BATCH AND COLUMN TRANSPORT STUDIES OF ENVIRONMENTAL FATE OF 3-NITRO-1,2,4-TRIAZOL-5-ONE (NTO) IN SOILS

by

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A Thesis Submitted to the Faculty of the
DEPARTMENT OF SOIL, WATER, AND ENVIRONMENTAL SCIENCE
In Partial Fulfillment of the Requirements For the Degree of

MASTER OF SCIENCE

In the Graduate College
THE UNIVERSITY OF ARIZONA

2014
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Acknowledgements

I would like to acknowledge Strategic Environmental Research and Development Program, SERDP (project ER-2220), who sponsored our work. I am very grateful to my advisors, Dr. Katerina Dontsova and Dr. Mark Brusseau, and Dr. Joan Curry for their intellectual guidance and support. A big thanks to members of the Contaminant Transport Group for being my springboard when I had an issue with my research. I’d like to thank my fellow biospherians, Dragos Zaharescu and Carmen Burghelea, for their advice and stimulating conversation. Thanks to Ed Hunt for his assistance in the lab; his excellent trouble-shooting abilities kept things moving forward. Last but certainly not least, thank you to my ‘lab spouse,’ Jennifer Arthur, who has been a wonderful colleague and friend these past two years.
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Terms, Abbreviations, and Acronyms
ARDEC – Armament Research, Development and Engineering Center
ATO – 5-amino-1,2,4-triazol-3-one
DNAN – 2,4-dinitroanisole
EC – electrical conductivity
HPLC – High Precision Liquid Chromatography
\( k \) – 1st order transformation coefficient
\( K_d \) – linear adsorption coefficient
\( K_f \) – Freundlich adsorption coefficient
IM – Insensitive Munition
NQ – nitroguanidine
NTO – 3-nitro-1,2,4-triazol-5-one
OC – organic carbon
OM – organic matter
RDX – high energy explosive, 1,3,5-hexahydro-1,3,5-trinitro-1,3,5-triazine
TNT – 2,4,6-trinitrotoluene
U.S. EPA – United States Environmental Protection Agency
Abstract

NTO (3-nitro-1,2,4-triazol-5-one) is one of the new explosive compounds used in insensitive munitions (IM) and developed to replace traditional explosives, TNT and RDX. Data on NTO fate and transport is needed to determine its environmental behavior and potential for groundwater contamination. In this study, we measured how NTO in solution interacts with different types of soils and related soil properties to transport and fate behavior. We conducted a series of kinetic and equilibrium batch soil sorption experiments and saturated column transport studies under steady-state and transient conditions. NTO adsorbed very weakly to the studied soils. Adsorption coefficients ($K_d$s) measured for NTO in a range of soils in batch experiments were less than 1 cm$^3$ g$^{-1}$. There was a highly significant negative relationship between measured NTO adsorption coefficients and soil pH ($P = 0.00011$). In kinetic experiments, first order transformation rate estimates ranged between 0.0004 h$^{-1}$ and 0.0221 h$^{-1}$. There was a general agreement between batch and column-determined fate and transport parameters. However, transport studies showed an increase in the NTO transformation rate as a function of time, possibly indicating microbial growth.
Chapter 1. Introduction

1.1 Context

Approximately half the United States population uses groundwater as their primary source of potable water (U.S. Geological Survey, 1999). Comprehensive monitoring of ground water quality is paramount to protecting and managing this resource. Water suppliers are required to meet specific drinking water standards under the Safe Drinking Water Act of 1986. The list of groundwater pollutants is constantly expanded as new synthetic compounds are introduced into the environment. Chemicals in water that were previously undetected or detected at unexpected levels are generally referred to as emerging contaminants of concern (CECs) because their risk to human and environmental health is not known or poorly characterized. CECs that have received recent attention by the EPA include pharmaceuticals and personal care products (PPCPs), perfluorinated compounds, and newly synthesized nitroaromatic and nitramine explosive mixtures. Soil and water contamination by nitroaromatic and nitramine explosives is present in training areas where armament testing occurs and industrial sites where explosives are developed and manufactured for military and commercial use. Toxicity information for traditional explosives (e.g. nitroaromatics) is robust; however, far less data describing novel explosives such as 3-nitro-1,2,4-triazol-5-one (NTO) and its degradation products exists. NTO is being used as the explosive filler in newly formulated munition mixtures but its fate and transport in the environment have been poorly described.

The U.S. Department of Defense (DoD) and the Department of Energy (DoE) have taken an active role in supporting research that seeks to investigate the effects of munition compounds, including NTO, on active firing ranges where munitions are tested before fielding. Soil as a mediator in NTO transport to groundwater is an important consideration at these testing ranges. In a soil system, NTO moving in solution can adhere to sediments (sorption) and/or be degraded by biotic and abiotic processes. Sorption delays transport of contaminants, whereas, biodegradation decreases the amount of contaminant available for transport. Soil-water partitioning coefficients are a chemical-specific measure of sorption. The linear form of this coefficient is called the distribution or adsorption coefficient, $K_d$. A decrease in residual mass of a contaminant in a soil system can result from degradation by chemical and biological mechanisms. Degradation can be described by first order degradation kinetics. The first order degradation coefficient, $k$ (in units of time$^{-1}$), describes the fraction of contaminant degraded per time. In the process of degradation, if this coefficient does not change, the mass of the contaminant will decrease exponentially with time.

Laboratory studies involving the interaction of a known amount of soil and NTO can be used to determine $K_d$ and $k$ values. Batch-type procedures for estimating soil sorption are a simple technique to evaluate the capacity of soils and soil components to remove chemicals from solution. Saturated flow experiments using flux-calibrated glass columns can be used to derive the same soil-interaction parameters measured in bath-type procedures and may further elucidate mechanisms involved in contaminant fate and transport. Both procedures vary considerably depending on research objectives and experimental conditions. Measured parameters such as $K_d$ and $k$ can be used in numerical models to estimate the potential extent of natural attenuation of
contaminants and their transport to groundwater. Furthermore, mechanisms of chemical interaction with soil can be derived by correlating $K_d$ and $k$ values to specific soil properties.

1.2 Literature Review

1.2.1 Explosive Compounds as Contaminants

Certain conventional explosives like RDX (1,3,5-trinitro-1,3,5-triazine) and TNT (2,4,6-trinitrotoluene) are susceptible to inadvertent detonation by unplanned stimuli like mechanical shock and high temperatures. For this reason, defense agencies are phasing out these explosives and replacing them with more stable formulations, termed insensitive munitions (IMs), due to safer transport, handling and storage advantages (Beyard, 2007). A series of new IM formulations (including IMX-101, 102, 104, and PAX-48) contain NTO (3-nitro-1,2,4-triazol-5-one) as one of the main ingredients.

IM formulations are tested on active firing ranges before fielding. In some cases, solid high explosives remain on range soils as a result of incomplete detonation. These solid explosives scattered on the range are transformed into solutions of IM components in a series of steps. This series includes dissolution (by rainfall or other precipitation events), photo-transformation (both of the solid and of aqueous solutions), transport to groundwater and interaction of the dissolved phase with soil constituents. If these compounds and their degradation products are shown to be toxic then transport to groundwater poses a threat to human health. Toxicology data for NTO shows low mammalian toxicity (London and Smith, 1985); however evidence suggests it can function as a male reproductive toxicant in rats (Wallace, 2011). The greater toxicity of TNT and other nitroaromatic explosives involves oxidative stress resulting from enzymatic formation of free radicals; these include formation of highly reactive nitroso and/or hydroxylamino metabolites and the direct oxidation of oxyhemoglobin into methemoglobin (Somerville et al., 1995; Vorbeck et al., 1998; Pak et al., 2000). Lower toxicity of nitrotriazole explosives like NTO may be attributed to their low electron-accepting properties (Sarlauskas et al., 2004).

In general, transformation products of a compound can have different environmental and toxicity properties than the original contaminant. NTO can form toxic transformation products (Le Campion et al., 1999b). For example, partial reduction of the nitro group can result in reactive intermediates, such as nitroso or hydroxylamino groups, that can interact with biomolecules to cause toxic or mutagenic effects (Hlavica, 1982).

1.2.2 Chemical Properties of NTO

NTO is readily soluble in water and its solubility is considerably higher than the solubility of 2,4-dinitroanisole (DNAN), TNT, and RDX (Table 1). As part of a formulation, NTO’s dissolution will depend not only on its solubility and dissolution rate, but also on the fraction of NTO exposed to water (Lever et al., 2005; Dontsova et al., 2006; Taylor et al., 2009b, a). IM formulations are produced by melt casting, where the explosive filler (e.g. RDX, NTO, or NQ) is mixed with a melted energetic binder (TNT, DNAN, or nitrate salts) (Pelletier et al., 2010; Rutkowski et al., 2010). The filler, NTO, has a higher solubility than the binder, DNAN. In formulation, initial loss of each compound during dissolution may not depend directly on the relative solubility of each component. For example, DNAN may protect NTO from dissolving. Also, the dissolution rate of solid explosives not only depends on solubility but particle size and climatic conditions (e.g. type of precipitation event and temperature) (Lever et al., 2005; Taylor et
In a situation where NTO is left undetonated on firing range soil, transport of NTO and its degradation products from the soil surface to groundwater begins with dissolution from a multicomponent solid. Once in solution, the mobility and persistence of NTO in the environment is in part determined by its inherent chemical and structural properties (Taylor et al., 2013).

Upon entering the subsurface, NTO and its transformation products will experience reactive transport through the soil. As a chemical compound migrates through the soil profile, it can undergo irreversible and reversible adsorption by different mineral and organic phases in the soil. It may also experience transformation, volatilization, and bio-uptake. The importance of each of these processes for the fate of a compound can be evaluated using the soil partition coefficient ($K_d$), soil organic carbon partition coefficient ($K_{oc}$), octanol-water partition coefficient ($K_{ow}$), Henry’s Law constant, the one-electron standard reduction potential, and transformation rate constant ($k$), among others. Values for solubility (mg L$^{-1}$), Log $K_{ow}$, and Log $K_{oc}$ for NTO, as well as for common explosives, TNT and RDX, and another component in IM formulations, 2,4-dinitroanisole (DNAN), can be found in Table 1.

**Table 1.** Selected environmentally relevant chemical and physical properties of NTO in comparison to DNAN, TNT, and RDX, including solubility at 25 °C, octanol-water partitioning coefficient ($K_{ow}$), and soil organic carbon partitioning coefficient ($K_{oc}$).

<table>
<thead>
<tr>
<th>Property</th>
<th>DNAN</th>
<th>NTO</th>
<th>TNT</th>
<th>RDX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility, mg L$^{-1}$</td>
<td>276.2$^{a1}$</td>
<td>16642.0$^{c2}$</td>
<td>100.5$^{a3}$</td>
<td>59.9$^{a3}$</td>
</tr>
<tr>
<td>Log $K_{ow}$</td>
<td>1.7 – 1.92$^{b}$</td>
<td>0.37 – 1.03$^{b4}$</td>
<td>1.6 – 1.84$^{3}$</td>
<td>0.81 – 0.87$^{5}$</td>
</tr>
<tr>
<td>Log $K_{oc}$</td>
<td>3.11$^{a6}$, 2.2$^{b7}$</td>
<td>3.03$^{a6}$, 2.1$^{b7}$</td>
<td>3.2$^{3}$</td>
<td>0.88 – 2.4$^{3}$</td>
</tr>
</tbody>
</table>

$^{a}$ measured; $^{b}$ estimated; $^{c}$ interpolated from measured values.

1 (Boddu et al., 2008); 2 (Spear et al., 1989); 3 (Brannon and Pennington, 2002); 4 (Sokkalingam et al., 2008); 5 (Yoon et al., 2005); 6 (Pesce-Rodriguez, unpublished); 7 (Chakka et al., 2008).

The affinity of NTO to be sorbed onto soil surfaces is characterized by the soil partition, or adsorption coefficient, $K_d$. Organic matter, phyllosilicate clays, iron and aluminum oxides and hydroxides, in particular, adsorb organic compounds due to their high surface areas (e.g. Dontsova et al., 2009). A high $K_d$ value indicates that the chemical will reside mostly on the solid phase and little is transported downward by infiltration of soil water. Theoretically, the majority of organic contaminants that undergo adsorption in soils can be attributed to soil organic carbon, therefore $K_d$ values are usually normalized to soil organic carbon content and the resulting parameter is called the organic carbon partition coefficient, $K_{oc}$. Once estimated for the compound, this parameter can be used to calculate adsorption to other soils depending on their carbon content. There is little information about soil adsorption coefficients for IM compounds. Recent work funded by Defense Research and Development Canada (Hawari et al., 2011; Hawari et al., 2012) reported low soil sorption for NTO ($K_d < 0.1$). Further studies are needed to measure how chemical and biological transformation would affect the measured $K_{oc}$ values and to determine the mechanisms involved. For example, RDX tends to partition to organic carbon (Tucker et al., 2002; Dontsova et al., 2009a), while TNT exhibits very complex sorption behavior, which includes adsorption in clay interlayer surfaces (Haderlein et al., 1996) and irreversible adsorption to organic matter following transformation (Thorn and Kennedy, 2002).
Unlike most other explosives, which are polar but non-ionic compounds, NTO is an acid with pK$_a$ of 3.7-3.76 (Chipen et al., 1966; Le Campion et al., 1997), and it will be negatively charged at environmentally relevant pHs (Smith and Cliff, 1999). Its weak acidic character is due to labile N—H bonds. Under normal conditions, a mechanism for sorption of organic anions is negative adsorption (e.g. Hirt and Schmitt, 1958; Bailey and White, 1970). At soil pH below the pK$_a$ value, the organic compound will exist primarily in the nondissociated form; however a fraction of the compound will be dissociated. Likewise, at soil pH above its pK$_a$, the organic compound will exist predominantly in the dissociated form, with less and less of the nondissociated form present as soil pH increases. As both organic and mineral soil surfaces tend to have net negative charge, we would predict low affinity of NTO for soils and greater mobility in the environment; however, negatively charged organics can be adsorbed in soils through cation bridging (e.g. Hyun and Lee, 2005). Ionizable nitroaromatic compounds showed a strong pH dependence of the apparent K$_d$ values in the pH region corresponding to the pK$_a$ of the compound (e.g. Haderlein et al., 1996).

The octanol-water partition coefficient is a measure of the tendency of the chemical to bio-accumulate (Boddu et al., 2008), and an indicator of its affinity for soil organic matter (OM). Relatively low reported K$_{ow}$s (Table 1) indicate that NTO would not be strongly adsorbed by OM in soils through non-polar interactions. However, it can still experience specific sorption, as have been shown for TNT and DNTs (Thorn et al., 2001; Thorn et al., 2008). Based on K$_{ow}$s one would expect NTO to be more mobile than DNAN and exhibit similar environmental behaviors to RDX. Relatively low volatility, as measured by Henry’s constants (log K$_H$ of -11.38 for NTO) (Sokkalingam et al., 2008), indicates that transport in a gas phase will not be important for the total mass flux of IM compounds in soils.

1.2.3 Microbial Breakdown of NTO

Nitroaromatic and nitramine compounds can be microbially degraded via oxidation-reduction (redox) reactions (Spain, 2000). Oxygen, nitrate, manganese, iron, sulfate, and carbon dioxide are primary electron acceptors. Oxygen as the electron acceptor yields the most energy to the microbe and carbon dioxide yields the least amount of energy to the microbe. Enclosed conditions may result in oxygen depletion and produce reducing (anaerobic) conditions, whereby
electron-accepting processes will proceed sequentially: the electron acceptor that will yield the next highest amount of energy to the microbe will be used. In general, anaerobic bacteria and fungi transform nitroaromatic compounds via fortuitous reactions, while aerobic microbes use nitroaromatic contaminants as growth substrate (Spain, 1995). Many organisms contain enzymes that can catalyze transformation of the aromatic nitro group. These include microbes that produce a variety of redox enzymes that can serve as nitroreductases. Enzymes that allow microbes to use nitroaromatic compounds as their source of carbon or nitrogen are less common but have been shown in studies involving microbially-mediated degradation of nitroaromatics under aerobic conditions.

The nitro group on NTO is a resonance hybrid: in a nitrogen-oxygen bond, the oxygen atom is more electronegative than the nitrogen atom; polarization causes the nitrogen to have a partial positive charge. Iron (II), other metals and reduced sulfur compounds can serve as non-enzymatic reductants of nitroaromatic compounds (Dunnivant et al., 1992; Heijman et al., 1993; Preuss, 1993). Biologically, the most common reaction of nitro groups is reduction of the electrophilic nitrogen, this can proceed by single electron mechanisms. One-electron standard reduction potential ($E_{\text{m}}$) is a measure of how easily nitro groups of nitroaromatic compounds reduce to amino groups (Uchimiya et al., 2010). Faster reduction was observed for explosives having more positive one-electron reduction potentials. Calculated electron standard reduction potential for NTO is -0.509 volts (Misevičienė et al., 2006), indicating that it would undergo reduction at a similar rate to the compound it is said to replace, RDX (-0.55 volts, experimental value) (Uchimiya et al., 2010).

Microbial degradation studies of NTO in industrial waste show nitroreduction, followed by the ring cleavage of the primary amine, 5-amino-1,2,4-triazol-3-one (ATO) (Le Campion et al., 1999). Many microorganisms reduced the nitro group on NTO; however, Bacillus licheniformis was the most efficient strain for NTO nitroreduction, and it was the only strain that degraded NTO to ATO. Microbial reduction of NTO was not affected by oxygen: oxygen saturated solutions and non-shaken incubations showed similar results for nitroreduction of NTO. Glucose played an important role in oxidoreduction by influencing enzymatic induction and cofactor supply. Highest nitroreduction rate for NTO and ATO was achieved at pH 6 and pH 8, respectively. At optimal conditions, the nitroreduction of NTO was complete in 24 h, while the degradation of ATO required 2 weeks of incubation. The end products of the biodegradation were carbon dioxide (40%), urea and a polar compound, assumed to be hydroxyurea.

1.3 Thesis Formatting

This thesis is intended to present rationale for methods and results of manuscripts found in Appendixes A and B. Manuscripts have been formatted for publication in Chemosphere. Ed Hunt analyzed non-reactive tracer for column experiments by ion chromatography. Soil particle-size and surface area analyses were performed by Mercer Medding. Anthony Di Stasio and Erika Rivera, US Army Armament Research, Development and Engineering Center (ARDEC), Picatinny Arsenal provided NTO. Bonnie M. Packer, Rosa Gwinn, Lisa DeGrazia, Bethany Keller, Jessica Milose, Amibeth Sheridan, Laurie Stenberg, Sarah Gettier from the Army National Guard–Environmental Directorate and URS Corporation (Germantown, MD) collected soil samples at the National Guard sites. Mike Heitmann (CH2M HILL, Englewood, CO) collected soil at Camp Butner, NC and Florence Military Reservation, AZ. Jennifer Arthur helped carry-out soil characterization procedures. Other manuscript co-authors aided in the experimental design of the
Chapter 2. Present Study

Motivations and rational to investigate the fate and transport of NTO in multiple soils having a range of properties are relayed in the following sections. A brief description of soils used in the study and discussion of major findings are presented. Full disclosure of methods, results and discussion are presented in appendixes. Batch-type experiments are presented in Appendix A and saturated flow column experiments are presented in Appendix B. Figures and tables not presented in manuscripts are included in Appendix C.

2.1 Rationale for Study

Studying the fate and transport of each component in an IM formulation will allow us to more accurately depict the observed behavior of the component when it is examined as part of a solid explosive mixture. For the purposes of this study, NTO is treated as a single component; if left on an active firing range as undetonated material, its release into the environment will involve dissolution from a solid explosive mixture. Each component in the mixture will have an associated dissolved phase as part of the overall mass flux in soil. Dissolved NTO can enter the subsurface where it has the potential to reach groundwater; however, NTO moving in solution may react with constituents in the soil, either adhering to sediments (sorption) and/or being degraded.

Currently, there is insufficient information about soil sorption for NTO, both the extent of sorption and the mechanisms involved. NTO $K_{ow}$ values have been both estimated and measured (Sokkalingam et al., 2008), but the $K_{oc}$ that are currently available were computationally derived (Chakka et al., 2008) from $K_{ow}$, which can result in unreliable values, particularly for polar compounds. Soil adsorption for NTO was measured for only two sandy soils (Hawari et al., 2011; Hawari et al., 2012). To address these knowledge gaps, NTO partition coefficients ($K_d$) for multiple soils having a range of properties were measured, and these values were normalized to their organic carbon content to determine $K_{oc}$. If these parameters are known, the fate of the chemical in the soil system can be modeled. Soil adsorption is an important mechanism for natural attenuation of organic contaminants. Values for $K_d$, $k$ and $K_{oc}$ are critical for understanding the fate and transport of these new IM compounds and can be used in transport models to assess the threat to potable groundwater sources.

2.2 Objectives and Approach

To measure soil adsorption properties and first-order degradation of NTO the following tasks were performed:

1) determine adsorption behavior of NTO in a range of soils;
2) characterize the transport behavior of NTO in soils and correlate its transport and fate to soil properties; and
3) model the results using a reactive transport model (HYDRUS 1-D) for simulating the environmental fate of NTO.

We conducted a series of kinetic and equilibrium batch soil sorption experiments to characterize the fate of NTO in soils and the effect of soil heterogeneity on NTO-soil interactions (Roy et al., 1992). Saturated column transport studies under steady-state and transient conditions were conducted to measure the transport of NTO in soils. Kinetic and column experiments were repeated using sterilized soils to evaluate contribution of biodegradation. Two soils of similar particle size but different organic carbon content were selected (Catlin and Sassafras soils) and sterilized by autoclave. For these experiments, mass recovery of NTO—extracted from soils and left in solution—were compared between untreated and sterilized soils.

In all experiments, NTO concentration was quantified using Dionex Ultimate 3000 high performance liquid chromatograph (HPLC) equipped with a diode array detector (ThermoFisher, MA). Adsorption isotherms were constructed from batch experiments yielding linear and Freundlich partition coefficients ($K_d$ and $K_f$), and kinetic experiments provide estimates for 1st order transformation rate constants ($k$). For column experiments, temporal moment analysis (TMA) was performed for breakthrough curve (BTC) data to provide mass balance ratio and initial parameters for the transport model. The HYDRUS-1D model was used to analyze breakthrough curves. First, the BTCs for a nonreactive tracer were modeled to determine physical parameters characterizing the column experiments. Physical parameters were fixed, then breakthrough curves of NTO were analyzed to determine chemical/reaction parameters involved. The reaction parameters determined using the inverse mode of HYDRUS-1D were compared to the same parameters independently determined in soil batch studies. Finally, batch derived soil adsorption and transformation rate constants were correlated to soil properties. Agreement between the batch and column derived parameters will provide an indication of how well the determined relationships characterize the transport and fate of NTO in soils.

### 2.3 Soils Selected for Study

Eleven different soils were used for this study. Ten of these soils were collected at military training ranges and Catlin silt loam was collected on the University of Illinois at Urbana-Champaign university farm.
The selected soils represent a range of climate types and conditions where personnel are undergoing training. They encompassed multiple soil orders, including Entisols, Inceptisols, Mollisols, Aridisols, and Ultisols with different moisture regimes—from arid to humid. The physical and chemical properties for the 11 soils selected for this study are summarized in Appendix A, Table 4. All studied soils had mixed particle size, ranged from fine (clay loam) to coarse (loamy sand). Soil pH ranged from 4 to 8 and soil organic carbon (OC) ranged from 0.34 to 5 %. Some of the soils used in the batch studies were selected to be used in column transport experiments (a subset of 8 soils).

2.4 Summary of Results

2.4.1 Batch adsorption studies

Kinetic experiments indicated that majority of soils reached equilibrium with NTO in solution at 24 hours. All determined adsorption coefficient ($K_d$) values were less than 1 cm$^3$ g$^{-1}$, with the largest observed $K_d$ equal to 0.51 cm$^3$ g$^{-1}$ in Camp Gruber soil. There was a highly significant negative relationship between linear adsorption coefficients and soil pH. Estimates of the first order transformation constant, $k$, ranged between 0.0004 h$^{-1}$ in Camp Guernsey soil and 0.0221 h$^{-1}$ in Catlin soil. Measured transformation rates were lower for soils with less organic carbon (OC). Kinetic experiments for Sassafras and Catlin comparing mass recovered in solution and extracted from soil showed significant differences between autoclaved and untreated soils. Mass loss was significantly greater for untreated soils and there was much greater loss for a soil with more organic carbon. No NTO transformation products were detected. A more detailed discussion of these results is presented Appendix A.
2.4.2 Saturated flow column studies

All NTO breakthrough curves (BTCs) exhibited no tailing. Some soils, Limestone Hills, Camp Butner, Arnold AFB, and Catlin, had breakthrough curves that indicated an increase in the transformation rate as a function of time and showed low recovery after interrupted flow. Batch and column (HYDRUS 1-D derived) NTO linear adsorption coefficients ($K_d$) correlated well ($P = 0.000576$, or highly significant). For the majority of soils, 24 hour flow interruption at steady-state resulted in a decrease in NTO outflow concentration. Autoclaved and untreated soils showed differences in mass balance ratio and response to 24 h flow interruption. Lower mass recovery was seen for untreated Catlin soil (5.28% OC). In sterilized Sassafrass soil (1.30% OC), 24 h flow interruption at steady state conditions did not cause a decrease in concentration as seen for the untreated soil. There was less NTO recovered in a soil with more organic carbon. No NTO transformation products were detected. See Appendix B for details.

2.5 Summary of Conclusions

NTO experienced adsorption and transformation in soils. NTO was weakly adsorbed, with adsorption coefficients lower than measured for RDX, the traditional explosive compound NTO is replacing. For most soils both Freundlich and linear adsorption isotherm described the observed adsorption well, but the linear equation resulted in a better fit. Soil pH was the strongest indicator of NTO affinity for soil surfaces. Adsorption decreased as soil pH increased. NTO transformation rates correlated positively with OC in the soil and greater mass loss was observed in untreated compared to sterilized soils for a soil with more organic carbon. This suggests that NTO is being biodegraded, possibly with OC acting as a substrate.

Column studies supported batch-determined parameters, but indicated that increase in transformation rates may be happening, possibly due to microbial growth. As was observed in batch-type experiments, saturated flow studies showed that NTO transformation rates correlated positively with OC in the soil. Soils with higher OC content exhibited non-first order transformation kinetics. Again, this suggests that OC could be acting as the carbon source in microbial degradation of NTO. Flow interruption tests showed a decrease in effluent NTO concentration, consistent with continued transformation.

There was general agreement between column and batch derived $K_d$; however, slope of the regression was greater than 1 indicating that there was a trend towards higher $K_d$ estimates in column experiments. Low inflow concentrations used in column experiments and slight non-linearity of adsorption with increase in concentration can account for this trend. The adsorption equilibrium HYDRUS-1D model best described NTO transport in selected soils, suggesting that contribution of non-equilibrium processes is small. Also, flow interruption tests did not show decreases in concentration at steady-state indicative of diffusion limited adsorption of the target analyte.
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Appendix A

Kinetic and Equilibrium Batch Soil Adsorption Studies of 3-nitro-1,2,4-triazol-5-one (NTO)

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Manuscript for submission to Chemosphere
Abstract

NTO (3-nitro-1,2,4-triazol-5-one) is one of the new explosive compounds used in insensitive munitions (IM) developed to replace traditional explosives, TNT and RDX. Data on NTO fate and transport is needed to determine its environmental behavior and potential for groundwater contamination. We conducted a series of kinetic and equilibrium batch soil sorption experiments to characterize the fate of NTO in soils and the effect of soil heterogeneity on NTO-soil interactions. Kinetic experiments were repeated using sterilized soils to evaluate the contribution of biodegradation. Adsorption coefficients ($K_d$) measured for NTO in a range of soils in batch experiments were less than 1 cm$^3$ g$^{-1}$. There was a highly significant negative relationship between measured NTO adsorption coefficients and soil pH ($P = 0.00011$). In kinetic experiments, first order transformation rate constant ($k$) estimates ranged between 0.0004 h$^{-1}$ and 0.0221 h$^{-1}$. NTO transformation rates correlated positively with organic carbon (OC) in the soil, and lower NTO recovery was observed in untreated versus sterilized samples for samples with high OC soil. This suggests that NTO is being biodegraded, possibly with OC acting as a substrate.

Introduction

Military services have been evaluating new explosive compounds termed insensitive munitions (IMs) to replace traditional explosives, RDX (1,3,5-trinitro-1,3,5-triazine) and TNT (2,4,6-trinitrotoluene). IMs are designed to perform their operational function on demand while responding less violently to unplanned stimuli, such as mechanical shock and high temperatures (Beyard, 2007). NTO (3-nitro-1,2,4-triazol-5-one) and 2,4-dinitroanisole (DNAN) are primary ingredients in a series of new IM formulations (including IMX-101, 104, PAX-21, 41 and 48) that are being, or soon will be fielded. Solid IM residues may be deposited on range soils as a result of incomplete detonation. It has been shown that these formulations readily dissolve releasing NTO first due to its high solubility (Taylor et al., 2013).

Toxicology data for NTO shows low mammalian toxicity (London and Smith, 1985; Nikolic et al., 1994), however evidence suggests it can function as a male reproductive toxicant in rats (Wallace, 2011). Furthermore, metabolic intermediates produced during nitroreduction of nitro-compounds exhibited neurotoxicity and cytotoxicity (Koch et al., 1979; Walton and Workman, 1987). Based on these risks, accurate fate and transport parameters, such as soil partition coefficients ($K_{ds}$) and degradation rates are necessary to model NTO fate and transport to groundwater.

There is little information about soil adsorption behavior for IM compounds. Hawari et al., (2011) and Hawari et al. (2012) reported low NTO soil sorption ($K_d < 0.1$) for two soils. Further studies are needed to measure how soil properties would affect the measured $K_d$ and $K_{oc}$ values and to determine the mechanisms involved NTO sorption. For example, RDX tends to partition to organic carbon (Tucker et al., 2002; Dontsova et al., 2009a), while TNT exhibits more complex sorption behavior involving adsorption in clay interlayer surfaces (Haderlein et al., 1996) and irreversible adsorption to organic matter following transformation (Thorn and Kennedy, 2002).

Unlike most other explosives, NTO is ionic and can act as an acid due to labile N—H bonds. It has a pKa of 3.7-3.76, and will be negatively charged at environmentally relevant pHs (Chipen et al., 1966; Le Campion et al., 1997; Smith and Cliff, 1999). As both organic and mineral soil surfaces tend to have net negative charge, we would predict low affinity of NTO for soils and
greater mobility in the environment; however, negatively charged organics can be adsorbed in soils through cation bridging (e.g. Hyun and Lee, 2005).

The octanol-water partition coefficient is a measure of the tendency of the chemical to bio-accumulate (Boddu et al., 2008), and an indicator of its affinity for soil organic matter (OM). Relatively low reported \( K_{ow} \) (Table 1) indicate that NTO would not be strongly adsorbed by OM in soils through non-polar interactions. However, NTO can still experience specific sorption, as have been shown for TNT, and DNTs (Thorn et al., 2001; Thorn et al., 2008). Based on \( K_{ow} \) one would expect NTO to be more mobile than DNAN and exhibit similar environmental behavior to RDX, such as low adsorption and high mobility in the environment (Table 1).

**Table 1.** Selected environmentally relevant chemical and physical properties of NTO and RDX, including solubility at 25 °C, octanol-water partitioning coefficient \( (K_{ow}) \), and soil organic carbon partitioning coefficient \( (K_{oc}) \).

<table>
<thead>
<tr>
<th>Property</th>
<th>NTO</th>
<th>RDX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility, mg L(^{-1})</td>
<td>16642.0(^{c})</td>
<td>59.9(^{c})</td>
</tr>
<tr>
<td>( \log K_{ow} )</td>
<td>0.37 – 1.03(^{a})</td>
<td>0.81 – 0.87(^{a})</td>
</tr>
<tr>
<td>( \log K_{oc} )</td>
<td>3.03(^{a}), 2.1(^{a})</td>
<td>0.88 – 2.4(^{a})</td>
</tr>
</tbody>
</table>

\(^{a}\) estimated; \(^{b}\) interpolated from measured values; \(^{c}\) measured.

\(^{1}\) (Spear et al., 1989); \(^{2}\) (Sokkalingam et al., 2008); \(^{3}\) (Pesce-Rodriguez, unpublished); \(^{4}\) (Chakka et al., 2008); \(^{5}\) (Brannon and Pennington, 2002); \(^{6}\) (Yoon et al., 2005).

Low volatility, as measured by Henry’s constants (log \( K_{H} \) of -11.38) (Sokkalingam et al., 2008), indicates that transport in a gas phase will not be important for the total mass flux of NTO in soils. One-electron standard reduction potential (\( E_{m} \)) is a measure of how easily nitro groups in these compounds reduce to amino groups—a common first step in transformation of many explosive compounds (Uchimiya et al., 2010). Faster reduction was observed for explosives having more positive one-electron reduction potentials. Calculated electron standard reduction potential for NTO was -0.509 volts (Misevičienė et al., 2006), indicating that it would undergo reduction at a rate similar to the compound it is said to replace, RDX (-0.55 volts, experimental value) (Uchimiya et al., 2010).

Microbial degradation studies of NTO in industrial waste show nitroreduction, followed by the ring cleavage of the primary amine, 5-amino-1,2,4-triazol-3-one (ATO) (Le Campion et al., 1999b). At optimal conditions, the nitroreduction of NTO was complete in 24 h, while the degradation of ATO required 2 weeks of incubation. The end products of the biodegradation were carbon dioxide (40%), urea and a polar compound, assumed to be hydroxyurea.

In summary, there is not enough information on NTO soil adsorption—both the extent of sorption and mechanisms involved. \( K_{ow} \) has been estimated and measured (Sokkalingam et al., 2008), but the \( K_{oc} \) that are currently available were computationally derived (Chakka et al., 2008) from \( K_{ow} \), which can result in unreliable values, particularly for polar compounds; and soil adsorption was measured for only two sandy soils (Hawari et al., 2012). To address these knowledge gaps, we experimentally determined NTO 1st order transformation rates \( (k) \) and partition coefficients \( (K_A \text{ and } K_F) \) for multiple soils having a range of properties and normalized the values to their organic carbon content to determine \( K_{oc} \). If these parameters are known, the fate of the chemical in the soil system can be modeled. Because soils are an important mechanism for
natural attenuation of organic and inorganic contaminants, values for $K_d$, $K_{oc}$ and $k$ are critical for understanding the fate and transport of NTO.

**Materials and methods**

**Soils**

Uncontaminated soils having a wide range of properties were collected on military installations across the United States. In addition, we included several soils that were previously used in studies of explosive and propellant formulation constituents (Dontsova et al., 2006; Dontsova et al., 2007; Dontsova et al., 2009a; Dontsova et al., 2009b; Taylor et al., 2012). Using the same soils allowed comparison of current results for NTO to those of prior research for traditional energetics. All soil samples were collected near the surface (top 12 inches or 30 cm).

The soils were air dried, sieved (< 2 mm), and characterized prior to the experiments. Organic carbon (OC) was analyzed using dry combustion according to EPA Standard Operating Procedure for sediments (U.S. Environmental Protection Agency, 2005). A known mass of soil was treated with 5% phosphoric acid (H$_3$PO$_4$) to ensure complete removal of inorganic carbon. A Shimadzu SSM-5000A Solid Sample Combustion Unit was used to analyze OC following pretreatment. Particle size was analyzed by Beckman Coulter LS 13 320 Laser Diffraction Particle Size Analyzer (Beckman Coulter, Inc., Fullerton, CA). Specific surface area (SSA) by N$_2$ adsorption was measured using Beckman Coulter SA 3100 and calculated by applying Brunauer-Emmett-Teller (BET) theory (Brunauer et al., 1938). A VWR SympHony SB70P Benchtop Digital pH and EC Meter was used to measure pH and electrical conductivity for a 1:1 soil to solution mixture.

Eleven different soils were used for this study. Ten of these soils were collected at military training ranges and Catlin silt loam was collected on the University of Illinois at Urbana-Champaign university farm.

Catlin and two additional soils, Plymouth sandy loam and Sassafras loam, were previously used to study soil interactions and transport of propellant constituents; Catlin and Plymouth soils had also been used in studies of high explosives (Dontsova et al., 2006; Dontsova et al., 2007; Dontsova et al., 2009a; Dontsova et al., 2009b). New soils were collected at training sites affiliated with the U.S. Army National Guard (Table 2). Facilities sampled included AZ National Guard Florence Military Reservation, Camp Butner National Guard Training Center North Carolina Army National Guard, Camp Swift Army National Guard Training Center, TX, Camp Guernsey a Premier Joint Training Center in Guernsey, WY, serving both a U.S. Air Force and National Guard. Samples were collected at two locations in Montana: Fort William Henry Harrison is the Montana National Guard training facility, where small arms, heavy machine gun, and pyrotechnics are fired; and Limestone Hills Training Area, where artillery and grenade training, as a well as Open Burn/Open Detonation is done. These soils belong to the same series but contain different amount of organic carbon, twice as much for Fort Harrison compared to Limestone Hills. Samples were also collected on the small arms range at Arnold Air Force Base, Tullahoma, TN.

Soils that were collected earlier are identified thereafter using their series name, while new soils are identified using the location where they were collected. Soil series name and classification for all soils are listed in Table 3.
Table 2. Soils used to study interaction of NTO with the soils. Series names, classification according to US Taxonomy and location where collected.

<table>
<thead>
<tr>
<th>Soil name</th>
<th>Soil classification</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catlin</td>
<td>Catlin silt loam, mixed, mesic, superactive Oxyaquic Argiudoll</td>
<td>Urbana, IL</td>
</tr>
<tr>
<td>Fort Harrison</td>
<td>Musselshell sandy loam, carbonatic, frigid Aridic Calciustepts</td>
<td>Fort William Henry Harrison, MT</td>
</tr>
<tr>
<td>Arnold AFB</td>
<td>Captina silt loam, siliceous, mesic, typic Fragiudults</td>
<td>Arnold Air Force Base, TN</td>
</tr>
<tr>
<td>Plymouth</td>
<td>Plymouth loamy sand, mesic, coated Typic Quartzipsamment</td>
<td>Massachusetts Military Reservation, MA</td>
</tr>
<tr>
<td>Camp Butner</td>
<td>Helena sandy loam, mixed, semiactive, thermic Aquic Hapludults</td>
<td>Camp Butner, NC</td>
</tr>
<tr>
<td>Limestone Hills</td>
<td>Musselshell sandy loam, carbonatic, frigid Aridic Calciustepts</td>
<td>Limestone Hills, MT</td>
</tr>
<tr>
<td>Sassafras</td>
<td>Sassafras loam, siliceous, mesic Typic Hapudult</td>
<td>Aberdeen Proving Ground, MD</td>
</tr>
<tr>
<td>Camp Gruber</td>
<td>Verdigris silt loam, mixed, superactive, thermic Cumulic Hapludolls</td>
<td>Camp Gruber, OK</td>
</tr>
<tr>
<td>Camp Guernsey</td>
<td>Keeline-Turnercrest loam, mixed, superactive, calcareous, mesic Ustic Torriorthents</td>
<td>Camp Guernsey, WY</td>
</tr>
<tr>
<td>Florence MR</td>
<td>Cherioni loam, mixed, superactive, hyperthermic, shallow Typic Haplodurids</td>
<td>Florence Military Reservation, AZ</td>
</tr>
<tr>
<td>Camp Swift</td>
<td>Bergstrom sandy clay loam, mixed, superactive, thermic Cumulic Haplustolls</td>
<td>Camp Swift, TX</td>
</tr>
</tbody>
</table>

Selected soils represent a range of states and climate types and conditions where personnel are undergoing training (Table 2). They encompassed multiple soil orders, including Entisols, Inceptisols, Mollisols, Aridisols, and Ultisols with different moisture regimes, from very dry to wet.
NTO

Technical grade NTO used in the experiments was obtained from US Army Armament Research, Development and Engineering Center (ARDEC), Picatinny Arsenal. Calibration standards for NTO were prepared using the same NTO.

Analytical methods

NTO concentration was quantified using Dionex Ultimate 3000 high performance liquid chromatograph (HPLC) equipped with a diode array detector (ThermoFisher, MA). Operational method was adapted from Le Campion et al. (1999a). Mobile phase, acetonitrile (ACN): deionized water (75:25) with 0.1 % TFA, was run isocractically at 1 mL min\(^{-1}\). Oven temperature was set at 32°C. NTO and its transformation products were separated using a Thermo Scientific Hypercarb Column. NTO was detected at approximately 2.5 mins using 315 nm wavelength. UV detector was set at 220 nm to monitor for presence of ATO (Le Campion et al., 1999a). No ATO was detected in analyzed samples.

Batch soil adsorption studies

To characterize fate of NTO in soils and effect of soil heterogeneity on NTO-soil interactions, we conducted a series of kinetic and equilibrium batch soil sorption experiments. In these experiments, soils were mixed with solutions of NTO and allowed to equilibrate for a predetermined amount of time. Afterwards, soil was separated from solution and supernatant analyzed to determine amount of NTO remaining in solution. Preliminary experiments to determine optimal soil to solution ratios of NTO and effect of background salt solution on adsorption of NTO were conducted. A range of soil to solution ratios was tested (Supplementary Materials, Table s1). Ratios that resulted in 70-90% of original NTO amount remaining in solution were selected for further studies. Experiments were conducted either in deionized water or in 0.005M CaCl\(_2\) solution to establish effect of background salt solution on adsorption.

To create a 1 mg L\(^{-1}\) input solution, 10 mL of a 100 mg L\(^{-1}\) stock solution of NTO was added to 990 mL of deionized water or 0.005M CaCl\(_2\) solution. Soils were weighed into clean, labeled 30 mL borosilicate glass centrifuge tubes according to Supplementary Materials, Table s1. Then 20 mL of 1 mg L\(^{-1}\) NTO in water or 0.005M CaCl\(_2\) solution was pipetted into each centrifuge tube. An aliquot of the input solution was collected and stored to determine the initial concentration of the solute. Duplicate or triplicate tests were conducted depending on observed variability. In addition, blanks were prepared by putting input solution (prepared either in water or 0.005M CaCl\(_2\)) into a centrifuge tube holding no adsorbent. Blanks and samples were continuously agitated on a reciprocating shaker at 110 rpm for 24 ± 0.5 hours. Experiments were conducted under constant conditions, away from light and at room temperature (22 ± 3°C). After 24 hours of agitation, any changes observed in the adsorbent or solution were recorded and tubes centrifuged for 20 minutes at 4700 rpm (4816 relative centrifugal force). A minimum of 3 mL of supernatant was filtered through 0.45 µm Millipore PVDF filter (EMD Millipore Darmstadt, Germany), and placed in labeled 4-mL amber vials. For HPLC analysis, 0.1 mL of this sample was pipetted into labeled autosampler vials for analysis and diluted to 1 mL with 75:25 acetonitrile (ACN):deionized water mixture. The supernatant solution was analyzed for target compounds, NTO and NTO transformation product, ATO, by HPLC. The pH and electrical conductivity (EC) of each sample was determined on an aliquot using a VWR SympHony SB70P Benchtop Digital pH and EC Meter.
All prepared solutions were wrapped in aluminum foil (to prevent photolysis) and sealed with parafilm. Samples and stock solutions were stored in a refrigerator at approximately 4°C.

To observe changes in NTO soil adsorption and transformation with time, a kinetic adsorption study was conducted, where treatment samples and blanks without soil were prepared in triplicate and equilibrated for 1, 4, 8, 24, 48, 72, 96, and 120 hours, then centrifuged, filtered, and stored as previously described. Aliquots were tested for pH and EC (Supplementary Materials, Table s2). The time interval when chemical equilibrium is attained was determined for each soil type. Equilibrium time was defined as the minimum time required to reach a rate of change in solute concentration of less than 5% in 24-hour interval (Roy et al., 1992). NTO transformation rates, $k$, were determined from the change in solute concentration over time, $dC / dt$. For 1st order rate the equation takes the following form

$$-dC/dt = kC.$$ 

The integrated form of the first-order rate law is

$$\ln C = -kt + \ln C_0$$

where $C_0$ is initial concentration of target analyte in solution. Slope of this plot was used to determine $k$.

The equilibrium time for NTO was 24 h for all soils, with the following exceptions: Catlin and Plymouth soil reached equilibrium with NTO in solution at 8 h and Limestone Hills soil at 48 h. After equilibrium time was determined, soil adsorption isotherms were constructed. Isotherms comprised eight input concentrations: 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 mg L$^{-1}$ NTO in 0.005M CaCl$_2$.

Adsorption coefficients were determined from measured solution concentrations and calculated sediment concentrations using linear and Freundlich adsorption isotherms. The linear isotherm is described by the following equation:

$$S = K_d C$$

where $S$ is the concentration in sediment (mg kg$^{-1}$), $K_d$ is the adsorption coefficient (mL g$^{-1}$), and $C$ is the contaminant concentration in solution (mg L$^{-1}$). The Freundlich isotherm was described by:

$$S = K_f C^n$$

where $K_f$ is the Freundlich adsorption coefficient and $n$ is an empirical parameter that indicates the affinity of the compound for the adsorbent. Regression analysis function in Windows Excel 2010 was used to determine linear distribution coefficients ($K_d$), confidence intervals for the estimate, $R^2$ and probabilities of parameters being significantly different from zero. Freundlich equation was used in linearized form to facilitate application of linear regression tools to determine adsorption parameters. Parameters from the equation that described the data the best (largest $R^2$) for majority of the treatments were compared between the treatments to elucidate the effect of treatments on adsorption. Determined parameters were related to measured soil properties, such as their particle size distribution, organic carbon content, and pH to determine effect of geochemical heterogeneity on adsorption of NTO and to help predict the environmental fate of NTO in soils.

Kinetic adsorption and transformation experiments were repeated for Catlin and Sassafras soils treated to kill microbiota to determine whether microbial degradation contributed to solute concentration loss. Soils were sterilized by autoclaving three times over three days with one hour exposure to 120°C each time. Two time intervals, 24 and 120 hrs, were used following the procedure previously outlined for kinetic adsorption. Following equilibration, the input solution
was decanted and analyzed using HPCL. To determine how much NTO remained in the soil, samples were extracted for 24 hours at a 1:4 soil to acetonitrile ratio. NTO extracts were diluted with deionized water to achieve a 1:3 ACN:DI water ratio, filtered through a 0.45μm filter, and analyzed using HPLC.

**Results and Discussion**

**Soils**

The physical and chemical properties for the 11 soils selected for this study are summarized in Table 3. All studied soils had mixed particle size, ranging from fine (clay loam) to coarse (loamy sand).

**Table 3.** Measured physical and chemical properties of soils used in adsorption and transport studies with NTO.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Texture</th>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
<th>pH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SSA&lt;sup&gt;c&lt;/sup&gt;</th>
<th>OC&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>µS cm&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>m&lt;sup&gt;2&lt;/sup&gt; g&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Catlin</td>
<td>silt loam</td>
<td>25.6</td>
<td>65.5</td>
<td>8.9</td>
<td>7.31</td>
<td>492</td>
<td>6.4</td>
<td>5.28</td>
</tr>
<tr>
<td>Fort Harrison</td>
<td>sandy loam</td>
<td>8.7</td>
<td>36.5</td>
<td>54.9</td>
<td>6.67</td>
<td>449</td>
<td>7.4</td>
<td>3.88</td>
</tr>
<tr>
<td>Arnold AFB</td>
<td>silt loam</td>
<td>11.4</td>
<td>65.5</td>
<td>23.1</td>
<td>6.66</td>
<td>131</td>
<td>7.8</td>
<td>2.68</td>
</tr>
<tr>
<td>Plymouth</td>
<td>loamy sand</td>
<td>4.4</td>
<td>20.4</td>
<td>75.2</td>
<td>4.23</td>
<td>206</td>
<td>1.7</td>
<td>2.45</td>
</tr>
<tr>
<td>Camp Butner</td>
<td>sandy loam</td>
<td>7.7</td>
<td>25.9</td>
<td>66.4</td>
<td>6.69</td>
<td>219</td>
<td>4.8</td>
<td>2.42</td>
</tr>
<tr>
<td>Limestone Hills</td>
<td>sandy loam</td>
<td>11.2</td>
<td>35.7</td>
<td>53.1</td>
<td>7.54</td>
<td>539</td>
<td>10.5</td>
<td>1.99</td>
</tr>
<tr>
<td>Sassafras</td>
<td>loam</td>
<td>16.4</td>
<td>42.3</td>
<td>41.4</td>
<td>4.40</td>
<td>212</td>
<td>NM&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.30</td>
</tr>
<tr>
<td>Camp Gruber</td>
<td>clay loam</td>
<td>32.3</td>
<td>44.9</td>
<td>22.8</td>
<td>5.39</td>
<td>74</td>
<td>38.3</td>
<td>0.83</td>
</tr>
<tr>
<td>Camp Guernsey</td>
<td>loam</td>
<td>4.1</td>
<td>12.5</td>
<td>83.4</td>
<td>8.21</td>
<td>477</td>
<td>3.9</td>
<td>0.77</td>
</tr>
<tr>
<td>Florence MR</td>
<td>loam</td>
<td>26.8</td>
<td>33.5</td>
<td>39.7</td>
<td>8.00</td>
<td>417</td>
<td>33.0</td>
<td>0.45</td>
</tr>
<tr>
<td>Camp Swift</td>
<td>sandy clay loam</td>
<td>23.7</td>
<td>20.8</td>
<td>55.6</td>
<td>7.83</td>
<td>203</td>
<td>15.1</td>
<td>0.34</td>
</tr>
</tbody>
</table>

<sup>a</sup> In 1:1 soil:water;  
<sup>b</sup> EC = Electrical conductivity;  
<sup>c</sup> SSA = specific surface area;  
<sup>d</sup> OC = organic carbon;  
<sup>e</sup> NM = not measured.
Batch soil adsorption studies

Preliminary experiments were designed to establish the effect of background electrolyte, 0.005M CaCl$_2$ solution, on sorption of NTO in soils and to determine what soil to solution ratio will result in target 10-30% retention of NTO on the soil, a value recommended to decrease the relative error in adsorption experiments (Roy et al., 1992; McDonald and Evangelou, 1997). No effect of background electrolyte at the concentration used in sorption experiments was observed (a possible contributor since NTO is negatively charged under environmental pH range), and 1:4 soil to solution ratio resulted in closest to the target solute removal. All remaining tests were performed in the presence of a 0.005M CaCl$_2$.

Kinetic experiments indicated that majority of soils reached equilibrium with NTO in solution at 24 h, with exception of Catlin and Plymouth soils that reached equilibrium at 8 h and Limestone Hills soil that required 48 h. Control NTO solution (blank) did not indicate decrease in concentration over 120 h kinetic sorption experiments. When soils were present, majority of soils had a faster decrease in solution concentrations over first 24 h followed by a slower decrease over the remaining time. No organic products of NTO transformation were detected.

To test for generic NTO mass loss, we conducted kinetic batch-type experiments in triplicate for 24 and 120 hrs using two soils of similar particle size but different OC content, Catlin (5.28% OC) and Sassafras (1.30% OC). After batch experiments were terminated, extraction of soil with 1:3 ACN:DI water resulted in low recovery of sorbed NTO in all treatments (≤1.3% mass recovered) (Fig. 4).

![Graph showing mass recovered for Sassafras and Catlin](image)

**Figure 4.** Batch-type experiments for Sassafras and Catlin comparing mass (%) associated with solution and soil for sterilized (autoclaved) versus untreated soils. Soil and NTO solution were reacted for 24 and 120 h. NTO mass associated with soil and solution are shown in light grey and dark grey, respectively. Error bars equal one standard error of the mean. Tan colored error bars indicate error for measured NTO mass in solution and black colored bars indicate error for measured NTO mass extracted from soil (n=9 with 3 independent replicate test tubes and 3 repeat HPLC measurements for each sample).
This suggests that NTO was undergoing mass loss. For Catlin and Sassafras, mass loss was significantly greater in untreated soil after interaction for 120 h. It is possible that soil microbes can degrade NTO using soil OC as substrate. Incomplete soil sterilization by autoclave or abiotic transformation may explain why NTO mass loss was still observed; however, there is also the possibility that NTO is irreversibly sorbed to the soil and therefore cannot be recovered by acetonitrile (ACN) extraction. No organic transformation products of NTO were detected by HPLC. Hawari et al., 2011 showed full mass recovery with minimal sorption and no transformation. Studied DRDC-08 and DRDC-09 soils were characterized as sandy with 0.36 and 2% OC, respectively, but may have lacked the conditions necessary for microbial degradation.

Figure 5 presents sample results for the kinetic experiment using Plymouth soil, including plot of concentrations normalized to input over time, and natural logarithm of this value plotted as a function of time. The slope of the linear regression between time and natural logarithm of the concentration is used to determine the rate of the first order transformation reaction ($k$). Estimates ranged between 0.0004 h$^{-1}$ in Camp Guernsey and 0.0221 h$^{-1}$ in Catlin. Rate estimates were highly significant, with 99% confidence interval, and different from zero for all soils, except Limestone Hills, where it was only significant at 95% level.

![Figure 5. Plot of NTO solution concentrations normalized by input over time in the presence of Plymouth soil (a) and the same concentrations plotted as natural logarithm of concentrations in](image-url)
order to determine transformation rate of NTO (b). Error bars equal one standard error of the mean (n=9 with 3 independent replicate test tubes and 3 repeat HPLC measurements for each sample).

**Figure 6.** Correlation between measured NTO transformation rates (ks) and soil OC. P= 0.02250.

Measured transformation rates were lower for soils with less OC, such as Camp Guernsey, Florence MR, and Camp Swift. There was a positive relationship between percent OC present in the soil and measured transformation rate, $k$ (P=0.02); however, $R^2$ was low at 0.46 (Fig. 6). Other soil parameters, such as clay content, pH, and specific surface area (SSA) poorly correlated with $k$. The measured NTO transformation rate in Plymouth soil, 0.0043 h$^{-1}$, was about three times slower than one observed previously for RDX (0.013 h$^{-1}$) (Dontsova et al., 2006).

**Table 4.** Freundlich adsorption parameters, $K_f$ and $n$, and $R^2$ values for the fits.

<table>
<thead>
<tr>
<th>Soil</th>
<th>$K_f$ (cm$^3$ g$^{-1}$)</th>
<th>$n$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catlin</td>
<td>0.21</td>
<td>1.03</td>
<td>0.94**</td>
</tr>
<tr>
<td>Fort Harrison</td>
<td>0.27</td>
<td>1.07</td>
<td>0.98**</td>
</tr>
<tr>
<td>Arnold AFB</td>
<td>0.58</td>
<td>0.86</td>
<td>0.98*</td>
</tr>
<tr>
<td>Plymouth</td>
<td>0.82</td>
<td>0.89</td>
<td>0.99</td>
</tr>
<tr>
<td>Camp Butner</td>
<td>0.74</td>
<td>0.54</td>
<td>0.77</td>
</tr>
<tr>
<td>Limestone Hills</td>
<td>0.33</td>
<td>0.88</td>
<td>0.92**</td>
</tr>
<tr>
<td>Sassafras</td>
<td>0.90</td>
<td>0.86</td>
<td>0.99</td>
</tr>
<tr>
<td>Camp Gruber</td>
<td>0.54</td>
<td>0.99</td>
<td>1.00**</td>
</tr>
<tr>
<td>Camp Guernsey</td>
<td>0.06</td>
<td>0.48</td>
<td>0.18*</td>
</tr>
<tr>
<td>Florence MR</td>
<td>0.09</td>
<td>0.77</td>
<td>0.55*</td>
</tr>
<tr>
<td>Camp Swift</td>
<td>0.10</td>
<td>0.84</td>
<td>0.92*</td>
</tr>
</tbody>
</table>

* P<0.05 (significant);  
** P<0.001 (very highly significant)
Table 5. Fate and transport parameters for NTO in studied soils: linear adsorption coefficient, $K_d$, adsorption coefficient normalized to fraction of organic carbon in soils, $K_{oc}$, transformation rate, $k$, and $R^2$ values for the fits. CI = 95% confidence interval.

<table>
<thead>
<tr>
<th>Soil</th>
<th>$K_d$ cm$^3$ g$^{-1}$</th>
<th>$R^2$</th>
<th>$K_{oc}$ cm$^3$ g$^{-1}$</th>
<th>$k$, h$^{-1}$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper CI</td>
<td>Lower CI</td>
<td>Upper CI</td>
<td>Lower CI</td>
<td>Upper CI</td>
</tr>
<tr>
<td>Catlin</td>
<td>0.21</td>
<td>0.24</td>
<td>0.18</td>
<td>0.92**</td>
<td>3.98</td>
</tr>
<tr>
<td>Fort Harrison</td>
<td>0.35</td>
<td>0.42</td>
<td>0.29</td>
<td>0.95**</td>
<td>9.02</td>
</tr>
<tr>
<td>Arnold AFB</td>
<td>0.34</td>
<td>0.38</td>
<td>0.29</td>
<td>0.94**</td>
<td>12.69</td>
</tr>
<tr>
<td>Plymouth</td>
<td>0.5</td>
<td>0.54</td>
<td>0.41</td>
<td>0.96**</td>
<td>20.41</td>
</tr>
<tr>
<td>Camp Butner</td>
<td>0.12</td>
<td>0.14</td>
<td>0.07</td>
<td>0.72**</td>
<td>4.96</td>
</tr>
<tr>
<td>Limestone Hills</td>
<td>0.21</td>
<td>0.26</td>
<td>0.17</td>
<td>0.92**</td>
<td>10.55</td>
</tr>
<tr>
<td>Sassafras</td>
<td>0.48</td>
<td>0.50</td>
<td>0.43</td>
<td>0.96**</td>
<td>36.92</td>
</tr>
<tr>
<td>Camp Gruber</td>
<td>0.51</td>
<td>0.55</td>
<td>0.47</td>
<td>0.99**</td>
<td>61.45</td>
</tr>
<tr>
<td>Camp Guernsey</td>
<td>0.02</td>
<td>0.04</td>
<td>0.01</td>
<td>0.21*</td>
<td>2.6</td>
</tr>
<tr>
<td>Florence MR</td>
<td>0.06</td>
<td>0.08</td>
<td>0.03</td>
<td>0.57*</td>
<td>13.33</td>
</tr>
<tr>
<td>Camp Swift</td>
<td>0.04</td>
<td>0.06</td>
<td>0.01</td>
<td>0.59**</td>
<td>11.76</td>
</tr>
</tbody>
</table>

* P<0.05 (significant);
** P<0.001 (very highly significant)
Figure 7. Adsorption isotherms for NTO in Plymouth, Sassafras, Camp Gruber, and Camp Swift soils. Grey dashed line indicates linear adsorption isotherm fit to the measured adsorption data (equation is in grey), while solid black line is a fit of Freundlich isotherm with equation presented in black.

NTO adsorbed very weakly to the studied soils, as can be expected for negatively charged compound in a matrix that also possesses a net negative charge. All adsorption coefficient ($K_d$) values were less than 1 cm$^3$ g$^{-1}$, with the largest observed $K_d$ equal to 0.51 cm$^3$ g$^{-1}$ in Camp Gruber soil. For most soils both Freundlich and linear adsorption isotherm described the observed adsorption well, but linear isotherm usually resulted in a slightly better fit (Fig. 7, Tables 4 and 5). The Freundlich parameter $n$ was not similar among the soils, with majority smaller than one, but above one for the two soils with the highest OC content, Catlin, and Fort Harrison. Fits to both linear and Freundlich isotherms were worse for soils that had very little adsorption (Camp Guernsey, Florence MR, and Camp Swift had $K_d$ less than 0.1 cm$^3$ g$^{-1}$, and Camp Butner 0.12 cm$^3$ g$^{-1}$) due to larger percentage error of these measurements.
Soil pH was the strongest indicator of NTO affinity for soil surfaces (Fig. 8). There was a highly significant negative relationship between linear adsorption coefficients and soil pH. The affect of soil pH on NTO sorption suggests that negatively charged NTO interacts with a protonated surface. The pH for all soils was greater than 4 (Supplementary Materials, Table s2). Under normal conditions, a mechanism for sorption of organic anions is negative adsorption (e.g. Hirt and Schmitt, 1958; Bailey and White, 1970). At a soil pH above pKa 3.76, NTO will exist both in the dissociated and nondissociated forms; however, NTO will be present predominantly in the dissociated form as soil pH increases well above its pKa. Highest $K_d$ values were measured when the fraction of NTO in the nondissociated form is said to be highest—though not predominant—over the tested soil pH range. A linear trendline has been ascribed to the measured data in Fig. 8; however, the relationship between $K_d$ and pH is likely sigmoidal, with asymptotic $K_d$ at soil pH greater than 8 and less than 4, as has been shown for other organic contaminants (e.g. Tulp et al., 2009). There was no relationship observed between $K_{ds}$ and OM, clay, or SSA. $K_f$ values had similar if slightly weaker ($R^2 = 0.7818$, P=0.00030) negative linear relationship with pH.

NTO was absorbed about 10 times less than RDX by Catlin soil (high OC, relativity high pH) (0.21 and 2.03 cm$^3$ g$^{-1}$, for NTO and RDX respectively) (Dontsova et al., 2009a), while in Plymouth soil (low pH, less OC), NTO was more strongly adsorbed than RDX ($K_f$ equal to 0.82 and 0.65 cm$^3$ g$^{-1}$, respectively) (Dontsova et al., 2006). Our results agreed with findings in Hawai et al., 2012. We suggest that NTO undergoes linear sorption, with poor soil affinity ($K_d < 0.1$) attributed to polarity of NTO molecule.

Conclusions

A series of kinetic and equilibrium batch soil sorption experiments were conducted to determine fate and transport parameters for NTO, including 1st order transformation rates ($k$) and partition coefficients ($K_d$ and $K_f$), using multiple soils with a range of properties. NTO was weakly adsorbed, with adsorption coefficients lower than measured for RDX (the conventional explosive compound it is replacing). Soil affinity was not influenced by OC in the soil, but was strongly
affected by soil pH. In general, adsorption decreased as soil pH increased. NTO transformation rates correlated positively with OC in the soil. Kinetic experiments for sterilized soils that differed in OC content (Catlin and Sassafras) showed significant differences in unaccounted for mass loss between autoclaved and untreated soils. This indicates that degradation was taking place in untreated soils. No NTO transformation products were detected.
Supplementary Materials

Table s1. Soil to solution ratios tested in NTO soil adsorption studies.

<table>
<thead>
<tr>
<th>Soil:solution ratio</th>
<th>Soil weight</th>
<th>Volume of solution containing solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>g mL⁻¹</td>
<td>g</td>
<td>mL</td>
</tr>
<tr>
<td>1:4</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>1:10</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>1:20</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>1:40</td>
<td>0.5</td>
<td>20</td>
</tr>
</tbody>
</table>

Table s2. Mean aliquot pH and electrical conductivity (EC) from adsorption isotherm experiments (a) aliquot measurements from blank (no soil, only NTO in 0.005M CaCl₂) test tubes. (b) aliquot measurements from test tubes with soil and NTO solution (1:4); mean includes measurements from NTO concentration (mg L⁻¹) range shown in (a) (n=9 with 3 independent replicate test tubes and 3 repeat HPLC measurements for each sample). Standard deviation shown (±).

(a)

<table>
<thead>
<tr>
<th>NTO, mg L⁻¹</th>
<th>pH</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.78</td>
<td>6.35±0.41</td>
<td>1084±1.0</td>
</tr>
<tr>
<td>1.56</td>
<td>6.05±0.26</td>
<td>1099±4.9</td>
</tr>
<tr>
<td>3.13</td>
<td>5.75±0.36</td>
<td>1103±9.8</td>
</tr>
<tr>
<td>6.25</td>
<td>4.64±0.32</td>
<td>1115±3.9</td>
</tr>
<tr>
<td>12.50</td>
<td>4.24±0.12</td>
<td>1127±4.9</td>
</tr>
<tr>
<td>25.00</td>
<td>3.82±0.15</td>
<td>1140±7.9</td>
</tr>
<tr>
<td>50.00</td>
<td>3.58±0.16</td>
<td>1178±9.8</td>
</tr>
<tr>
<td>100.00</td>
<td>3.41±0.00</td>
<td>1223±10.8</td>
</tr>
</tbody>
</table>
(b)

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catlin</td>
<td>7.13±0.02</td>
<td>1159±7.1</td>
</tr>
<tr>
<td>Fort Harrison</td>
<td>6.17±0.08</td>
<td>1276±13.9</td>
</tr>
<tr>
<td>Arnold AFB</td>
<td>6.06±0.37</td>
<td>1183±10.7</td>
</tr>
<tr>
<td>Plymouth</td>
<td>3.90±0.02</td>
<td>1113±6.6</td>
</tr>
<tr>
<td>Camp Butner</td>
<td>6.43±0.12</td>
<td>1096±45.7</td>
</tr>
<tr>
<td>Limestone Hills</td>
<td>7.22±0.09</td>
<td>1336±28.3</td>
</tr>
<tr>
<td>Sassafras</td>
<td>4.06±0.03</td>
<td>1310±17.4</td>
</tr>
<tr>
<td>Camp Gruber</td>
<td>4.92±0.10</td>
<td>1195±14.3</td>
</tr>
<tr>
<td>Camp Guernsey</td>
<td>8.20±0.18</td>
<td>1115±23.3</td>
</tr>
<tr>
<td>Florence MR</td>
<td>7.90±0.25</td>
<td>1220±27.7</td>
</tr>
<tr>
<td>Camp Swift</td>
<td>7.51±0.17</td>
<td>1148±5.1</td>
</tr>
</tbody>
</table>
References Cited


Spear, R.J., Louey, C.N., Wolfson, M.G., 1989. A Preliminary Assessment of 3-Nitro-1,2,4-Triazol-5-One (NTO) as an Insensitive High Explosive. DSTO Materials Research Laboratory, Maribyrnong, Australia, p. 38.


Appendix B

Column Transport Studies of 3-nitro-1,2,4-triazol-5-one (NTO) in Soil

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Manuscript for submission to Chemosphere
Abstract

NTO (3-nitro-1,2,4-triazol-5-one) is one of the new explosive compounds used in insensitive munitions (IM) and developed to replace traditional explosives, TNT and RDX. Data on NTO fate and transport is needed to determine its environmental behavior and potential for groundwater contamination. To measure transport of NTO in soils, we conducted saturated column studies under steady state and interrupted flow conditions and modeled breakthrough curve data using HYDRUS 1-D to better understand mechanisms of sorption and transformation. Six soils used in Mark et al (unpublished) were selected for repacked soil column experiments. All NTO BTCs exhibited no tailing. Based on HYDRUS 1-D modeling, we suggest that NTO transport is best described by adsorption equilibrium and uniform flow (variable flow if flow is interrupted) for transport in soils whose behavior generally matched batch studies. We compared batch and column study determined parameters for the same soils and correlated these values to soil properties. Batch and column (HYDRUS 1-D derived) NTO linear adsorption coefficients ($K_d$) correlated well ($P = 0.000576$, or very highly significant); there was a trend towards higher $K_d$ estimates in column experiments. For the majority of soils, 24 hour flow interruption at steady-state resulted in a decrease in NTO outflow concentration, indicating rate-limited degradation. Autoclaved and untreated (non-sterilized) soils with different organic carbon content showed differences in mass balance ratio. NTO mass balance was lowest for an untreated soil with more organic carbon.

Introduction

The U.S. military has been testing new explosive compounds to replace traditional explosives like RDX (1,3,5-trinitro-1,3,5-triazine) and TNT (2,4,6-trinitrotoluene) with more stable formulations called insensitive munitions (IMs). IMs retain the performance of traditional explosives but are better able to tolerate unplanned stimuli, such as mechanical shock and high temperatures (Beyard, 2007). NTO (3-nitro-1,2,4-triazol-5-one) is one of the primary components in a series of new IM formulations (including IMX-101, 102, 104, PAX-21, 41 and 48) that are being, or soon will be fielded. As a result of incomplete detonation, solid IM explosives containing NTO may remain on range soils, and after dissolution (Taylor et al., 2013) can potentially reach groundwater. Complex interaction of the dissolved IM component with soil constituents is key to understanding the threat of groundwater contamination. NTO transport to potable groundwater sources could result in exposure to a chemical that is said to be a male reproductive toxicant in rats (Wallace, 2011). Also, NTO can form toxic transformation products (Le Campion et al., 1999b). Metabolic intermediates of nitro-compounds have been linked to local damage in the liver (McCoy et al., 1981). Based on these risks, accurate soil partition coefficients ($K_{d,D}$) and degradation rates ($k$) are necessary to model NTO fate and transport to groundwater.

NTO has a relatively high aqueous solubility ranging from 16642.0 mg/L at 25 °C (Spear et al., 1989). Unlike most other explosives, NTO is polar and ionic. It function as an acid with pKa of 3.7-3.76, therefore, we would expect NTO to be negatively charged at environmentally relevant pHs (Le Campion et al., 1997; Smith and Cliff, 1999). Octanol-water partition coefficient ($K_{ow}$) indicates the tendency of a chemical to bioaccumulate and approximates its affinity for soil organic matter (OM). Low reported $K_{ow}$s (log $K_{ow}$ of 0.02-1.03, estimated) suggest that NTO would not strongly adsorb to soil OM (Brannon and Pennington, 2002; Hawari et al., 2011; Hawari et al., 2012); however, specific sorption to soils, as has been shown for TNT and DNT, is possible (Thorn...
et al., 2001; Thorn et al., 2008). Given the $K_{ow}$, NTO would likely be more mobile than other components in IM mixtures, such as DNAN, and may exhibit similar environmental behaviors to RDX. Gas phase transport will not be important for the total mass flux of NTO in soils due to relatively low volatility, as measured by Henry’s constant (log $H$ of -11.38 for NTO) (Sokkalingam et al., 2008). Microbial degradation studies have shown that NTO is capable of undergoing nitroreduction, followed by ring cleavage of the primary amine to form 5-amino-1,2,4-triazol-3-one (ATO) (Le Campion et al., 1999a). At optimal conditions, complete nitroreduction of NTO occurred in 24 hours. A two week incubation period was necessary to fully degrade ATO, resulting in the following end products, carbon dioxide (40%), urea and a polar compound, assumed to be hydroxyurea.

In Mark et al. (unpublished), a series of kinetic and equilibrium batch soil sorption experiments were conducted to determine 1st order transformation rates ($k$) and partition coefficients ($K_d$ and $K_f$) for NTO using multiple soils with a range of properties. NTO was weakly adsorbed, with linear soil adsorption coefficients, $K_d$, ranging from 0.51 cm$^3$ g$^{-1}$ to 0.02 cm$^3$ g$^{-1}$. Soil affinity was not influenced by OC in the soil, but was strongly affected by soil pH. Adsorption decreased as soil pH increased, probably due to protonation of soil surfaces. Estimates for NTO 1st order transformation rate constants ($k$) ranged between 0.0004 h$^{-1}$ in Camp Guernsey soil and 0.0221 h$^{-1}$ in Catlin soil. There was a significant positive relationship between OC in soils and measured transformation rate. Kinetic experiments were repeated for soils of similar particle size but different organic carbon (OC) content (Sassafras and Catlin with 1.30 and 5.28% OC, respectively). Mass loss was significantly greater for untreated soils and there was much greater loss for a soil with higher organic carbon; this suggests microbial degradation, possibly with organic carbon as the energy source. NTO extracted from soil grains by acetonitrile was insignificant and no organic transformation products were detected by high performance liquid chromatography (HPLC) with diode array.

To further elucidate processes involved in the reactive transport of NTO in soils, we performed saturated repacked soil column experiments under steady state and transient flow conditions. A subset of eight soils used in Mark et al. (unpublished) were selected. NTO 1st order transformation rate constants ($k$) and partition coefficients ($K_d$) were determined using temporal moment analysis (TMA) and HYDRUS 1-D solute transport modeling software. To assess the impact of experimental approach, transport parameters ($k$ and $K_d$) were determined and compared to the results of the batch studies (Mark et al., unpublished).

**Materials and Methods**

**Soils**

Uncontaminated soils having a wide range of properties were collected on military installations across the United States. In addition, we included soils that were previously used in studies of explosive and propellant formulation constituents (Dontsova et al., 2006; Dontsova et al., 2007; Dontsova et al., 2009a; Dontsova et al., 2009b; Taylor et al., 2012). Using the same soils allowed comparison of current results for NTO to those of prior research for traditional energetic. All used materials were surface soils (top 12 inches or 30 cm).

Collected soils were air dried, sieved (< 2 mm), and characterized prior to the experiments. Pretreatment of soil with 5% phosphoric acid ($\text{H}_3\text{PO}_4$) ensured complete removal of inorganic carbon. After pretreatment, Shimadzu SSM-5000A Solid Sample Combustion Unit was used to
analyze organic carbon (OC) following EPA Standard Operating Procedure for sediments (U.S. Environmental Protection Agency, 2005). Particle size was analyzed by Beckman Coulter LS 13 320 Laser Diffraction Particle Size Analyzer (Beckman Coulter, Inc., Fullerton, CA). Specific surface area (SSA) by N₂ adsorption was measured using Beckman Coulter SA 3100 and calculated by applying Brunauer-Emmett-Teller (BET) theory (Brunauer et al., 1938). A VWR SympHony SB70P Benchtop Digital pH and EC Meter was used to measure pH and electrical conductivity from a 1:1 soil to solution mixture.

The physical and chemical properties for the 8 soils selected for this study are summarized in Table 1. All studied soils had mixed particle size, ranging from fine (clay loam) to coarse (loamy sand).

**Table 1.** Measured physical and chemical properties of soils used in adsorption and transport studies with NTO.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Texture</th>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
<th>pH⁰</th>
<th>ECᵇ</th>
<th>SSAᶜ</th>
<th>OCᵈ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catlin</td>
<td>silt loam</td>
<td>25.6</td>
<td>65.5</td>
<td>8.9</td>
<td>7.31</td>
<td>492</td>
<td>6.4</td>
<td>5.28</td>
</tr>
<tr>
<td>Arnold AFB</td>
<td>silt loam</td>
<td>11.4</td>
<td>65.5</td>
<td>23.1</td>
<td>6.66</td>
<td>131</td>
<td>7.8</td>
<td>2.68</td>
</tr>
<tr>
<td>Camp Butner</td>
<td>sandy loam</td>
<td>7.7</td>
<td>25.9</td>
<td>66.4</td>
<td>6.69</td>
<td>219</td>
<td>4.8</td>
<td>2.42</td>
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<td>Limestone Hills</td>
<td>sandy loam</td>
<td>11.2</td>
<td>35.7</td>
<td>53.1</td>
<td>7.54</td>
<td>539</td>
<td>10.5</td>
<td>1.99</td>
</tr>
<tr>
<td>Sassafras</td>
<td>loam</td>
<td>16.4</td>
<td>42.3</td>
<td>41.4</td>
<td>4.40</td>
<td>212</td>
<td>NMᵉ</td>
<td>1.30</td>
</tr>
<tr>
<td>Camp Guernsey</td>
<td>loam</td>
<td>4.1</td>
<td>12.5</td>
<td>83.4</td>
<td>8.21</td>
<td>477</td>
<td>3.9</td>
<td>0.77</td>
</tr>
<tr>
<td>Florence MR</td>
<td>loam</td>
<td>26.8</td>
<td>33.5</td>
<td>39.7</td>
<td>8.00</td>
<td>417</td>
<td>33.0</td>
<td>0.45</td>
</tr>
<tr>
<td>Camp Swift</td>
<td>sandy clay loam</td>
<td>23.7</td>
<td>20.8</td>
<td>55.6</td>
<td>7.83</td>
<td>203</td>
<td>15.1</td>
<td>0.34</td>
</tr>
</tbody>
</table>

⁰ In 1:1 soil:water;
ᵇ EC=Electrical conductivity;
ᶜ SSA – specific surface area;
ᵈ OC = organic carbon;
ᵉ NM – not measured.

**NTO**

Technical grade NTO used in the experiments was obtained from US Army Armament Research, Development and Engineering Center (ARDEC), Picatinny Arsenal. Calibration standards for NTO were prepared using the same NTO.
Analytical methods

NTO concentration was quantified using Dionex Ultimate 3000 high performance liquid chromatograph (HPLC) equipped with a diode array detector (ThermoFisher, MA) as described in Mark et al. (unpublished). Running method was adapted from Le Campion et al. (1999a). Mobile phase, acetonitrile (ACN): deionized water (75:25) with 0.1 % TFA, was run isocractically at 1 mL min\(^{-1}\). Oven temperature was set at 32°C. NTO and its transformation products were separated using a Thermo Scientific Hypercarb Column. NTO was detected at approximately 2.5 mins using 315 nm wavelength. UV detector was set at 220 nm to monitor for presence of ATO (Le Campion et al., 1999a). No ATO was detected in any analyzed samples.

Saturated Flow Experiments

To measure the transport of NTO in soils, we conducted saturated miscible displacement experiments under steady-state and transient conditions. Solutions of NTO were used as the source. Some of the soils used in the batch studies (Mark et al., unpublished) were selected for column transport experiments. Concentrations of NTO (presence of ATO was monitored but not observed), as well as conservative tracer (bromide, Br\(^-\)) used to characterize water flow through the columns were determined in the column effluent and used to construct breakthrough curves.

Saturated flow column experiments were conducted using Supelco (Bellefonte, PA) glass tubes (7 cm length with 1.18 cm internal diameter) with PTFE caps. Small columns minimize the amount of source material needed and also reduce hazardous waste produced in the study. 11-12 g of soil was packed homogenously over a bottom layer of silanized glass wool that prevented migration of particles. The mass of air-dried soil used to pack a column of known volume was determined for bulk density. Properties are summarized in Table 1. Average packed bulk density (\(\rho\)) ranged from 1.71±0.06 g cm\(^{-3}\) (Camp Guernsey soil) to 1.31±0.02 g cm\(^{-3}\) (Arnold AFB soil). Saturated flow parameters water content (\(\theta\)) and packed bulk density (\(\rho\)) for all studied soils are shown in Table 3.

A layer of glass wool overlaid the packed soil and acted to evenly distribute incoming flow. To avoid air entrapment, columns were saturated from the bottom up with a 0.005M CaCl\(_2\) solution for approximately 1 to 1.5 h. After saturation, pore volume (PV) was determined as the volume of solution required to saturate the packed column. Tygon microbore tubing connected to a Cole-Parmer (Vernon Hills, IL) Master flex peristaltic pump was attached to the top of each column to supply 1 mg L\(^{-1}\) NTO solution with 0.005M CaBr\(_2\) tracer at a given flux. A 0.02 mL min\(^{-1}\) flow rate or 1.1 cm h\(^{-1}\) flux was used (measured average flow rate was 0.0197±0.0019 mL min\(^{-1}\), or solution flux of 1.083±0.0017 cm h\(^{-1}\)). Outflow (effluent) was collected continuously into 4 mL amber vials using Teledyne ISCO (Lincoln, NE) Foxy 200 Fraction collector with a 200 vial capacity. Pump flow was calibrated for constant, target flow prior to the start of the experiment. Volumetric flow rate was monitored based on collected outflow. A maximum of ten column experiments could be conducted simultaneously. After about 6 to 16 PV, depending on the soil, inflow was switched to 0.005M CaCl\(_2\) solution and the flow continued for another 6-16 PV to evaluate NTO elution from the soil. A conservative tracer (bromide, Br\(^-\)) was analyzed using Ion Chromatography (Dionex ICS 5000 with diode array) to distinguish between non-equilibrium processes attributed to physical versus chemical properties and to determine dispersion and diffusion in the columns. After termination, soil in the columns was subdivided into thirds: top, middle, bottom, and weighed. Soil extractions were performed on each third using acetonitrile (1:4 soil to acetonitrile ratio was used with samples agitated for 24 h before centrifugation and filtering)
to determine how much NTO remained in the soil (U.S. Environmental Protection Agency, 2007). A minimum of 3 mL of supernatant was filtered through 0.45 µm Millex-HV PVDF filter (EMD Millipore Darmstadt, Germany), and placed in labeled 4-mL amber vials.

As the same soils were used in batch adsorption and transformation studies, we were able to compare parameters determined in batch and column experiments allowing verification of observed processes responsible for NTO attenuation. In addition, these soils have been used in previous explosive and propellants studies (Dontsova et al., 2006; Dontsova et al., 2007; Dontsova et al., 2008; Dontsova et al., 2009a; Dontsova et al., 2009b; Taylor et al., 2012), so we would be able to compare the transport of IM compounds to that of other energetics.

Flow-interruption tests, wherein flow was stopped for 24 h, were performed for some experiments to quantify mass-transfer processes, such as diffusion and sorption (Brusseau et al., 1989; Brusseau et al., 1997). When flow is stopped the system is allowed to equilibrate. This is reflected in decreases in concentration if degradation or kinetic sorption is taking place, or in increases in concentration if diffusion is limiting desorption of the target analyte. Transient flow experiments were repeated for Catlin and Sassafras soils to determine whether solute concentration loss resulted from microbial degradation. Catlin and Sassafras differ in organic carbon content, 5.28 and 1.30%, respectively. Soils were sterilized by autoclaving three times over three days with one hour exposure to 120°C each time.

**Temporal Moment Analysis**

Temporal moment analysis (TMA) is a non-parametric, statistical tool for quantifying solute transport properties such as dispersivity, mass balance ratio and retardation factor. It is unable to predict solute concentrations based on physical processes; however, it does not rely on an underlying transport model (e.g. chemical non-equilibrium model). For a continuous function \( f(t) \), the moment is generated by,

\[
M_p = \int t^p f(t) \, dt 
\]  

[Eq. 1]

where the subscript \( p = 0, 1, 2 \ldots \) represents the zeroth, first and second order absolute moments. Solute transport studies use the dimensionless form to represent breakthrough curves:

\[
f(t) = \frac{C(z,t)}{C_0} \]  

[Eq. 2]

*Where* \( C_0 \) *is the initial solution concentration at time* \( t = 0 \). The zeroth order moment, \( M_0(T) \), represents the dimensionless mass of the solute,

\[
M_0 = \frac{C(z,t)}{C_0} \, dT 
\]  

[Eq. 3]

The mass balance ratio, \( r \), is given by

\[
r = \frac{M_0}{M_0^{in}} 
\]  

[Eq. 4]

Where \( M_0^{in} \) *denotes the mass of the input pulse*. The mass ratio describes the percentage recovery of the solute at depth, \( L \); therefore, the difference \((1-r)\) represents the percentage lost to sorption, degradation and other processes.
The normalized moment, $\mu_p^n$, is defined as

$$\mu_p^n = M_p/M_0$$  \hspace{1cm} [Eq. 5]

The first normalized moment represents the mean concentration breakthrough, $\tau (T)$:

$$\tau = \mu_1^n M_1/M_0$$  \hspace{1cm} [Eq. 6]

Central moments, $\mu_p$, are defined as

$$\mu_p = 1/M_0 \int (T- \mu_1^n)^p C (z, t)/ C_0 \, dT$$  \hspace{1cm} [Eq. 7]

$p = 0, 1, 2…$

The second central moment $\mu_2 (T^2)$, quantifies the typical spread of the BTC in relation to the mean breakthrough time, referred to as variance. The longitudinal dispersivity, $\lambda (L)$, is given as:

$$\lambda = (L/2)(\mu_2/ \tau_2)$$  \hspace{1cm} [Eq. 8]

The retardation factor, $R$, (which is equal to $1+ (\rho/\theta) K_d$, where $\rho$ is the soil bulk density, $K_d$ is the linear partition coefficient, and $\theta$ is the water content) is defined as:

$$R = M_1/M_0 - (0.5*M_0)$$  \hspace{1cm} [Eq. 9]

The first-order transformation rate, $k (T^{-1})$ can be obtained by:

$$k = (1/t_2 - t_1) * \ln (M_0^1/M_0^2)$$  \hspace{1cm} [Eq. 10]

where $t_1$ and $t_2$ indicate two different time levels at relatively steady state effluent concentration, while $M_0^1$ and $M_0^2$ indicate the corresponding zeroth moments.

The temporal moments and associated parameter were calculated using the trapezoidal or Simpson's formulae of numerical integration of concentration distribution at selected times. To obtain mean temporal moments, first each individual realization was calculated then the mean for all realizations was determined.

**Numerical Modeling Using HYDRUS 1-D**

HYDRUS-1D model (Šimůnek et al., 2008a; Šimůnek and van Genuchten, 2008) was used in the inverse mode to analyze breakthrough curves obtained in column experiments. Water flow through all studied soils was constant

Solute transport was modeled using the standard advection-dispersion equation:

$$\delta \theta c/\delta t + \rho(\delta s/\delta t) = \delta /\delta z [\theta D (\delta c/\delta z)] - qc/\delta z - \phi$$  \hspace{1cm} [Eq. 11]
where $c$ is the solution concentration $[\text{ML}^{-3}]$, $s$ is the sorbed concentration $[\text{MM}^{-1}]$, $D$ is the dispersion coefficient which includes both molecular diffusion and hydrodynamic dispersion $[\text{LT}^{-1}]$, $q$ is the volumetric fluid flux density $[\text{LT}^{-1}]$ evaluated using the Darcy-Buckingham law, and $\phi$ is a sink-source term which includes various zero- and first-order reactions $[\text{ML}^{-3}\text{T}^{-1}]$. In our model, $\phi$ indicates first-order solution reaction only (referred to as sink liquid term, SNKL1). Constant pressure head was used to model continuous flow experiments, while variable pressure head was used to model flow interruption tests.

First, the HYDRUS-1D model was used to analyze breakthrough curves of a nonreactive tracer to determine physical parameters characterizing the column experiments, namely longitudinal dispersivity, $\lambda$. Then with physical parameters fixed, it was used to analyze breakthrough curves of reactive compounds to determine chemical/reaction parameters involved. The reaction parameters determined using the inverse mode of HYDRUS-1D were compared to the same parameters independently determined in soil batch studies. Agreement between the two sets of values will provide an indication of how well the determined relationships characterize the transport of NTO in soils.

**Parameter Estimation**

Temporal moment analysis (TMA) was used to confirm behavior of the conservative tracer (bromide). HYDRUS-1D estimates for tracer longitudinal dispersivity, $\lambda$ (cm), used TMA calculated values of $\lambda$ as initial inputs. TMA was performed on NTO breakthrough curve (BTC) data to provide initial estimates for solute transport parameters, including longitudinal dispersivity, $\lambda$ (cm), linear adsorption coefficient, $K_d$ $(\text{g}^{-1}\text{cm}^3)$, and 1st order rate coefficient for dissolved phase (degradation rate), $\mu_w$ (h$^{-1}$). Parameters calculated by TMA for NTO were inputted into the chemical equilibrium model in HYDRUS 1-D. Longitudinal dispersivity ($\lambda$) for the tracer (estimated by HYDRUS 1-D) better served as initial estimates for predicting NTO dispersivity values, therefore, tracer dispersivity values estimated in HYDRUS 1-D were fixed for NTO. Overall, bromide breakthrough curves provided an estimate of the total movement of solution and HPLC measurements gave an estimate of the NTO transport. Mass balance calculations were performed for NTO by mass balance ratio, $r$. NTO mass balance estimates were compared to recovery of the conservative tracer, bromide (Table 2).

In Tables 3 and 4, $R^2$ values for fitted parameters show the correspondence between measured and fitted breakthrough concentrations and confidence intervals show the behavior of the objective function around its minimum. Parameter estimates were considered significant if confidence intervals did not intersect with zero. Insignificant parameter estimates were not used to draw conclusions.

Interrupted and continuous flow experiments were treated as replicates for the purposes of statistical analysis. Differences between treatments were considered significant if confidence intervals did not overlap at 95% probability.

**Results and Discussion**

**Conservative Tracer**

In general, longitudinal dispersivity was larger for coarser soils such as Camp Butner and Camp Guernsey ($0.1\pm0.02$ and $0.13\pm0.05$ cm, respectively) than for the finer Catlin and Camp
Swift soils (0.03±0.008 and 0.07±0.04 cm, respectively) (Tables 1 and 3), but relatively small as expected for short repacked columns. Breakthrough of the conservative tracer occurred at one pore volume (~140 min), which indicates a lack of preferential flow. Air entrainment within the column was not observed. Tracer breakthrough curves were plotted on a pore volume basis to compare behavior to NTO. After flow interruption, effluent concentrations of bromide resumed at similar values, indicating limited or no physical non-equilibrium in the column. Mass balance calculations showed full recovery of the tracer (Table 2).

**Table 2.** Mass recovered (%) in outflow of column transport experiments involving NTO and conservative tracer, bromide. FI = flow interruption for 24 h.

<table>
<thead>
<tr>
<th>Soil</th>
<th>NTO</th>
<th>Bromide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnold AFB</td>
<td>54</td>
<td>106</td>
</tr>
<tr>
<td>Arnold AFB FI</td>
<td>51</td>
<td>97</td>
</tr>
<tr>
<td>Butner</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Butner FI</td>
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<td>102</td>
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<tr>
<td>Catlin</td>
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<td>110</td>
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<td>116</td>
</tr>
<tr>
<td>Florence MR FI</td>
<td>95</td>
<td>102</td>
</tr>
<tr>
<td>Camp Guernsey</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>Camp Guernsey FI</td>
<td>93</td>
<td>107</td>
</tr>
<tr>
<td>Limestone Hills</td>
<td>73</td>
<td>99</td>
</tr>
<tr>
<td>Limestone Hills FI</td>
<td>61</td>
<td>99</td>
</tr>
<tr>
<td>Sassafras</td>
<td>89</td>
<td>108</td>
</tr>
<tr>
<td>Sassafras FI</td>
<td>82</td>
<td>99</td>
</tr>
<tr>
<td>Camp Swift</td>
<td>93</td>
<td>105</td>
</tr>
<tr>
<td>Camp Swift FI</td>
<td>99</td>
<td>102</td>
</tr>
</tbody>
</table>

**NTO**

Experiments were conducted in duplicate. Mass balance calculations for NTO were relatively consistent between treatments (Table 2). No NTO or transformation products were recovered from soil by acetonitrile extraction following termination of column experiments. Figures 1 and 2 present breakthrough curves for Camp Swift and Camp Guernsey soils. Majority of the soils, Camp Swift, Camp Guernsey, Sassafras, as well as Camp Butner and Florence MR, exhibited expected behavior based on the batch studies. For example, Camp Swift, steady-state concentrations similar to inflow concentrations observed in breakthrough curves (BTC) (Figure 1) were consistent with low $K_d$ and $k$ measured in batch studies, while Sassafras BTCs (Fig. 3) were consistent with higher $K_d$ and $k$ measured in batch studies.
Figure 1. Breakthrough curves for NTO in Camp Swift soil. Dashed grey vertical line indicates timing of 24-hour flow interruption. Tan vertical line indicates time, when solution was changed back to saturating solution to observe desorption phase of the isotherm.
Autoclaved and untreated soils showed a significant difference in mass loss for soil with higher OC content, suggesting biodegradation (Fig. 3 and 4). Mass balance ratio showed 75.4% NTO recovery in the column effluent for autoclaved Catlin soil and 33.2% NTO mass recovery in outflow of untreated Catlin soil. 95% NTO was recovered from autoclaved Sassafras soil and 88.6% was recovered from untreated Sassafras soil. In Fig. 3, flow interruption had no effect at steady-state NTO concentration for autoclaved Sassafras soil. This indicates that the decrease in concentration following flow interruption is due to rate-limited degradation. No transformation products were observed, and no mass was recovered by acetonitrile extraction.

**Figure 2.** Breakthrough curves for NTO in Camp Guernsey soil. Dashed grey vertical line indicates timing of 24-hour flow interruption. Tan vertical line indicates time, when solution was changed back to saturating solution to observe desorption phase of the isotherm.
Figure 3. Breakthrough curves for NTO in untreated and autoclaved Sassafras soil. Dashed grey vertical line indicates timing of 24-hour flow interruption. Tan vertical line indicates time, when solution was changed back to saturating solution to observe desorption phase of the isotherm.
Figure 4. Breakthrough curves for NTO in untreated and autoclaved Catlin soil. Dashed grey vertical line indicates timing of 24-hour flow interruption. Tan vertical line indicates time, when solution was changed back to saturating solution to observe desorption phase of the isotherm.

Some soils, Limestone Hills, Camp Butner, Catlin (Fig. 4) and Arnold AFB (Fig. 5) had breakthrough curves that indicated an increase in the transformation rate as a function of time and low recovery after interrupted flow. Both trends indicate that microbial growth in the column maybe occurring with time. A change in mechanisms of transformation, i.e. increasing anaerobic conditions with time, is not likely happening since the reservoir was periodically refilled while solution was being supplied to the columns.
**Figure 5.** Breakthrough curves for NTO in Arnold AFB soil. Dashed grey vertical line indicates timing of 24-hour flow interruption. Tan vertical line indicates time, when solution was changed back to saturating solution to observe desorption phase of the isotherm.
Solid triangles represent measured values for NTO concentration for experiments with and without flow interruption (Fig. 1 to 5). High mobility was supported by mass balance (Table 2). NTO values for longitudinal dispersivity ($\lambda$) were relatively similar to bromide. NTO breakthrough was observed after breakthrough of the conservative tracer, suggesting adsorption to the solid phase. A decrease in effluent NTO concentration after 24 h flow interruption then return to steady state was shown for Camp Swift and Camp Guernsey soils (Fig. 1 and 2), as well as Sassafras and Florence soils, therefore, sorption and/or degradation processes are said to be rate-limited. Good fit for majority of breakthrough curves was achieved using equilibrium adsorption and transformation model suggesting that kinetic sorption is likely negligible. The $k$ values determined from HYDRUS-1D matched well with TMA derived values and fell within 95% confidence intervals, with the exception of Limestone Hills and Camp Swift (Table 4). Also, $K_d$ values determined from HYDRUS-1D matched well with TMA derived values and fell within 95% confidence intervals, with the exception of Florence MR and Camp Guernsey (Table 4). Irreversible attenuation and transformation as indicated by $k$ was statistically significant in TMA and when modeled by HYDRUS-1D; however, the lower CI for Camp Swift fell below zero for TMA derived $k$ and $K_d$ values and is therefore considered insignificant. Catlin and Arnold AFB soils have higher OC content and showed non-first order, irreversible attenuation as indicated by lower NTO recovery in outflow and change in transformation rate with time.

Overall, results show that advection-dispersion equation predicted the solute parameters reasonably well. Values for NTO and bromide dispersivity seem to be an order of magnitude too large compared with the TMA, though both TMA and HYDRUS-1D derived parameters suggest the same correlation: that NTO dispersivity is relatively similar to the conservative tracer, bromide.
Table 3. Solute transport parameters obtained using HYDRUS 1-D from saturated flow experiments involving NTO and conservative tracer, Bromide. CI = 95% confidence interval. Bulk density (ρ) was determined for mass of air-dried soil used to pack a column of known volume. Saturated water content (θ) was determined from the weight of the water needed to saturate the column. Standard deviation shown (±). NA indicates that results are not available.

<table>
<thead>
<tr>
<th>Soil</th>
<th>ρ</th>
<th>θ</th>
<th>λ, cm</th>
<th>R²</th>
<th>K_d, cm³/g⁻¹</th>
<th>λ, cm</th>
<th>k, h⁻¹</th>
<th>R²</th>
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<td>Arnold AFB</td>
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<td>0.117</td>
<td>0.044</td>
<td>0.011</td>
<td>0.98</td>
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<td>NA</td>
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<td></td>
<td></td>
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<td>0.442</td>
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<td>0.86</td>
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<td>NA</td>
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<td>0.100</td>
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<td>0.97</td>
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<td>0.152</td>
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<td>0.068</td>
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<td>0.21</td>
</tr>
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<td></td>
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<tr>
<td>Sassafras</td>
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<td>0.45</td>
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<tr>
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<td>0.02</td>
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<td></td>
<td></td>
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<td>0.052</td>
<td>0.042</td>
<td>0.98</td>
<td>0.05</td>
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</tr>
</tbody>
</table>
Table 4. Comparison of fate and transport parameters, linear adsorption coefficients, $K_d$ and 1st order transformation rates, $k$, for NTO determined from temporal moment analysis (TMA) and HYDRUS 1-D. CI = 95% confidence interval. TMA results include data from both interrupted flow (FI) and continuous flow experiments. HYDRUS 1-D results show interrupted flow (FI) and continuous flow experiments separately. NA indicates that results are not available.

<table>
<thead>
<tr>
<th>Soil</th>
<th>TMA</th>
<th>HYDRUS 1-D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$, cm$^3$g$^{-1}$</td>
<td>$k$, h$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>Estimate</td>
<td>Upper CI</td>
</tr>
<tr>
<td>Arnold AFB</td>
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<tr>
<td>Arnold AFB FI</td>
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<td>NA</td>
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<tr>
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<td>Camp Butner FI</td>
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</tr>
<tr>
<td>Catlin</td>
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<td>0.56</td>
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<tr>
<td>Catlin FI</td>
<td>NA</td>
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</tr>
<tr>
<td>Florence MR</td>
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<td>0.28</td>
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<tr>
<td>Florence MR FI</td>
<td>0.04</td>
<td>0.083</td>
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<tr>
<td>Camp Guernsey</td>
<td>0.17</td>
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<td>Camp Guernsey FI</td>
<td>0.06</td>
<td>0.081</td>
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<tr>
<td>Limestone Hills FI</td>
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<tr>
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<td>0.06</td>
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</table>

NOTE: For Limestone Hills and Camp Butner, non-first order transformation occurred following 24 hour flow interruption but did not occur under continuous flow conditions. In all cases, TMA estimates of $k$ were determined for the portion of the BTC where steady-state influent concentration was achieved.
All NTO BTCs exhibited no tailing, and through modeling and analysis of conservative tracer indicated that immobile water was not likely present, and therefore, our medium in all cases is said to be homogenous and unstructured. Based on HYDRUS 1-D modeling, we suggest that NTO transport is best described by sorption equilibrium and uniform flow (variable flow if flow is interrupted) for transport in soils whose behavior generally matched batch studies.

Figure 6 and 7 show a comparison of linear adsorption coefficients, \( K_d \), for NTO determined from batch and column experiments. There was a general agreement between batch and column determined fate and transport parameters. Correlation between linear adsorption coefficients \( (K_d) \) derived from temporal moment analysis of BTC data and measured in batch experiments was highly significant \( (P = 0.00185) \) (Fig. 6). In Figure 7, correlation between batch and HYDRUS 1-D derived column NTO linear adsorption coefficients \( (K_d) \) was very highly significant \( (P = 0.000576) \). Both Figures 6 and 7 show that slope of the regression was higher than 1 indicating that there was a trend towards higher \( K_d \) estimates in column experiments. Confidence interval for HYDRUS 1-D derived and measured batch \( K_d \)s overlapped for only four of six soils: Limestone Hills, Florence MR, Camp Guernsey and Camp Swift, indicating that estimates were similar at 95%. The presence of immobile domains was tested using a conservative tracer; however, since column studies have the disadvantage of comingling physical and chemical processes, it is possible that diffusion-governed release of NTO in undetected immobile domains led to greater retardation and consequently higher \( K_d \) values, but this explanation is unsubstantiated. 1\textsuperscript{st} order transformation rate constant, \( k \), estimates using batch and column experiments were significantly different from each other for each of eight soils that were used in both batch and column experiments. A slope of the correlation between \( k \) estimates using batch and column experiments was significantly different from one.

\[ y = 1.99x \]
\[ R^2 = 0.79 \]

**Figure 6.** Correlation between column (derived from temporal moment analysis) and batch NTO linear adsorption coefficients \( (K_d) \). \( P = 0.00185 \) for \( K_d \), highly significant. Includes values from both continuous and flow interrupted experiments.
Figure 7. Correlation between continuous flow column (HYDRUS-1D derived) and batch linear adsorption coefficients ($K_d$). $P = 0.000576$ for $K_d$, very highly significant relationship. Includes values from continuous flow experiments only.

**Conclusion**

We conducted a series of saturated flow experiments with and without flow interruption (for 24h equilibration period) for subset of eight soils previously used to determine 1st order transformation rates ($k$) and partition coefficients ($K_d$) (Mark et al., unpublished). Column studies generally supported batch-determined parameters, but indicated that increase in transformation rates may occur if the soil becomes slightly anaerobic. As was observed in batch-type experiments, saturated flow studies showed that NTO transformation rates correlated positively with OC in the soil. Soils with higher OC content showed non-first order transformation. For some soils, flow interruption tests showed a decrease in effluent NTO concentration, therefore, degradation processes are said to be rate-limited.
**Supplementary Materials**

*Table s1.* Retardation factor (R) calculated using Eq. 9 in “Temporal Moment Analysis” section under Materials and Methods. Interrupted and continuous flow experiments were treated as replicates for the purposes of statistical analysis. CI = 95% confidence interval.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Estimate</th>
<th>Upper CI</th>
<th>Lower CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnold AFB</td>
<td>3.17</td>
<td>4.45</td>
<td>1.90</td>
</tr>
<tr>
<td>Butner</td>
<td>2.43</td>
<td>3.04</td>
<td>1.81</td>
</tr>
<tr>
<td>Catlin</td>
<td>2.09</td>
<td>2.30</td>
<td>1.87</td>
</tr>
<tr>
<td>Florence MR</td>
<td>1.93</td>
<td>2.35</td>
<td>1.52</td>
</tr>
<tr>
<td>Camp Guernsey</td>
<td>1.79</td>
<td>1.86</td>
<td>1.71</td>
</tr>
<tr>
<td>Limestone Hills</td>
<td>1.59</td>
<td>1.85</td>
<td>1.33</td>
</tr>
<tr>
<td>Sassafras</td>
<td>3.78</td>
<td>5.18</td>
<td>2.38</td>
</tr>
<tr>
<td>Camp Swift</td>
<td>1.18</td>
<td>1.40</td>
<td>0.96</td>
</tr>
</tbody>
</table>
References Cited


Spear, R.J., Louey, C.N., Wolfson, M.G., 1989. A Preliminary Assessment of 3-Nitro-1,2,4-Triazol-5-One (NTO) as an Insensitive High Explosive. DSTO Materials Research Laboratory, Maribyrnong, Australia, p. 38.


Appendix C: Additional Figures and Tables

**Figures 1-10.** Plot of mean NTO solution concentrations normalized by input over time in the presence of Fort Harrison, Catlin, Camp Gruber, Camp Guernsey, Camp Swift, Limestone Hills, Sassafras, Arnold AFB, and Florence MR soil (a) and the same concentrations plotted as natural logarithm of concentrations in order to determine transformation rate of NTO (b). (n=9 with 3 independent replicate test tubes and 3 repeat HPLC measurements for each sample).
\[ y = -0.0221x + 0.0751 \]
\[ R^2 = 0.8458 \]
Camp Gruber

\[ y = -0.0025x - 0.0842 \]
\[ R^2 = 0.9837 \]
Camp Guernsey

\[ y = -0.0004x - 0.0082 \]

\[ R^2 = 0.7559 \]

Camp Guernsey
Blank
The graph shows the relationship between time (in hours) and NTO concentration (normalized by the initial concentration, $C/C_0$) for two conditions: Camp Swift and Blank. The data points and line of best fit are indicated for both conditions.

For Camp Swift, the equation of the line is $y = -0.009x - 0.0234$ with a goodness of fit $R^2 = 0.9326$. This indicates a strong linear relationship between time and NTO concentration for Camp Swift conditions.
Limestone Hills

$y = -0.0037x + 0.0013$

$R^2 = 0.9342$
$y = -0.008x - 0.0204$

$\text{R}^2 = 0.9969$
Arnold AFB

\[ y = -0.0044x - 0.0131 \]

\[ R^2 = 0.9376 \]
y = -0.0021x - 0.0457
R² = 0.9765

Camp Butner

Camp Butner

Camp Butner

Camp Butner

Camp Butner

Camp Butner

Camp Butner

Camp Butner

Camp Butner
\[ y = -0.0005x - 0.0324 \]

\[ R^2 = 0.9137 \]
Figures 12-18. Adsorption isotherms for NTO in Catlin, Fort Harrison, Camp Butner, Limestone Hills, Florence MR, Camp Guernsey and Arnold AFB soils. Grey dashed line indicates linear adsorption isotherm fit to the measured adsorption data (equation is in grey), while solid black line is a fit of Freundlich isotherm with equation presented in black.

**Catlin**

\[ y = 0.21x \]
\[ R^2 = 0.94 \]

**Fort Harrison**

\[ y = 0.35x \]
\[ R^2 = 0.95 \]
Camp Guernsey

\[ y = 0.02x \]
\[ R^2 = 0.21 \]

\[ y = 0.06x^{0.48} \]
\[ R^2 = 0.18 \]

Arnold AFB

\[ y = 0.34x \]
\[ R^2 = 0.97 \]

\[ y = 0.58x^{0.86} \]
\[ R^2 = 0.98 \]
Figure 19-23. Breakthrough curves for NTO in Limestone Hills, Camp Butner, Florence MR, Sassafras and Catlin soil. Dashed grey vertical line indicates timing of 24-hour flow interruption. Tan vertical line indicates time, when solution was changed back to saturating solution to observe desorption phase of the isotherm.
Camp Butner

- Fitted NTO
- Measured NTO
- Fitted Bromide

Camp Butner FI

- Measured NTO
- Fitted Bromide
- Start 24 hr Flow Interruption
- Switched to Sat. Solution
Sassafras

Catlin

C/C₀ vs Pore Volume

Fitted NTO
Measured NTO
Fitted Bromide

Measured NTO
Fitted Bromide