017), the yield of ATO production as a fraction of NTO removed is stoichiometric, averaging $95.3 \pm 9.4\%$. Therefore, transformation yield coefficients of 105% and 85% were used to evaluate the relationship between the dissolved water concentration and yield coefficient. **Figure 49** and **Figure 50** present time series of computed transformation fluxes from NTO into ATO under different yield coefficients. NTO concentrations also decreased due to

transformation. This indicates the potential for natural attenuation of IM constituents in soils through adsorption and transformation.

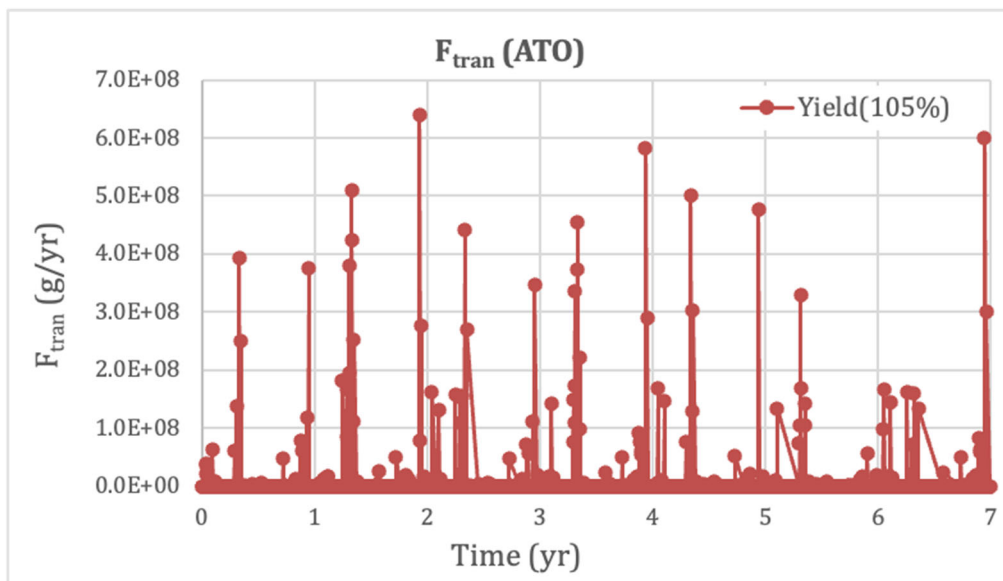


Figure 49. Computed transformation flux from NTO into ATO versus time at 105% yield fraction.

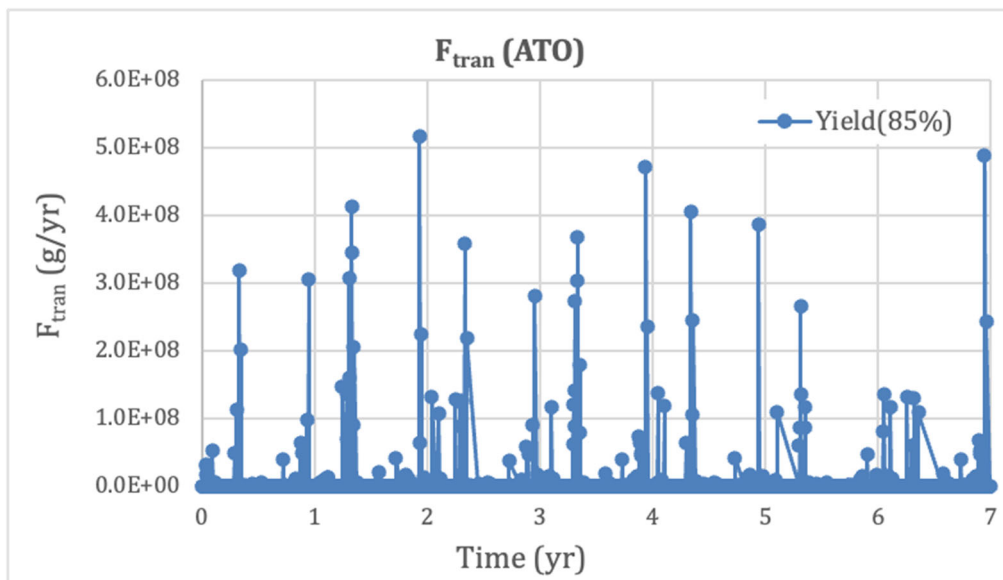


Figure 50. Computed transformation flux from NTO into ATO versus time at 85% yield fraction.

Figure 47 presents model computed dissolved water concentrations (C_{dp}) of ATO versus time for this application. There is no significant difference in the dissolved water concentration of ATO between two transformation yield coefficients specified in the model.

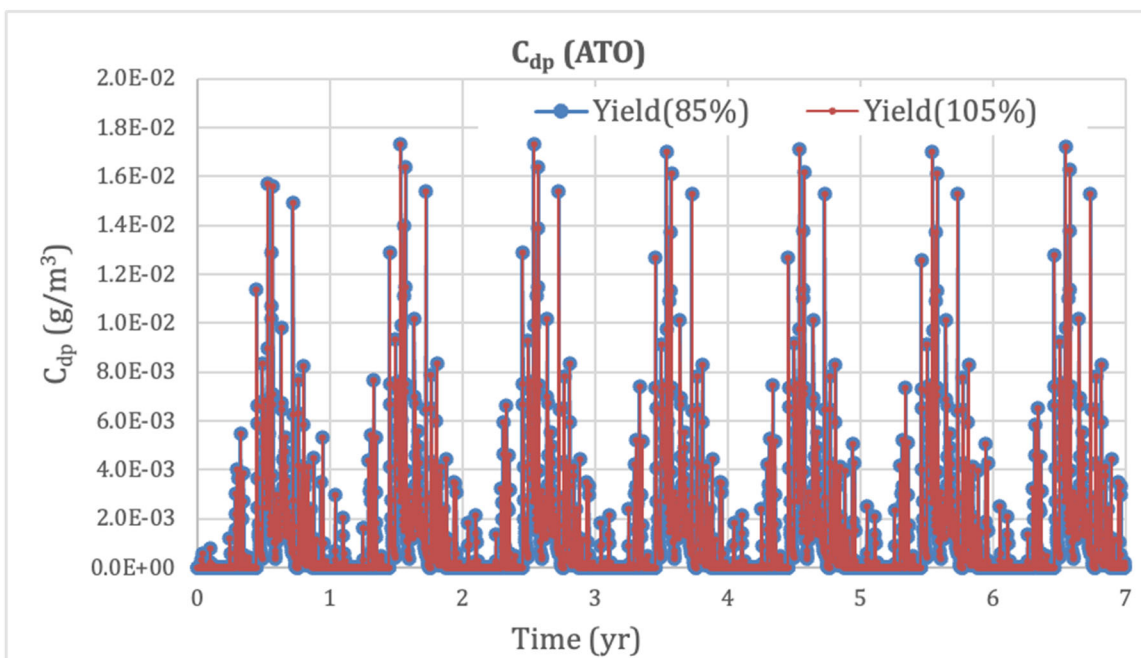


Figure 51. Computed dissolved water concentration of ATO versus time.

The model results are presented below for the second POC application. **Figure 52** shows model computed dissolved water concentrations of DNAN versus time for this application which includes two degradation pathways as shown in **Figure 40**. **Figure 53** shows computed degradation fluxes of DNAN versus time. The first order degradation is the main reaction process for this application and determines the fate and concentration of DNAN in soils.

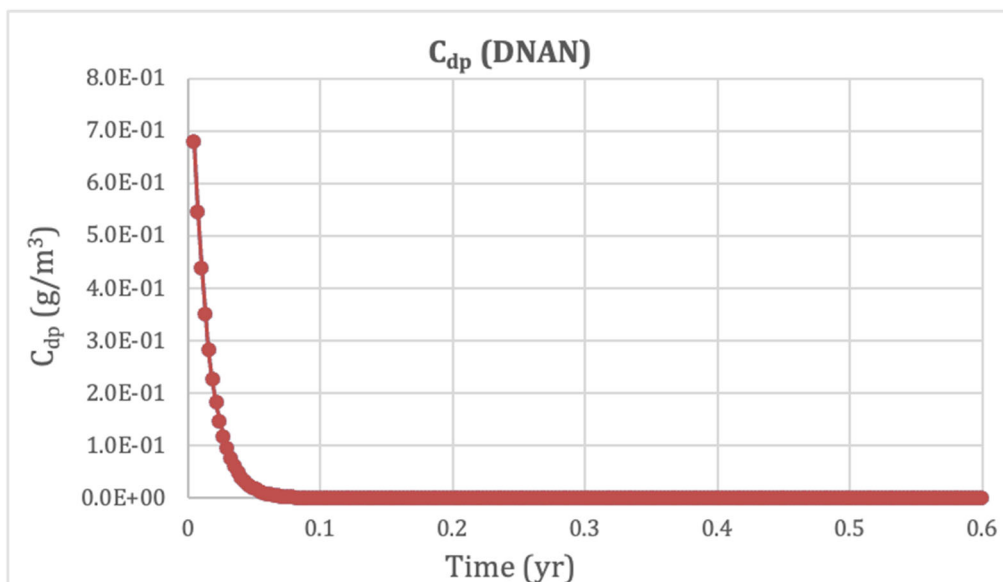


Figure 52. Computed dissolved water concentration of DNAN versus time.

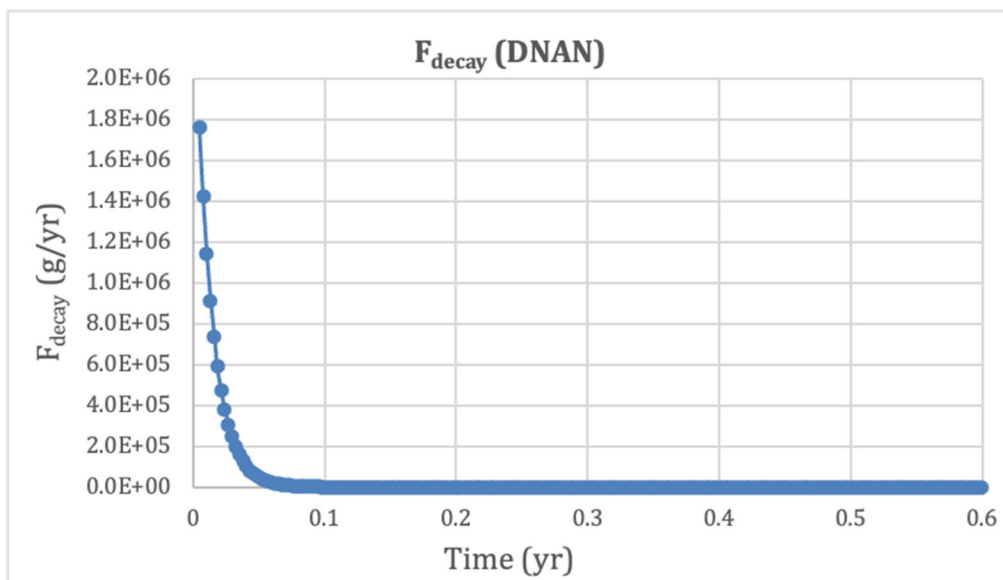


Figure 53. Computed degradation flux of DNAN versus time.

Figure 54 shows model computed dissolved water concentrations of 2-ANAN versus time for this application. Since the degradation rate is uncertain, we ran the model with two specified degradation rates, one 50% smaller than the other. As expected, the reduced degradation rate produced higher concentrations of 2-ANAN at each time step. This difference in decay rates in turn affects the fraction of 2-ANAN degraded to DAAN as shown in **Figure 45**. Both degradation and transformation of DNAN affect the fate of 2-

ANAN. The transformation process of DNAN only has less impacts than the degradation of 2-ANAN for this application.

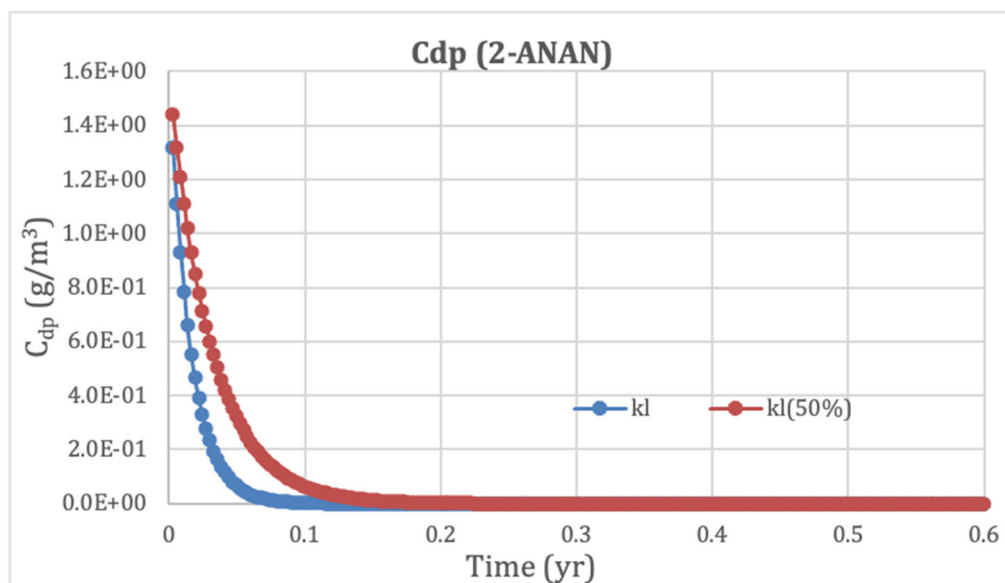


Figure 54. Computed dissolved water concentration of 2-ANAN versus time.

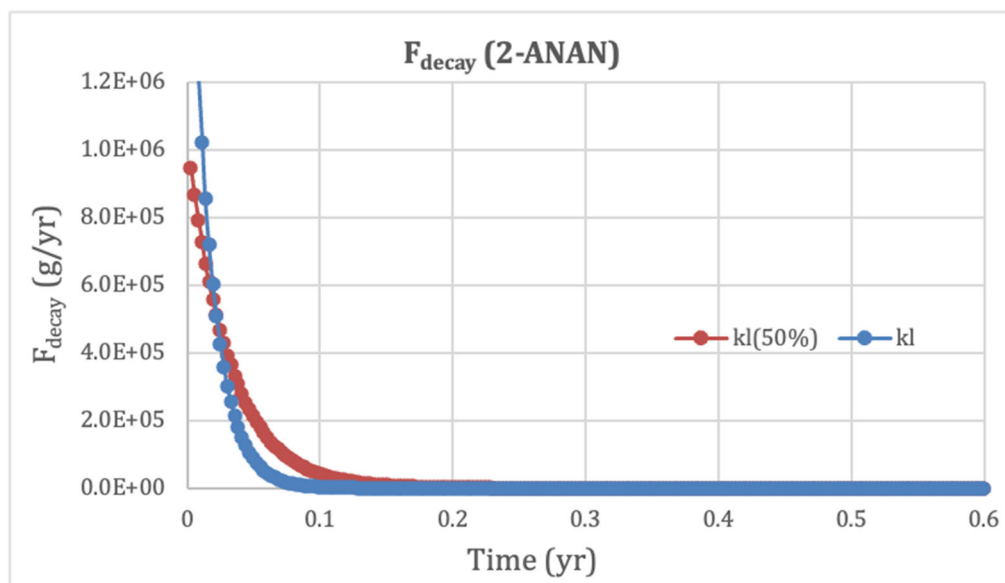


Figure 55. Computed degradation flux of 2-ANAN versus time.

To check the mass balance computed from the model, **Figure 56** shows the comparison of model computed degradation flux of 2-ANAN and transformation flux of DAAN

versus time for this application. The magnitude of both fluxes is identical and verifies the source and sink terms are correctly computed by the model.

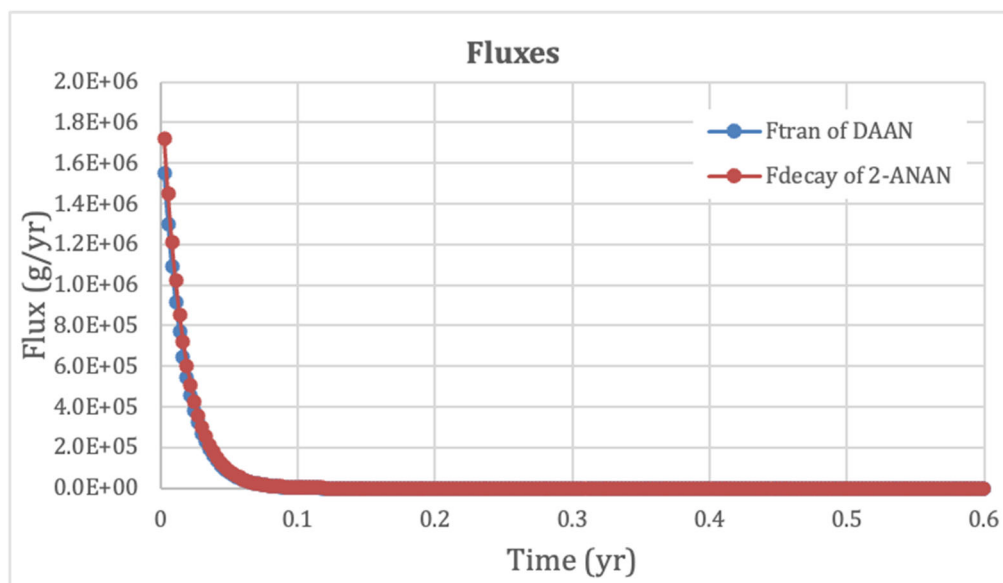


Figure 56. Comparison of degradation flux of 2-ANAN and transformation flux from 2-ANAN into DAAN versus time

Figure 57 shows model computed dissolved water concentrations of DAAN versus time for this application. The fate of DAAN affected by the transformation process from 2-ANAN is insignificant for this application. The degradation of DAAN is a dominant process in soils.

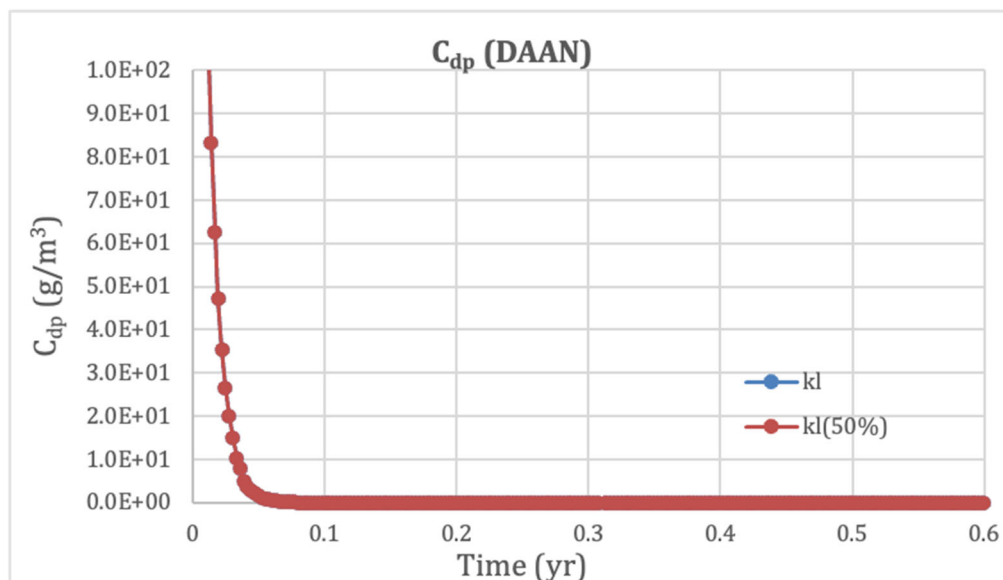


Figure 57. Computed dissolved water concentration of DAAN versus time.

A transformation capability feature has been implemented into the surface soil contaminant fate model in TREECS™ and has been inherited into TREECS successor, FRESCO v1.1 (available by email to jodi.l.ryder@usace.army.mil). When this feature is used, additional chemical parameters that characterize transformation process are required for the model inputs. The enhanced soil model generates yearly or daily fate and export fluxes for MC. Additional output includes a transformation flux. Testing of transformation capability in SSCFM with daily hydrology was performed by applying the model to a small arms firing range located at FLW, MO and running the same site conditions conducted previously for RDX and Pb. This POC application showed that enhanced SSCFM performed well for tracking transformation flux from one MC to its daughter products and computing soil concentrations and transport and reaction process fluxes of daughter products

Application of the transformation feature in SSCFM for tracking transformation of MC and its daughter products is able to address the range of possible environmental scenarios that can be met once the IM formulation reaches the environment. However, the ability to predict soil concentrations and transformation fluxes of MC in the AOI has not been validated due to a lack of field data at this time. Users of the SSCFM supply degradation and yield rates as inputs to the model thus they can be generated from experiment, field observations, or computational modeling approaches. However, although there exists literature describing explosive transformations, far less data is available for transformation rates to be used in the modeling framework. Considerable

research remains to be conducted to measure the values of these parameters in order to know the importance of these processes. Processes and parameterizations developed under idealized conditions at the laboratory scale are not likely to accurately depict the field scale situation. Thus, there exists the need for data collection at the field scale.

The kinetics describing the transformation processes in SSCFM were lumped into a first-order degradation because transformation mechanisms are poorly understood for MC. Translating the current understanding and laboratory data into quantitative mathematical process descriptors for MC is impeded by the nature of the information available and will require additional process level research. It is important to determine the transformation kinetics of individual compounds and those of explosive formulations as well. The soil contaminant model should be improved by incorporating better process descriptors of MC.

12. OVERALL CONCLUSIONS AND IMPLICATIONS

When this project was designed, there was very little data on the environmental fate and effects of any IMC compounds and information on their transformation products was almost completely lacking. Therefore, this project was designed to provide models that could predict pathways of IMC transformation, their resulting reaction products, and the physico-chemical properties of those products. We identified several existing models—with on-going development—that offered frameworks for pathway prediction (esp. CTS and EAWAG-BBD), and we evaluated the capabilities of these models for IMCs. These models predict the most well-studied transformation pathway of explosives compounds (reduction of nitro aromatic and nitramine moieties) but were not able to extrapolate their rules for nitro reduction to more novel IMCs, like NTO. In principle, these models can be expanded to cover all IMCs, but this will require considerable manual curation for transformation rules. Another limitation to these models is their very limited ability to prioritize major reaction steps over minor steps.

Some of the limitations of rules-based model are overcome by the approach developed as part of this project, which involved data-base enabled quantum chemical calculation of reaction energetics using Arrows. This allows thermodynamic weighting to determine the relative importance of steps in a reaction network and can be applied to any (IMC) molecule for which a molecular structure can be specified (not just those for which training data are available). Arrows was applied to develop more complete understanding of several IMC transformation pathways, such as hydrolysis of DNAN. In principle, Arrows can be used to predict reaction networks for a more diverse range of compounds than rules-based models, but the former require greater operator expertise and computing power, so will remain as research tools for the time being.

Once transformation products are identified, their physico-chemical properties can be determined. We found that few measured data were available, so we adapted several existing property prediction models and applied them to IMCs and their expected transformation products. In general, the predicted properties suggest that the IMCs and products will be more mobile in the environment than traditional explosives compounds, which may become an issue in the management of training range sites in the future.

The transformation pathway and property data obtained in this study were integrated in a reactive-transport model, to provide a tool that is directly applicable to training range site management. For this, we extended the Soil Surface Contaminant Fate Model within the TREECSTM model developed previously by USACE. This gives the TREECS model

the capability to (i) track mass from parent to daughter species and through multiple succeeding reaction pathways and (ii) transmit fluxes of multiple tracked species to downgradient media (vadose zone, surface water). The modeling framework used for these developments was designed to allow efficient expansion to cover additional transformation pathways, novel IMCs, etc.

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14. APPENDICES

14.1. Supporting Data

None provided here.

14.2. List of Scientific/Technical Publications

Peer-Reviewed:

- Bylaska, E. J., Tratnyek, P. G., Torralba-Sanchez, T., Edwards, K. C., Dixon, D. A., Pignatello, J. J., & Xu, W. (2022). Computational Predictions of the Hydrolysis of 2,4,6-Trinitrotoluene (TNT) and 2,4-Dinitroanisole (DNAN). *Journal of Physical Chemistry, B*, submitted.
- Bylaska, E. J., Song, D., Ilton, E. S., O’Leary, S., Torralba-Sánchez, T. L., & Tratnyek, P. G. (2021). Chapter Five - Building toward the future in chemical and materials simulation with accessible and intelligently designed web applications. In D. A. Dixon (Ed.), *Annual Reports in Computational Chemistry* (Vol. 17, pp. 163-208). Elsevier. <https://doi.org/10.1016/bs.arcc.2021.09.003>

In Preparation (largely written, but not yet submitted):

- Ryder, J.L., Zhang, Z., Gerald, J.A., and B.E. Johnson (2023) "Contaminant Transformation Capabilities in TREECS™." U.S. Army Engineer Research and Development Center, Environmental Laboratory, ERDC/EL TR-XX-2023
- Torralba-Sanchez, T. L., Bylaska, E. J., & Tratnyek, P. G. (2022). Estimating transformation products and their physicochemical properties using computational methods with insensitive munitions compounds as a model system. *Environmental Science and Technology*, in prep.

14.3. Other Supporting Materials

14.3.1. White Paper on Microcosm Design for Experiments on IMC Transformation Products

The first IPR for this project (Winter 2018) resulted in an action item and new Subtask 4.3 titled “White Paper on Microcosm Protocol (v1)”. The description was “provide a white paper to describe the protocol for microcosm testing including the rationale for conducting the study, a definition of the study objectives and the experimental plan for how the testing will be conducted.”

This follows from an issue that I raised during the IPR, which pertains mainly to Task 1.2 (Microcosm Experiments) and to a lesser degree Task 2.1 (Measured Properties). The general issue is how to best formulate microcosms for batch tests, such that they represent the range of relevant environmental conditions both sufficiently and efficiently.

In the proposal, the description of methods to be used in Tasks 1.2 and 2.1 is fairly detailed with respect to the later steps in the process (dosing, mixing, sampling, analysis, and even fitting). However, little detail was provided regarding the setup of the reactors, including the environmental samples to be used as the reactor medium.

The question of how to select environmental samples for the reactor medium is the main issue that I raised during the IPR presentation. To maximize the overall impact of this project, we need the selected materials to produce reactor conditions that cover the range that is most relevant to DoD training sites. This could require more material samples than can be utilized within the time and resources allocated to the experimental tasks in this project.

Even if a sufficient number of site samples could be obtained and utilized in microcosm experiments, the “shot gun” approach to covering relevant conditions is inefficient and unsatisfying in that it does not offer a systematic rationale for sample selection. Instead, we propose to develop an approach that is systematic in a way that provides efficiency, ensures balanced coverage, and provides a rational basis extrapolating results to conditions not tested.

The usual ways to approach this can be described as “*bottom up*” and “*top down*”. Bottom-up involves building complex environmental materials using individual components and top-down involves fractionating complex environmental into materials that are (partially) purified components. Variations on these approaches include *amending* environmental samples with concentrated components or *blending* environmental samples to obtain intermediate conditions.

An additional characteristic of the above (approaches to formulating materials to represent environmental conditions in microcosm experiments) is that the ingredients are often assumed to combine *directly* to form conditions that are *static*. However, the reality is that the ingredients usually interact to some degree and the resulting reactions (mainly biotic, but also abiotic) are the main determinant of the overall conditions. In the latter case, the ingredients contribute *indirectly* to conditions that are *dynamic*.

There is no doubt that all of these approaches to microcosm experimental design have been used in past work, but there is no reliable way to search the literature for this, so we

have not tried to do a systematic analysis of prior work in this regard, and we have not found any such efforts (e.g., review papers) by others. We implied during the IPR that we might take on this challenge, but have decided—for now—that it would divert too much of our efforts from the core tasks of the project.

To our knowledge, there is no consensus that any of the above approaches to microcosm experimental design are generally preferred over the others. In some cases, the best choice is determined by circumstantial constraints (e.g., when treatability at a particular site is the focus), but in other cases the choice seems to be based mainly by convenience (e.g., using resources already at hand). As part of the experimental tasks in this project, we aim to define a more rigorous selection process by placing greater emphasis on fundamental biogeochemical considerations.

The approach to microcosm design that we are planning to utilize in this project will employ whole samples from a small number of “standard” sites, amended and incubated in such a way that a range of redox conditions is achieved: aerobic for oxic conditions, anoxic with sulfate for sulfidic conditions, etc. Operationally, this is the same approach that has been used in many studies of biodegradation in soil/sediment, and it currently is being used by Rick Johnson et al. in the column studies they are doing under ER-2621.

A major reason for selecting this approach is that it should allow efficient testing of a range of “dynamic” redox conditions while keeping other “static” physico-chemical properties (mineral composition, organic matter content, etc.) constant. This seems like the right configuration of experimental variables to validate our model predictions with respect to both IMC degradation pathways and the properties of the resulting products without requiring an excessively large number of experiments.

The main variable involved in implementation of this approach is selection of the “standard” site materials. The criteria we are using for this selection include (i) relevance to DoD training ranges, (ii) suitability of the physico-chemical characteristics, (iii) abundance of previously-reported characterization data, and (iv) availability of the material from a reliable source. We have surveyed a wide range of possible choices, and one emerged as the clear first choice, given the overall goals and constraints of this project.

The first site material that we plan to use in these experiments will be *Sassafras Sandy Loam (SSL)*. This soil is part of the Sassafras Series of Official/Standard soils established by the U.S. Department of Agriculture (soilseries.sc.egov.usda.gov), which also is the official soil of the state of Maryland, and therefore has been used in a vast

number of studies of soil properties. More importantly, SSL has been used as a model soil in many SERDP funded, or SERDP relevant, studies done by Herb Allen and Dominic Di Toro (U. Delaware), Roman Kuperman (Edgewood Chemical Biological Center), and Jalal Hawari (while at CNRC) ((Gonzalez-Forero, 2013; Kuperman et al., 2013; Miglino, 2015; Monteil-Rivera et al., 2004; Rocheleau et al., 2011; Rocheleau et al., 2010; Rocheleau et al., 2008)). In particular, SSL was among the soils used to calibrate the predictive models for IMC sorption developed under ER-1688 ((Gonzalez-Forero, 2013; Miglino, 2015)) and in bioavailability/toxicity testing on MCs done for CU-1221 and ER-2724. Collectively, this body of highly relevant prior work on SSL should be highly advantageous in designing and interpreting the experiments to be performed in this study. SSL is also included in the USDA database of soils that is utilized by TREECS, which should facilitate testing our pathway prediction models into TREECS, which is Task 3.3 of this project.

The Allen Laboratory at the University of Delaware has been experimenting with SSL from a library of soil samples that they have been maintaining for decades. We plan to obtain some of this sample for use in this study, to minimize differences due to sample source heterogeneity or handling. We will then subdivide this sample for use in the microcosm experiments for this study, add amendments (nitrate, sulfate, etc.) and incubate (aerobic or anaerobic) with very gentle mixing until the targeted conditions stabilize. The time required to reach stable, target conditions will be determined by continuous monitoring of Eh and pH, and periodic measurements of $\text{Fe(II)}_{\text{aq}}$ and $\text{S(II)}_{\text{total}}$. The equilibrated microcosms will then be spiked with the target compound (IMC or pure standard or a putative degradation product) and the results (degradation, partitioning, etc.) will be measured as described in the original proposal.

In addition to the microcosm experiments done as conventional well-mixed batch reactors (which are preferred for efficiency of replication, and ease of isolating experimental variables such as the effect of mass transport), we may also operate some bench-scale microcosm experiments as lysimeters (to simulate soil and vadose zone conditions) or columns (to simulate groundwater conditions). The latter approach could consume more time than the experimental tasks of this project allow, so we will start with the batch reactor approach to ensure that we have time to obtain a self-consistent dataset of validating the predictive models developed under the other tasks.

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14.4. Outreach, Training, Other Technology Transfer

14.4.1. Short Courses and Webinars

- None specific to this project.

14.4.2. Workshops

- None.

14.4.3. Conference Presentations (Keynote and Invited)

- SERDP Symposium 2017; Tratnyek, Johnson, Bylaska, Hawari; Incorporating Transformation Products into Models of the Environmental Fate of Insensitive Munition Constituents (ER-2725)
- Battelle Chlorinated Conference 2018; Paul G. Tratnyek, Tiffany L. Torralba-Sanchez, Eric J. Bylaska, Jalal Hawari, Billy E. Johnson; Incorporating Transformation Products into Models of the Environmental Fate of Insensitive Munition Constituents

- Army Research Office (ARO) Workshop on deep learning for multiphase chemistry 2018; Paul Tratnyek; In Silico Environmental Chemical Science: Contaminant Properties from Statistical and Computational Modeling; September 27-28, 2018
- Gordon Research Conference, Holderness, NH, 2018; Tiffany L. Torralba-Sanchez, Paul G. Tratnyek, Eric J. Bylaska; Incorporating Transformation Products into Models of the Environmental Fate of Insensitive Munitions Constituents
- Society for Environmental Toxicology and Chemistry (SETAC) National Meeting 2018, Sacramento, CA. November 2018; Tiffany L. Torralba-Sanchez, Paul G. Tratnyek, Eric J. Bylaska; Prediction of Transformation Products of Insensitive Munition Constituents
- SERDP Symposium 2018; Modeling Transformation Pathways, Products, and Physicochemical Properties of Insensitive Munition Constituents; Tiffany L. Torralba-Sanchez, Paul G. Tratnyek, Eric J. Bylaska
- American Chemical Society, Northwest Regional Meeting (NORM) 2019; Tiffany L. Torralba Sánchez, Paul G. Tratnyek, Eric J. Bylaska; Predicting the environmental risk of insensitive munition constituents and their transformation products using in silico methods; June 16-19, 2019 Portland State University
- 10th National Conference on Environmental Chemistry, Nankai University, Tianjin, China, August 15-19, 2019; In Silico Environmental Chemical Science: Contaminant Properties from Statistical and Computational Modeling; Paul G. Tratnyek, Tiffany Torralba-Sánchez, Eric J. Bylaska
- SERDP Symposium 2019; Tiffany L. Torralba-Sanchez, Eric J. Bylaska, and Paul G. Tratnyek; Prediction of Transformation Products of Insensitive Munition Constituents
- SERDP Symposium 2020; Ryder, J.L. (2020) "The Challenges of Modeling Munition Constituent Risks to Support Range Assessment". Virtual.
- E. J. Bylaska, NWChemEx AIMD Development (PWDFT), Virtual workshop, MolSSI Workshop on HPC in Computational Chemistry and Materials Science, December 13-15, 2021.
- Battelle Chlorinated Conference, Palm Springs, CA (2022); Paul G. Tratnyek, Tiffany L. Torralba-Sanchez, Eric J. Bylaska, Billy E. Johnson, Jodi L. Ryder; Degradation of Insensitive Munitions Constituents (IMCs) in the Environment: Predicting the Products and their Properties Using In Silico Methods

14.4.4. Special Projects

- None.

14.4.5. Social Media

- Nothing specific to this project.