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Determination of Toxicological Effects of Insensitive Munitions Compounds DNAN and NTO on Soil Invertebrates Enchytraeid Worm and Collembola

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14. ABSTRACT
The military services have been developing and evaluating several insensitive munitions (IMs) for future weapon systems, to replace present munitions that contain highly sensitive explosives. The overall objective of the present research was to develop soil invertebrate-based toxicity benchmarks acceptable for deriving draft ecological soil screening levels (Eco-SSLs) for IM compounds 2,4-dinitroanisole (DNAN) and 3-nitro-1,2,4-triazol-5-one (NTO). Ecotoxicological testing was specifically designed to meet the criteria for Eco-SSL derivation. Toxicity studies were conducted using two soil invertebrate species, including the enchytraeid worm *Enchytraeus crypticus* and the collembolan *Folsomia candida*. Ecotoxicological data developed in this research will provide critical information for installation managers to gauge the ecotoxicological impacts of military operations that involve the use of DNAN and NTO, and ultimately will promote the sustainable use of testing and training ranges.

15. SUBJECT TERMS

2,4-Dinitroanisole (DNAN)	Soil invertebrate toxicity
3-Nitro-1,2,4-triazol-5-one (NTO)	*Enchytraeus crypticus*
Insensitive munitions (IMs)	*Folsomia candida*

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PREFACE

The work described in this report was authorized under project no. SERDP-ER-2724. The work was started in April 2017 and completed in January 2020.

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This report has been approved for public release.

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1. INTRODUCTION

Unintended detonation of munitions and munition stockpiles has caused losses of human life, infrastructure, and materiel. The military services, therefore, are developing and evaluating several insensitive munitions (IMs) for future weapon systems, to replace present munitions that contain highly sensitive explosives such as 1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT) and improve the safety of munitions (Powell, 2016). Among the IM compounds being developed are 2,4-dinitroanisole (DNAN; Chemical Abstracts Service [CAS] no. 119-27-7) and 3-nitro-1,2,4-triazol-5-one (NTO; CAS: 932-64-9) used as components of several insensitive munition explosive compositions (IMXs). IMX-101 contains significant proportions of DNAN, NTO, as well as other explosives compounds.

DNAN was historically used as an explosive in warheads containing Amatol 40 and is being investigated as a substitute for TNT in IM formulations (Viswanath et al., 2018). DNAN is a nitroaromatic compound with physicochemical properties similar to those of TNT, such as solubility in water and hydrophobicity (Viswanath et al., 2018). DNAN also has the potential to undergo photochemical transformation (Taylor et al., 2017a). It is toxic to a wide variety of aquatic species (Dodard et al., 2013; Kennedy et al., 2015; Stanley et al., 2015; Gust et al., 2018) and soil invertebrates (Dodard et al., 2013; Gong et al., 2018). Lotufo et al. (2016) conducted bioaccumulation studies involving the uptake of DNAN by earthworms in amended soil and showed that the DNAN concentration in tissue exceeded the concentration of DNAN in soil. The bioaccumulation factors ranged from 1.2 to 4.3 kg of dry soil per kilogram of wet tissue.

NTO has been used since the 1980s in multiple energetic formulations. It has excellent qualities as an explosive, such as insensitivity and thermal and mechanical stability, and it is considered a potential insensitive replacement for RDX in various formulations (Viswanath et al., 2018). NTO has relatively low toxicity to tadpoles, fish, aquatic invertebrates, and soil nematodes and was considerably less toxic than TNT and DNAN in comparative studies (Lotufo et al., 2015; Stanley et al., 2015; Kennedy et al., 2017; Pillard et al., 2017; Gong et al., 2018; Gust et al., 2018; Madeira et al., 2018). The low estimated octanol–water partition coefficient (log K_{ow}) values for NTO (from 0.89 to 1.19; Toghiani et al., 2010) indicate a low propensity to adsorb to organic carbon through hydrophobic interactions. NTO adsorbs very weakly to soils (Mark et al., 2017), as expected for a negatively charged compound in a matrix that also possesses a net negative charge, and it has been shown that the organic carbon content in soils is not a good predictor of NTO adsorption (Taylor et al., 2017b). Hawari et al. (2014) reported that earthworms accumulated NTO from amended soil at lower concentrations than those found in the soil. Other studies have shown that NTO was not detected in plant roots and shoots in exposures to amended soils (Richard and Weidhaas, 2014).

During live-fire training, IMs are scattered by partial detonations and may weather and dissolve once on the soil surface (Taylor et al., 2017a), thereby posing a potential risk to soil invertebrates and terrestrial plants. Although information on IM concentrations in natural soils was not publicly available at the start of this research, and no real-world exposure information could be inferred, the manufacturing and use practices of the materials coupled with the chemical properties conducive to environmental transport warranted further investigation. Furthermore, ecotoxicological data were needed to address a clear gap in current knowledge of the potential risks associated with the release of DNAN and NTO into terrestrial environments. The overall objective of the present research was to develop ecotoxicological data for the IM compounds DNAN and NTO in soil. This data can then be used to derive risk-based levels that meet regulatory requirements for developing ecological soil screening levels (Eco-SSL). Eco-SSLs can be applied in screening-level ecological risk assessments (SLERAs) for key soil ecological receptors.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

Both DNAN and NTO were obtained from BAE Systems, Ordnance Systems (Falls Church, VA) with a listed purity of 99.9%. High-performance liquid chromatography (HPLC)-grade acetonitrile (ACN; certificate of analysis listed the purity as ≥99.9%) was obtained from Fisher Scientific (Fair Lawn, NJ). HPLC-grade methanol was obtained from Pharmco (Brookfield, CT). Formic acid was obtained from Sigma-Aldrich (St. Louis, MO; product number 33015, lot SZBD2830V, purity 99.3%). 2-Amino-4-nitroanisole (2-ANAN) was obtained from Sigma-Aldrich (product number 161195, lot S76153V). The certificate of analysis listed the purity (gas chromatography [GC] area percentage) as 99.6%. 4-Amino-2-nitroanisole (4-ANAN) was obtained from Apollo Scientific (Stockport, UK; product number OR0934, lot AS433424). The certificate of analysis listed the purity as greater than 95%. 1,3-Dinitrobenzene (1,3-DNB) was obtained from Avocado Research Chemicals (Heysham, UK; product number A11518, lot B7488C) with a listed purity of 97%.

2.2 Soil Collection and Characterization

Soil clay and organic matter content have been identified as the key soil constituents that adsorb explosives and thus affect the bioavailability and toxicity of energetic materials (EMs) for soil organisms (Haderlein et al., 1996; Pennington and Brannon, 2002; Jaenig, 2006; Singh et al., 2008, 2010; Dontsova et al., 2009; Kuperman et al., 2013; Arthur et al., 2017). For ecological risk assessment (ERA), particularly for developing ecotoxicological values protective of soil biota, we used a natural soil Sassafras sandy loam (SSL; fine-loamy, siliceous, semiactive, mesic Typic Hapludult). Additional soil invertebrate studies were conducted in Webster clay loam (WCL; fine-loamy, mixed, superactive, mesic Typic Endoaquoll). The qualitative relative bioavailability (QRB) scores for organic chemicals in natural soils were considered “very high” for SSL, and “medium” for WCL, according to the U.S. Environmental Protection Agency (USEPA) Eco-SSL criteria (USEPA, 2005). Studies with WCL soil were used to confirm that toxicity benchmarks developed using a soil type that supports the high relative bioavailability of IM compounds are sufficiently conservative for use in SLERA.

SSL soil was collected from an open grassland field in the coastal plain on the property of the U.S. Army Aberdeen Proving Ground in Harford County, MD. WCL was collected in Story County, IA. During soil collection in the field, vegetation and the organic horizon were removed, and the top 12 cm of the A-horizon was then collected. The soil was sieved through a 5 mm screen, air-dried for at least 72 h, mixed periodically to ensure uniform drying, passed through a 2 mm sieve, then stored at room temperature before use in testing. Random soil samples were collected and analyzed for physical and chemical characteristics by the Agricultural Analytical Services Laboratory at the Pennsylvania State University (University Park, PA; Table 1).

Table 1. Physical and Chemical Characteristics of SSL and WCL Soils

Soil Parameter	SSL	WCL
Sand (%)	55	33
Silt (%)	28	39
Clay (%)	17	28
Texture	Sandy loam	Clay loam
Cation exchange capacity ($cmol \cdot kg^{-1}$)	9.3	21
Organic matter (%)	2.3	5.3
pH	4.9	5.9
Water holding capacity (%)	18	23
QRB*	Very high	Medium

*Based on QRB scores for nonionizing organic contaminants in natural soils (USEPA, 2005).

The selected SSL soil had sufficiently low organic matter and clay contents to fulfill the USEPA requirement for using soils with characteristics that support high or very high relative bioavailability of organic pollutants, for developing realistic conservative Eco-SSL values (USEPA, 2005).

2.3 Soil Amendment Procedures

Soil was weighed separately in a glass container for each IM compound treatment and was spread from 2.5 to 4 cm thickness. Each IM compound amendment was prepared separately in a glass volumetric flask and dissolved in acetone as the carrier in studies with DNAN and methanol as the carrier in studies with NTO, to produce a more homogeneous mixture in soil than would the addition of solid DNAN or NTO. Soil treatments were individually amended with DNAN or NTO. The IM compound–carrier solution was quantitatively transferred to the soil. It was added evenly across the soil surface, to ensure that the volume of solution added at any one time did not exceed 15% (v/w) of the dry mass soil. The same total volume of the carrier was added to every DNAN or NTO treatment, which equaled the volume of carrier required to dissolve DNAN or NTO at the highest concentration tested. The amended soils were then air-dried for a minimum of 18 h in a darkened chemical fume hood. Each amended soil sample was transferred into a high-density polyethylene container coated with a fluoropolymer and mixed for 18 h using a three-dimensional soil mixer in darkness to prevent photolysis of the IM compounds. Four to nine concentrations of each IM compound were used in addition to controls (negative, positive, and carrier). All treatments were

appropriately replicated. After three-dimensional mixing, samples of freshly amended soil were collected from each soil treatment for analytical determinations of the initial DNAN or NTO concentrations. The remaining soil in each batch was hydrated with ASTM Type I water to 60% of the respective soil's water-holding capacity (WHC), thereby initiating the IM compound weathering-and-aging procedure in soil.

2.4 Weathering and Aging of EMs in Soils

Special consideration in assessing IM compound toxicity for Eco-SSL development was given to the inclusion of weathering and aging of contaminant explosives in soil in the assessment of the IM compound effects on terrestrial receptors. This more closely approximates the exposure effects in the field and is more relevant for ERA because Eco-SSL development by the USEPA was specifically undertaken for use at Superfund sites (locations where contaminants have been long-present). Weathering and aging of chemicals in soil may reduce exposure of terrestrial plants and soil invertebrates to EMs due to photodecomposition, hydrolysis, reaction with organic matter, sorption or fixation, precipitation, immobilization, occlusion, microbial transformation, and other fate processes that commonly occur at contaminated sites. This can result in a dramatic decrease in the amount of parent compound that is bioavailable, compared to tests conducted with recently amended chemicals or those tested after a short equilibration period (e.g., 24 h) and can affect the IM compound toxicity, as was demonstrated in our previous studies.

The weathering-and-aging procedure of DNAN and NTO in soil was performed at the U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD) in preparation for definitive toxicity testing with soil invertebrates. It was conducted to simulate, at least partially, the weathering-and-aging process in field soils, and to more closely approximate the exposure effects on soil biota at contaminated sites. These procedures included the exposure of amended and control soils, initially hydrated to 60% of the WHC of SSL or WCL soil, in open glass containers in the greenhouse at ambient temperature to alternating moistening and air-drying cycles for 10 days. During the weathering-and-aging procedure, all soil treatments were weighed and readjusted to their initial mass by periodically adding ASTM Type I water to the soil. Soil samples collected from each treatment after the weathering-and-aging procedure, which corresponded to the beginning of the definitive toxicity tests, were processed for analytical determinations of DNAN and NTO concentrations.

2.5 Extraction and Analytical Determinations of DNAN and NTO

DNAN was extracted from SSL or WCL soil by placing 1 g soil samples from the soil batch treatments and controls into 50 mL polypropylene centrifuge tubes and then adding 5 mL of ACN to each tube. Samples were vortexed with the ACN for 1 min and then sonicated in darkness for 18 h at 20 °C. After sonication, 5 mL of $CaCl_2$ solution (5 g/L) was added to each of the 50 mL polypropylene centrifuge tubes. The tubes were vortexed for 1 min and centrifuged for an additional 1 min. The supernatant was filtered through a 0.45 µm polytetrafluoroethylene (PTFE) syringe cartridge, and 1 mL of each filtered solution was transferred into an HPLC vial.

Either 1 or 3 µL of the internal standard (4.97 mg of 1,3-DNB per liter) was added to each HPLC vial. The vials were vortexed for 15 s before being analyzed and quantified by HPLC. Confirmation was conducted using GC and tandem mass spectrometry (MS/MS).

2.5.1 Analytical Methods: DNAN and Transformation Products in SSL Soil

The analytical determinations of DNAN were performed using an 1100 series HPLC system (Agilent; Wilmington, DE) equipped with diode array detection (DAD). Separation was achieved using a 250 × 4.6 mm Supelco Discovery C18 column (Merck; Darmstadt, Germany) at 35 °C. The isocratic mobile phase consisted of 50% water and 50% methanol running at a rate of 1 mL/min. The injection volume was 10 µL. Detection and subsequent concentration determinations of DNAN were performed at a 298 nm wavelength. The limit of detection (LOD) under these experimental conditions was 0.049 µg/mL and the limit of quantitation (LOQ) was 0.173 µg/mL. A 10-point calibration curve (0.173, 0.347, 0.695, 1.39, 2.79, 5.58, 11.17, 22.35, 44.70, and 89.40 µg/mL) was used to determine DNAN concentrations in soil extracts. The calibration curve correlation yielded a coefficient of determination (R^2) of 0.9999.

DNAN transformation products included 2-ANAN and 4-ANAN. The analytical determinations of 2-ANAN and 4-ANAN were performed using the 1100 Series HPLC system equipped with DAD. Separation was achieved using a 250 × 4.6 mm Supelco Discovery C18 column at 35 °C. A gradient mobile phase (1 mL/min) was used for separation of the two transformation products. The injection volume was 25 µL. Detection and subsequent concentration determinations of 2-ANAN and 4-ANAN were performed at a 240 nm wavelength. The LOD for 2-ANAN under these experimental conditions was 0.01 µg/mL, and the LOQ was 0.146 µg/mL. A stock solution of 2-ANAN (1.1164 mg/mL in ACN) was diluted with 50% ACN to develop a seven-point calibration curve (0.146, 0.291, 0.582, 2.91, 5.82, 29.10, and 58.20 µg/mL) that was used to determine 2-ANAN concentrations in soil extracts. The LOD for 4-ANAN under these experimental conditions was 0.011 µg/mL, and the LOQ was 0.075 µg/mL. A stock solution of 4-ANAN (1.2108 mg/mL in ACN) was diluted with 50% ACN to develop an eight-point calibration curve (0.075, 0.151, 0.302, 0.605, 3.03, 6.05, 30.27, and 60.54 µg/mL) that was used to determine 4-ANAN concentrations in soil extracts. The calibration curve correlation for 2-ANAN and 4-ANAN yielded R^2 values of 1.0000 and 0.9999, respectively.

2.5.2 GC–MS/MS Confirmation for Analytes in SSL Soil

Stock solutions of DNAN (1.788 mg/mL in ACN), 2-ANAN (1.116 mg/mL in ACN), and 4-ANAN (1.211 mg/mL in ACN) were used to prepare the calibration standards. Samples of the stock solutions were diluted with 50% ACN to obtain the following six nominal concentration points: 1, 5, 10, 25, 50, and 100 µg/mL. Each calibration standard also contained 25 µg/mL 1, 3-DNB diluted from the stock solution as an internal standard. All calibration standards were stored at –20 °C until needed.

Analytical confirmation was achieved using a 7000A Triple Quad GC–MS instrument (Agilent Technologies; Santa Clara, CA). GC separations were achieved using an RTx-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Restek Corporation; Bellefonte, PA). The carrier gas was helium, and the flow rate was 1 mL/min. Injections of 1.0 or 3.0 µL were made using an Agilent 7693 automatic liquid sampler autoinjector into a splitless injector port at a temperature of 300 °C. The initial oven temperature of 100 °C was held for 2 min, then ramped to 280 °C at 20 °C/min, and held for 1.5 min. After each analysis was complete, the column was back-flushed at 280 °C for 2.1 min at the reduced inlet pressure (0.5 psi).

Samples (1 mL) of the soil extracts were spiked with 5.2 µL of 1,3-DNB (used as the internal standard) stock solution (4.798 mg/mL in ACN) to yield a final internal standard concentration of 25 µg/mL.

Samples were ionized by positive-ion chemical ionization with methane reagent gas. Chemical ionization source conditions were optimized using fluoroether E3 (CAS registry number 3330-16-3; Agilent Technologies) tuning compound. Mass spectra were obtained at a dwell time of 0.1 s for each transition in the multiple-reaction monitoring mode. Helium was used as the collision gas with a collision energy of 20 V. The collision energy was optimized for the mass-to-charge ratio (*m/z*) 199 > 182 transition for DNAN, the *m/z* 169 > 108 transition for 2-ANAN, the *m/z* 169 > 94 transition for 4-ANAN, and the *m/z* 169 > 122 transition for 1,3-DNB. MassHunter software, provided with the Agilent 7000A system, was used to process and analyze the data. The software provides automated peak detection, calibration, and quantitation.

2.5.3 Extraction of NTO from SSL or WCL Soil

NTO was extracted from SSL or WCL soil by placing 1 g soil samples from the soil treatments and controls into respective 50 mL polypropylene centrifuge tubes and then adding 5 mL of 50% methanol to each tube. The tubes were vortexed for 1 min and placed into an ultrasonic water bath in darkness for 18 h at 20 °C. After sonication, 5 mL of $CaCl_2$ solution (5 g/L) was added to each of the 50 mL polypropylene centrifuge tubes. The tubes were vortexed for 1 min and centrifuged for an additional 1 min. The supernatant was filtered through a 0.45 µm PTFE syringe cartridge, and 1 mL of each filtered solution was transferred into an HPLC vial. Three microliters of the internal standard (4.97 mg 1,3-DNB/L) was added to each HPLC vial just before analytical determination. The vials were vortexed for 15 s and then analyzed and quantified by HPLC.

2.5.4 Analytical Methods: NTO and Transformation Products in SSL Soil

A primary stock solution of NTO was prepared by dissolving crystalline NTO in 50% ACN with 0.1% trifluoroacetic acid (TFA). This stock solution was then serially diluted using 50% ACN with 0.1% TFA to create analytical standards of 0.401, 0.802, 1.61, 3.21, 6.42, 12.84, 25.68, 51.37, 102.75, and 205.5 µg/mL (R^2 values for NTO standards ranged from 0.9998 to 0.9999). The standards were stored in darkness at 4 °C.

The analytical determinations of NTO in soil extracts were conducted using the Agilent 1100 series HPLC system with UV DAD. The wavelength was set at 315 nm for NTO or 230 nm for 1,3-DNB. Separation was achieved using a 150 × 4.6 mm Hypercarb column (Thermo Scientific; Waltham, MA). The initial mobile phase was isocratic and consisted of a 25:75 ratio of ACN containing 0.1% TFA and water containing 0.1% TFA. The flow rate was 1 mL/min, and the injection volume was 25 µL. The LOD for NTO under these experimental conditions was 0.005 µg/mL (peak to peak). The isocratic mobile phase was run for 4 min to elute the NTO. The mobile phase was then changed to a gradient to remove the 1,3-DNB internal standard.

2.5.5 Analytical Methods: DNAN, NTO, and Transformation Products in WCL Soil

The analytical determinations of DNAN, 2-ANAN, 4-ANAN, and NTO in WCL soil were performed using an Agilent 6410 triple-quadrupole liquid chromatography–MS system equipped with DAD. Separation was achieved using a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 µm pore size) at a temperature of 25 °C. The isocratic mobile phase consisted of 60% aqueous (0.1% formic acid in deionized water) and 40% organic (0.1% formic acid in methanol) with a flow rate of 1 mL/min. Injection volumes of either 10 or 50 µL were made. Detection and subsequent concentration determinations of DNAN were performed at a 300 nm wavelength, and determinations of 2-ANAN and 4-ANAN were performed at a 240 nm wavelength. The internal standard 1,3-DNB was also detected and determined at a 240 nm wavelength. NTO detection and concentration determination was performed at a 315 nm wavelength. Analytical confirmation was accomplished using positive electrospray ionization MS with a fragmentor voltage of 125 V. Mass spectra were acquired by scanning from 100 to 500 amu with a capillary voltage of 4000 V and a drying gas flow rate of 12 L/min at 350 °C.

Soil extracts (1 mL) were taken for analysis. Internal standard (5.2 µL) was added to each extract, and vials were vortexed for 15 s. A 10 or 50 µL (for greater sensitivity) injection was made and analyzed as described in Section 2.5.2 for the SSL soil. Replicate analyses were performed for each extract. Select blank extracts (both negative controls and method controls) were spiked with DNAN and NTO at high levels (100 ng/µL), medium levels (10 ng/µL), and low levels (1 ng/µL). For DNAN, recoveries were 97 ± 1.2% for the high level, 99 ± 1.3% for the medium level, and 129 ± 5.5% for the low level. For NTO, recoveries were 83 ± 1.2% for the high level, 104 ± 8.9% for the medium level, and 77 ± 10.5% for the low level.

Separate linear internal standard calibration curves were generated for DNAN and its transformation products 2-ANAN and 4-ANAN and for NTO using calibration standard concentrations of 0.5, 1, 2.5, 5, 10, 25, 50, and 100 ng/µL. Each calibration standard also contained 25 ng/µL 1,3-DNB as an internal standard. Relative responses (λ $Response_{Analyte}$/λ $Response_{Internal\ standard}$) were plotted against relative concentrations ($Conc_{Analyte}$/$Conc_{Internal\ standard}$), where λ Response equals the area under the peak at the detection wavelength. The calibration curve correlations typically yielded an R^2 of 0.9978. Under the experimental conditions previously described and with 50 µL injections, the LOD for DNAN and its transformation products was 0.03 ng/µL, while the LOD for NTO was 0.1 ng/µL.

2.5.6 Quality Control

USEPA Method 8000C (USEPA, 2003) was used for guidance in determining acceptable calibration and quality control requirements. Briefly, each set of 20 samples of either DNAN or NTO contained a blank, laborator y control sample (LCS), a matrix spike(MSp), and a matrix spike duplicate (MSD). The LCS consisted of hydromatrix (diatomaceous earth sorbent) spiked with the analytes of interest. The MSp consisted of an actual sample spiked with the analytes of interest. Sets of samples with very limited volume did not have a MSp and a MSD. In this case, the LCS and LCS duplicates were used as substitutes. The LCS, MSp, and MSD of a given set were all spiked at the same concentration. Spike recoveries were considered acceptable between 70 and 130%.

2.6 Soil Invertebrate Toxicity Studies

2.6.1 Potworm Toxicity Test

The Enchytraeid toxicity test was used to assess the individual effects of IM compounds on the enchytraeid worm (potworm) *Enchytraeus crypticus*. The test is an adaptation of an International Organization for Standardization (ISO) bioassay ISO 16387 (ISO, 2023), *Soil Quality: Effects of Pollutants on Enchytraeidae (*Enchytraeus *sp.): Determination of Effects on Reproduction and Survival*. This test was selected based on its ability to measure chemical toxicity to ecologically relevant test species during chronic assays and its inclusion of at least one reproduction component among the measurement endpoints. The ISO guideline for this assay was originally developed for use with Organisation for Economic Co-operation and Development (OECD, 1984) artificial soil (a similar soil formulation was later adapted for USEPA Standard Artificial Soil or SAS; USEPA, 1996). However, several studies demonstrated that this test could also be conducted using natural soils (Amorim et al., 2005a,b; 2009; Dodard et al., 2005; Kuperman et al., 2003, 2004, 2005, 2006a,b). The ISO 16387 was initially developed using the enchytraeid worm species *Enchytraeus albidus.* Results of our previous studies using *E. albidus* showed that this species requires soils containing high organic matter content with a soil pH 6 (±0.5) for optimal test conditions. This species performed poorly in natural soils having physical and chemical characteristics that support a higher level of EM bioavailability (Amorim et al., 2009; 2005a; Kuperman et al., 1999; 2006a). The *E. crypticus* species of genus *Enchytraeidae*, which is listed in the ISO protocol as an acceptable alternative to *E. albidus*, was selected for toxicity testing.

Potworms were bred in 4.3 L clear plastic boxes (34 × 20 × 10 cm) filled with 2 kg (dry mass) of SSL soil. The culture was kept in an environment-controlled incubator under a 16 h light–8 h dark photoperiod cycle. The mean photosynthetically active radiation light intensity was 12.8 ± 0.7 µM/m/s (985 ± 52 lux) and the mean temperature was 21.6 ± 0.1 °C (with standard errors [SEs]). The soil moisture level was adjusted to 100% of the WHC of SSL soil and was maintained by periodic (once per week) mass checks and water adjustments. The soil in the breeding culture was aerated by carefully mixing it once each week. The potworms were fed approximately twice each week with ground oats spread onto the soil surface. If food from the previous feeding date remained on the soil surface, the amount of food added was adjusted. Every 6 weeks, the worms were transferred into a freshly prepared culture substrate.

Cultures were synchronized so that all worms used in each test were approximately the same age. The potworm culture was considered healthy if worms were whitish, reproduced continuously, did not try to leave the soil, and exhibited a shiny outer surface with no soil particles clinging to them.

Glass jars (42 mm i.d.; 45 mm deep) were used as test containers. They were rinsed with carrier, tap water, and ASTM Type I water (ASTM, 2004) before the tests. Twenty grams (dry mass basis) of test soil and 0.05 g of ground oats were added to each test container, then mixed and hydrated to 100% of the WHC of each soil. The mass of each container with soil was recorded.

Adult potworms with eggs in the clitellum region were used for testing. They were collected from culture and were placed in a Petri dish filled with a small amount of ASTM Type I water for examination using a stereomicroscope. Potworms with no eggs were discarded. Any invertebrates living in the cultures (such as mites) were also removed. Ten potworms selected for uniformity (approximately 1 cm in length) were placed on top of the soil in each test container. Plastic wrap was stretched over the top of each container and secured with a rubber band. Three pinholes were made in the plastic wrap to facilitate air exchange. All containers were placed in an environment-controlled incubator under the same conditions as described above for the maintenance of the potworm culture. The containers were weighed once a week, and the mass loss was replenished with the appropriate amount of ASTM Type I water. Ground oats (0.05 g) were added to each test container at that time.

After two weeks, the soil in each test container was carefully searched and adult potworms were removed and counted. Potworms were examined for any morphological or behavioral changes. The remaining test substrate, including any cocoons laid during the first two weeks of the test, was incubated for an additional two weeks. After four weeks from the start of the test, soil in the test containers was fixed with 70% ethanol, and nine drops of Rose Bengal biological stain (1% solution in ethanol) were added. Staining continued for a minimum of 24 h. The content of each test container was wet-sieved using a no. 100 mesh sieve (150 µm), and retained contents were transferred to a counting tray, where potworms were counted. Measurement endpoints included the number of surviving adults after 14 days and the number of juveniles produced after 28 days.

Treatment concentrations for definitive tests with each IM compound were selected based on the results of the range-finding tests to bracket the 20 and 50% inhibition in the production of juveniles, as compared with the production of juveniles in carrier control for each soil. All definitive tests included negative controls (no chemicals added) and carrier controls.

Toxicity tests with the reference toxicant boric acid (positive control) were conducted using SSL soil to assess changes in sensitivity, health, and performance of *E. crypticus* maintained in DEVCOM CBC laboratory culture. Test treatments were prepared by adding appropriate solutions of boric acid in ASTM Type I water to SSL soil to obtain nominal concentrations of 0 (negative control), 20, 30, 50, 80, 100, and 200 mg/kg. Nonlinear regression analyses of toxicity data from independent studies were used to establish the respective median effective concentration (EC50) values and corresponding 95% confidence limits (CLs) for

juvenile production. These values were plotted on a boric acid warning chart, using modified procedures described by Environment Canada (EC, 2005) to monitor the condition of the potworms and precision within laboratory culture. The modification included using calculations based on arithmetic (untransformed) EC50 values instead of logarithmic concentrations for boric acid concentrations.

Four replicates of each IM compound treatment or control were used in the definitive tests. Validity criteria for the negative controls in toxicity tests included the following performance parameters (ISO 16387, 2023):

1. The adult mortality does not exceed 20% after 14 days.
2. The average number of juveniles is greater than 25 per test container at the end of the test, assuming that 10 adult worms per test container were used.
3. The coefficient of variation (CV) for the mean number of juveniles is ≤50%.

2.6.2 *Folsomia* Toxicity Test

The *Folsomia* toxicity test was used to assess the individual effects of DNAN and NTO on the reproduction of the collembolan *Folsomia candida*. The test is an adaptation of the bioassay, ISO 11267 (ISO, 2014). The measurement endpoints for the test included the production of juveniles and the survival of *F. candida* as adults. Collembolans were exposed to a range of DNAN or NTO concentrations, which were mixed into the soil. The total number of juvenile *F. candida* produced (i.e., an indicator of effective reproduction) and the number that survived as adult *F. candida* were determined by counting the live organisms after the 28 day test duration. The effective reproduction and survival of adult *F. candida* exposed to DNAN or NTO were compared with those of the carrier (solvent) control treatment to quantify ecotoxicological parameters. These parameters included the no-observed-effect concentration (NOEC), the lowest-observed-effect concentration (LOEC), and the effective concentration that causes a *p* percentage reduction (ECp) in the production of juveniles compared with those in the carrier controls (e.g., EC20 or EC50; 20 or 50% effect concentration, respectively), and the number of *F. candida* surviving as adults on day 28.

Toxicity tests with reference toxicant boric acid (positive control) were conducted using SSL soil to assess changes in sensitivity, health, and performance of *F. candida* maintained in DEVCOM CBC laboratory cultures. Test treatments were prepared by adding appropriate solutions of boric acid in ASTM Type I water to SSL soil to obtain nominal concentrations of 0 (negative control), 30, 50, 80, 100, and 200 mg/kg. Nonlinear regression analyses of toxicity data from independent studies were used to establish the respective EC50 values and corresponding 95% CLs for juvenile production. These values were plotted on a boric acid warning chart using modified procedures described by Environment Canada (EC, 2005) to monitor the condition of the potworms and precision within the laboratory culture. The modification included using calculations based on arithmetic (untransformed) EC50 values instead of logarithmic concentrations for boric acid concentrations.

Five replicates of each IM compound treatment or controls were used in the definitive tests. Validity criteria for the negative controls in the toxicity tests included the following performance parameters (ISO, 2014):

1. The adult *F. candida* mortality should not exceed 30% at the end of the test.
2. The average number of juvenile *F. candida* per chamber should reach 80 instars (nymphs) at the end of the 28 day test.
3. The CV for reproduction should not exceed 30% at the end of the test.

Glass jars (42 mm i.d.; 45 mm deep) were used as test containers. They were rinsed with ASTM Type I water before the tests. To prepare each treatment in the range-finding tests, 100 g of each air-dried treatment soil was hydrated to 88% of the WHC of SSL soil. Then one-fifth of the weight of each batch of hydrated treatment soil was transferred into a test container, and 0.05 g of baker's yeast was added to the soil surface. In the definitive tests with weathered-and-aged treatments, 20 g of test soil hydrated to 60% of the WHC and 0.05 g of baker's yeast were added to each test container. The container contents were then mixed and hydrated to 88% of the WHC of SSL soil by adding 1 g of ASTM Type I water. The mass of each container with soil was recorded to monitor soil moisture loss during the test. Ten 10–12 day old *F. candida* juveniles were placed in each test container. A piece of plastic food wrap was placed on each container and held in place with a rubber band. Five replicates were used for each treatment concentration and for the control treatments.

All containers were placed in an environment-controlled incubator under a 16 h light–8 h dark photoperiod cycle with a mean photosynthetically active radiation light intensity of 12.8 ± 0.7 µmol/m^2/s^{-1} (985 ± 52 lux) and a mean temperature of 21.6 ± 0.1 °C. The containers were weighed once a week, and the mass loss was replenished with the appropriate amount of ASTM Type I water. Baker's yeast (0.05 g) was added to each test container at that time.

To terminate a test, water was added to a test container, gently mixed with a spatula, and examined under a dissecting microscope (at 15× magnification) for the presence of *F. candida* juveniles and adults. The juvenile and adult *F. candida* that floated to the surface were counted.

2.7 Data Analyses

Ecotoxicological data were analyzed using regression models selected from among those described in the Environment Canada Guidance Document (EC, 2005) to estimate the effective concentration for a specified percentage effect (ECp) and the corresponding 95% confidence intervals (CIs). During the model selection process, compliance with the normality assumptions and homoscedasticity of the residuals were determined by examining the stem-and-leaf graphs and histograms of the residuals. The best fit was evident when the regression lines generated by the models were closest to the data points, the regression coefficients for point estimates were the greatest, the residuals were homoscedastic (i.e., had the most random scattering), and the means, standard errors, and variances of the residuals were the smallest. The ECp parameters included the IM concentrations producing 20% (EC20) and 50% (EC50) decreases in the measurement endpoint compared with carrier control. Measurement

endpoints included adult survival and reproduction in studies with soil invertebrates. The EC20 parameter based on reproduction (soil invertebrates) endpoint is the preferred parameter for deriving Eco-SSL values (USEPA, 2005). The models selected for data analyses in these studies were logistic (Gompertz; eq 1), and logistic hormetic (eq 2):

$$Y = a \times \mathrm{e}^{\{[\log(1-p)]\times(\mathrm{C}\div\mathrm{ECp})^b\}} \quad (1)$$

$$Y = \frac{a\times[1+(h\times\mathrm{C})]}{1+[(p+(h\times\mathrm{C}))\div(1-p)]\times[\mathrm{C}\div\mathrm{ECp}]^b} \quad (2)$$

where *Y* is the dependent variable for a measurement endpoint (e.g., number of juveniles or adults); *a* is the *y* axis intercept (i.e., the control response); e is the exponent of the base of the natural logarithm; *p* is the desired value for *p* effect (e.g., 0.50 for a 50% decrease from the control response; EC50); C is the exposure concentration in test soil; ECp is the estimate of concentration for a specified percent effect; *h* is the hormetic effect parameter; and *b* is a scale parameter that defines the shape of the equation.

Data that exhibited hormesis, a concentration-response phenomenon characterized by a low-dose stimulation followed by a high-dose inhibition (Calabrese, 2008), were fitted to the hormetic model. The ECp parameters used in these studies included the EM concentration producing a 50% (EC50) decrease in the measurement endpoint compared with the negative control. The 95% confidence intervals (CIs) associated with the point estimates were determined.

Analysis of variance was used to determine the bounded (when possible) NOEC and LOEC values for survival and reproduction data using Systat 13 software (Grafiti; Palo Alto, CA). Mean separations in the invertebrate tests were determined using Fisher's least-significant difference (FLSD) pairwise comparison test. A significance level of $p \leq 0.05$ (95% confidence level) was accepted for all statistical tests. All toxicological benchmarks were developed on the measured chemical concentration basis.

3. RESULTS

3.1 DNAN Concentrations in SSL Soil

Nominal DNAN concentrations selected for the definitive tests in SSL soil across all test organisms were 0, 30, 40, 60, 80, 120, 160, 320, and 640 mg/kg (Table 2). DNAN concentrations were analytically determined in all treatment and control soils at the beginning and end of the weathering-and-aging period. Initial analytically determined soil concentrations of DNAN before weathering and aging in SSL were 31, 44, 63, 82, 125, 158, 334, and 648 mg/kg, respectively. DNAN concentrations were fairly stable during the 10 day weathering and aging in SSL soil. The percentages of DNAN remaining in the soil at the end of the weathering-and-aging period were 81, 78, 88, 89, 87, 92, 88, and 100, respectively, compared with initial concentrations. Statistical analysis showed a significant (*t* test, $p < 0.02$) decrease in DNAN concentration in 30, 80, and 120 mg/kg nominal treatments. DNAN concentrations were not statistically different in the remaining treatments on day 10 compared with initial concentrations on day 0. DNAN was not detected above the LOQ in the control soils.

DNAN concentrations in SSL soil were also determined in low-, medium-, and high-treatment concentrations on days 3 and 7 of the weathering-and-aging period to determine trends in transformation rates (Table 2 and Figure 1). For 10 days of weathering and aging of DNAN in SSL, all but the 640 mg/kg treatment group showed a loss in extractable DNAN detected in the soil extracts. The average amount of DNAN loss was 14% (SE, 1.9%), while the 640 mg/kg treatment group did not show any losses (based on the SE). The transformation products of DNAN (2-ANAN and 4-ANAN) at 0 and 10 days were below detection limits for HPLC and GC–MS/MS.

Table 2. DNAN Concentrations During Weathering and Aging in Amended SSL Soil*

Nominal Concentration (mg/kg)	DNAN (mg/kg) [SE]			
	0 Days	**3 Days**	**7 Days**	**10 Days**
30	31 [0.39]	28 [0.79]	31 [0.43]	25 [1.07]†
40	44 [1.76]	ND	ND	34 [1.48]
60	63 [1.65]	ND	ND	55 [1.69]
80	82 [1.91]	ND	ND	73 [1.92]†
120	125 [0.95]	116 [4.01]	102 [9.42]	108 [1.81]†
160	158 [6.95]	ND	ND	145 [5.21]
320	334 [7.10]	ND	ND	295 [9.87]
640	648 [39.49]	621 [29.1]	609 [16.61]	649 [6.96]

*2-ANAN and 4-ANAN were below detection limits for HPLC and GC–MS/MS methods.
†Significant decrease (*t* test, $p < 0.02$) .
ND, not determined; treatments were not used for analytical determinations.

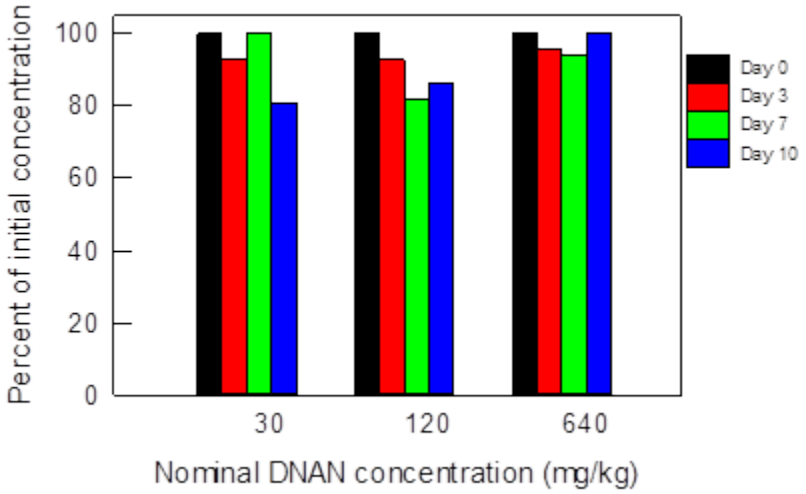

Figure 1. DNAN concentrations recovered from three treatments monitored over the 10 day period during weathering and aging in SSL soil.

The GC confirmation analysis of DNAN and 2-ANAN were comparable to the HPLC analysis. However, the 4-ANAN was not detected in any of the extract samples when analyzed on GC–MS/MS.

3.2 NTO Concentrations in SSL

Concentrations of NTO in the SSL soil treatments used in the soil invertebrate studies are shown in Table 3. Results of chemical analyses showed a rapid decrease (mean of 68% from initial on day 0) in NTO concentrations during the first 24 h. The decrease was likely due to the formation of non-identified transformation products. Measured NTO concentrations were relatively stable for the rest of the eight-day weathering-and-aging process in SSL soil (Figure 2), which suggests that chemical exposure conditions did not change appreciably during the toxicity tests.

Table 3. NTO Concentrations During Weathering and Aging in Amended SSL Soil

Nominal Concentration (mg/kg)	**NTO (mg/kg) [SE]***			
	0 Days	**1 Day**	**4 Days**	**8 Days**
50	42.72 [1.08]	34.07 [0.77]	36.40 [1.23]	31.29 [0.57]
178	176.83 [4.94]	48.43 [7.19]	47.32 [7.98]	39.58 [1.22]
300	293.95 [3.83]	107.02 [11.15]	119.86 [7.36]	91.64 [5.40]
400	387.78 [20.40]	162.57 [6.61]	146.15 [23.98]	124.99 [9.23]
600	567.71 [10.69]	232.65 [20.25]	187.03 [8.70]	218.85 [17.90]
800	819.84 [6.75]	293.93 [35.13]	252.33 [5.11]	272.50 [64.11]
1600	1634.07 [45.89]	727.95 [72.29]	467.64 [34.49]	547.35 [37.01]
2400	2359.57 [167.05]	1199.04 [55.82]	1029.57 [100.80]	785.09 [139.23]

*For SEs, $n = 3$.

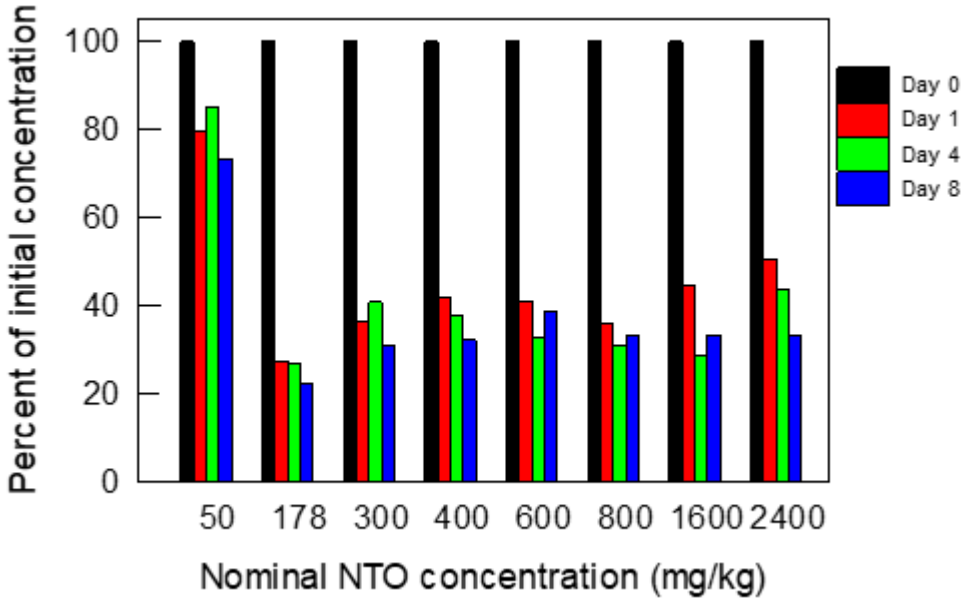

Figure 2. Recovered NTO concentrations during weathering and aging in SSL soil.

3.3 DNAN Concentrations in WCL Soil

Nominal DNAN concentrations selected for the definitive tests in WCL soil for all test invertebrate species were 0, 32, 42, 63, 84, 126, 168, 337, and 674 mg/kg (Table 4). DNAN concentrations were analytically determined in all treatment and control soils at the beginning and end of the weathering-and-aging period. Initial analytically determined soil concentrations of DNAN before weathering and aging in SSL were 0, 34, 42, 59, 92, 142, 174, 403, and 774 mg/kg, respectively. The percentages of DNAN remaining in the soil at the end of the weathering-and-aging period were 70, 88, 73, 76, 73, 86, 78, and 90, respectively, compared with initial concentrations (Figure 3). The average amount of DNAN loss was 21% (SE, 1.9%). DNAN was not detected above the detection limits in the control soils. The transformation products of DNAN (2-ANAN and 4-ANAN) were below detection limits for GC–MS/MS at day 0. 4-ANAN was not detected at day 10.

Table 4. Concentrations of DNAN and Transformation Products Extracted from WCL Soil Before (0 Days) and After (10 Days) the Weathering-and-Aging Procedure*

Nominal Concentration (mg/kg)	DNAN (mg/kg) [SE]		2-ANAN (mg/kg) [SE]	4-ANAN (mg/kg) [SE]
	0 Days	10 Days	10 Days	10 Days
32	34 [1.49]	24 [1.67]	BLQ*	BLQ*
42	42 [1.85]	37 [0.77]	0.44 [0.07]	BLQ
63	59 [1.40]	43 [2.78]	1.03 [0.10]	BLQ
84	92 [1.81]	70 [0.39]	1.76 [0.14]	BLQ
126	142 [6.94]	103 [2.34]	3.38 [0.10]	BLQ
168	174 [3.44]	150 [5.98]	4.59 [0.09]	BLQ
337	403 [19.28]	313 [9.44]	7.99 [0.12]	BLQ
674	774 [18.90]	693 [42.61]	13.85 [1.11]	BLQ

*2-ANAN and 4-ANAN were BLQ at day 0.
BLQ, below limit of quantitation.

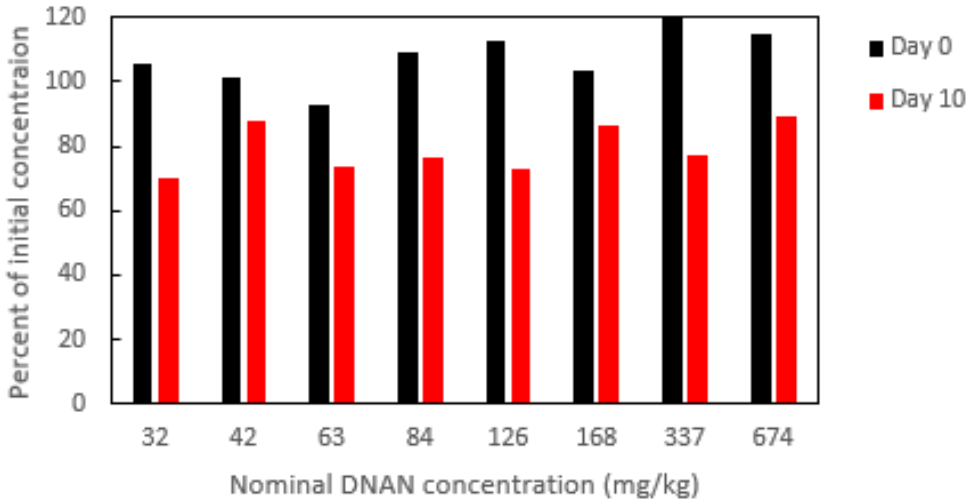

Figure 3. Recovered DNAN concentrations following weathering and aging in WCL soil.

3.4 NTO Concentrations in WCL Soil

Results of chemical analyses showed a rapid decrease (mean of 76% from initial on Day 0) in NTO concentrations during the 10 day weathering and aging in WCL soil (Table 5 and Figure 4).

Table 5. NTO Concentrations During Weathering and Aging in Amended WCL Soil

Nominal Concentration (mg/kg)	NTO [SE] (mg/kg)	
	0 Days	10 Days
100	101.48 [6.05]	21.08 [0.38]
200	206.66 [8.52]	45.78 [0.49]
300	289.73 [9.60]	71.94 [1.53]
400	417.06 [16.66]	93.59 [0.70]
600	645.60 [18.41]	151.96 [2.93]
800	799.55 [30.16]	201.04 [6.64]
1200	1161.79 [70.96]	296.11 [8.27]
1600	1728.66 [75.79]	459.34 [7.28]

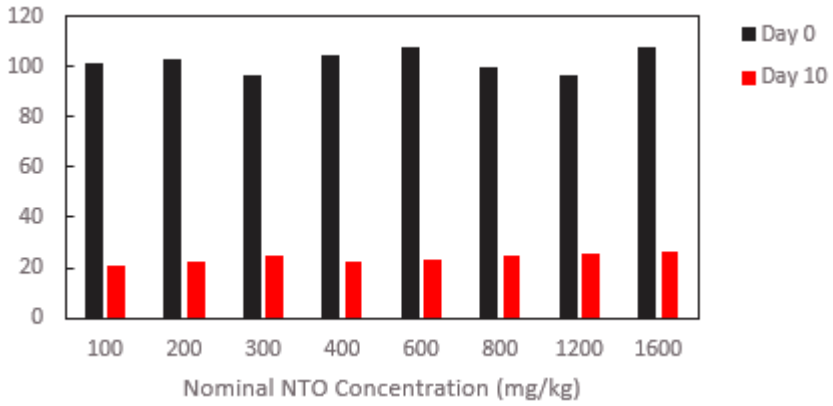

Figure 4. Recovered NTO concentrations following weathering and aging in WCL soil.

3.5 Enchytraeid (Potworm) Toxicity Studies

3.5.1 Positive Control

Toxicity tests with boric acid (reference toxicant) were conducted in SSL soil to obtain EC50 values and the corresponding 95% CLs. Nonlinear regression analyses of reproduction toxicity data established for seven testing dates produced the following EC50 values and their corresponding CLs (in parentheses) for juvenile production: 56 (48–63), 60 (47–77), 46 (36–56), 55 (44–65), 52 (39–66), 55 (46–65), and 55 (46–64) mg of H_3BO_3/kg of soil. These EC50 values were plotted on a boric acid warning chart to monitor the condition of the potworm culture. All resulting EC50 values were within both the warning limits and the

95% CLs that were established for the *E. crypticus* culture in tests with boric acid (Figure 10). These charted results confirmed that the condition of the *E. crypticus* culture met the validity requirements of the test protocol.

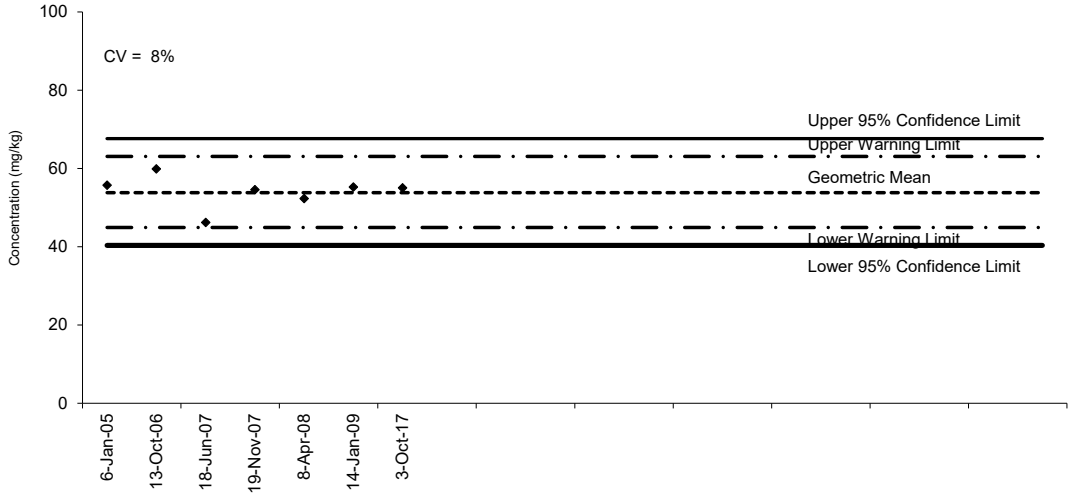

Figure 5. Warning chart for the *E. crypticus* culture showing the EC50 values for juvenile production established in definitive tests with the reference toxicant (boric acid) in SSL soil.

3.5.2 Ecotoxicological Effects of DNAN on Potworm *E. crypticus* in SSL Soil

The definitive toxicity test with soil invertebrate *E. crypticus* exposed to DNAN in freshly amended SSL soil was conducted to assess the acute (adult mortality) and chronic (juvenile production) effects of DNAN on *E. crypticus* in SSL soil and to determine test concentrations for the definitive study using DNAN weathered and aged in SSL soil. Measurement endpoints were assessed using treatment concentrations that were based on the results of the range-finding studies. Measurement endpoints included the number of surviving adults after 14 days and the number of juveniles produced after 28 days. Exposure concentrations for each soil were selected for definitive tests to achieve bracketing of significant effects on reproduction endpoints (i.e., production of juveniles).

Results of toxicity tests with DNAN freshly amended or weathered and aged in SSL soil complied with the validity criteria defined in the ISO 16387 test guideline (ISO, 2023). The validity criteria (mean adult survival, mean number of juveniles produced, and CVs) for test results from the negative-control treatment were 98%, 354, and 21%, respectively in freshly amended treatment, and 93%, 776, and 17%, respectively, in the weathered-and-aged treatment. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the DNAN treatments.

The bounded NOEC and LOEC values for DNAN effects on adult survival were 63 and 82 mg/kg in freshly amended soil and 73 and 108 mg/kg for DNAN weathered and aged in SSL soil, respectively. The logistic Gompertz model had the best fit for the adult survival data (acute toxicity). The EC20 and EC50 values were 82 and 225 mg/kg, respectively, for DNAN

freshly amended in SSL, and 128 and 180 mg/kg, respectively, for DNAN weathered and aged in SSL soil (Table 6). Evaluation of these data showed that weathering and aging of DNAN in SSL soil did not significantly (95% CI basis) affect acute toxicity for *E. crypticus.*

Table 6. Acute Ecotoxicological Benchmarks for DNAN Freshly Amended or Weathered and Aged in SSL Soil Determined for Survival of Adult *E. crypticus*

Ecotoxicological Parameter	**DNAN (mg/kg)**	
	Freshly Amended	**Weathered and Aged**
NOEC	63	73
p	0.145	0.205
LOEC	82	108
p	0.004	<0.0001
EC20	83	128
CI (95%)	34–131	108–147
EC50	225*	180
CI (95%)	57–392	156–203
Model used	Gompertz	Gompertz
R^2	0.988	0.989

*Estimated outside the tested range.

Juvenile production was the more sensitive measurement endpoint for assessing DNAN toxicity for *E. crypticus* compared with adult survival, based on the EC50 values. These data for DNAN freshly amended or weathered and aged in SSL soil are summarized in Table 7. The logistic Gompertz model had the best fit for reproduction data (R^2 = 0.922) from toxicity tests with DNAN freshly amended in SSL soil (Figure 6). The numbers of juveniles were significantly (p = 0.016) greater in the first positive-treatment concentration, producing an unbounded LOEC value of 31 mg/kg and bounded no-observed-adverse-effect concentration (NOAEC) and lowest-observed-adverse-effect concentration (LOAEC) values of 44 and 63 mg/kg, respectively.

Table 7. Chronic Ecotoxicological Benchmarks for DNAN Freshly Amended or Weathered and Aged in SSL Soil Determined for Production of Juveniles by *E. crypticus*

Ecotoxicological Parameter	DNAN (mg/kg)	
	Freshly Amended	Weathered and Aged
NOAEC	44	73
p	0.126	0.205
LOAEC	63	108
p	0.029	<0.0001
EC20	40	70
CI (95%)	21–59	46–93
EC50	57	106*
CI (95%)	44–70	89–123
Model used	Gompertz	Gompertz
R^2	0.881	0.956

*Statistically significant (95% CI basis) decrease in toxicity following weathering and aging of DNAN in soil.

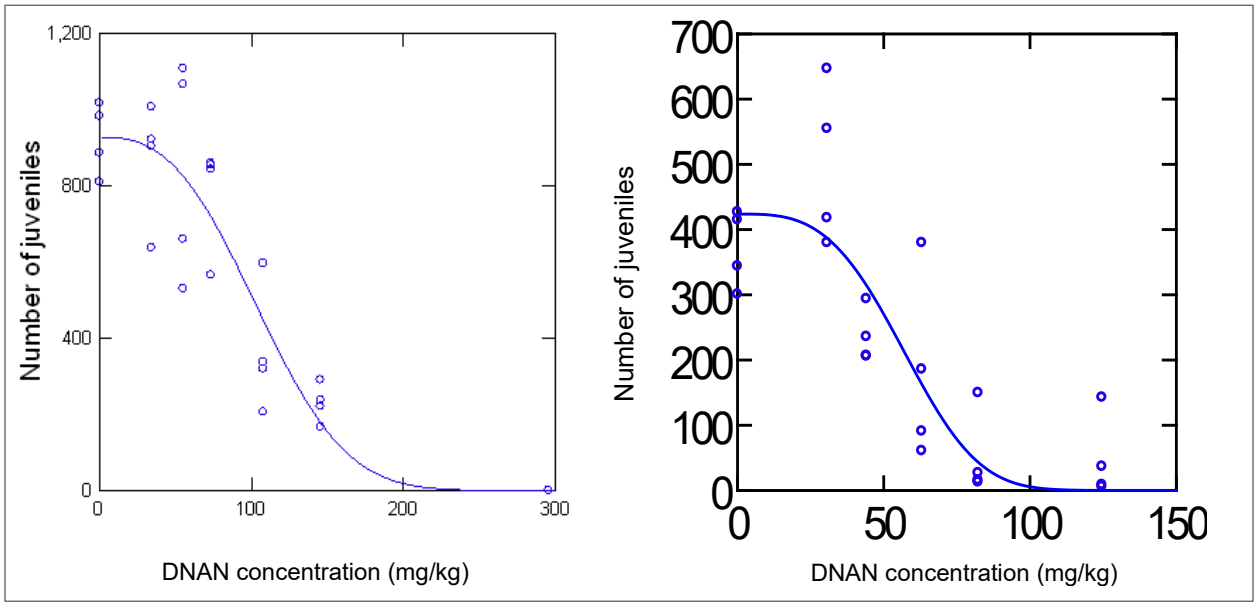

Figure 6. Effect of DNAN freshly amended (right) or weathered and aged (left) in SSL soil on the production of juveniles by *E. crypticus*.

The bounded NOAEC and LOEC values for DNAN weathered and aged in SSL soil were 73 and 108 mg/kg, respectively. The logistic Gompertz model had the best fit for the reproduction data in the study. The EC50 and EC20 values (and corresponding 95% CIs) for the production of juveniles in weathered and aged treatment of SSL soil were 106 (89–123) and 70 (46–93) mg/kg, based on analytically determined DNAN concentrations. Weathering and aging of DNAN in SSL soil significantly decreased reproduction toxicity for *E. crypticus* based on the EC50 values and their respective 95% CIs (Table 7).

3.5.3 Ecotoxicological Effects of NTO on the Potworm *E. crypticus* in SSL Soil

There were no statistically significant differences between the numbers of surviving adults or juveniles produced in negative and solvent (methanol) controls in studies with NTO freshly amended or weathered and aged in SSL soil (*t* test, $p = 0.604$ and 0.569, respectively, for adults, and $p = 0.746$ and 0.648, respectively, for juveniles). Therefore, the results for the two control treatments were combined to evaluate the compliance with the validity criteria defined in the ISO 16387 test guideline (ISO, 2023). Results of toxicity tests with NTO freshly amended or weathered and aged in SSL soil complied with the validity criteria. The mean adult survival, mean number of juveniles produced, and CVs for combined controls results were: 84%, 274, and 47%, respectively in freshly amended treatment, and 99%, 857, and 12%, respectively, in weathered-and-aged treatment. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the NTO treatments.

The bounded NOEC and LOEC values for NTO effects on adult survival were 388 and 568 mg/kg, respectively in freshly amended soil. The logistic Gompertz model had the best fit for the adult survival data in the study with NTO freshly amended in SSL and established the EC20 and EC50 values 540 and 620 mg/kg, respectively.

The numbers of surviving adults were not significantly different from carrier control up to and including 272 mg/kg treatment for NTO weathered and aged in SSL, followed by 100% mortality in the next tested concentration of 547 mg/kg. This lack of a gradual concentration–response relationship precluded the determination of the adult survival EC20 and EC50 values for NTO weathered and aged in SSL. The NOEC and LOEC values for NTO weathered and aged in SSL were 272 and 547 mg/kg, respectively (Table 8).

Table 8. Acute Ecotoxicological Benchmarks for NTO Freshly Amended or Weathered and Aged in SSL Soil Determined for Survival of Adult *E. crypticus*

Ecotoxicological Parameter	NTO (mg/kg)	
	Freshly Amended	**Weathered and Aged**
NOEC	388	272
p	0.303	0.106
LOEC	568	547
p	0.002	≤0.0001
EC20	540	>272
CI (95%)	420–661	ND
EC50	620	>272
CI (95%)	407–834	ND
Model used	Gompertz	ND
R^2	0.968	ND

ND, not determined; could not be determined within the concentration range tested.

Chronic ecotoxicological benchmarks for NTO freshly amended or weathered and aged in SSL soil are summarized in Table 9. The bounded NOEC and LOEC values were 294 and 388 mg/kg, respectively for NTO freshly amended in SSL soil. Production of juveniles by *E. crypticus* was significantly decreased in the first positive concentration of NTO weathered and aged in SSL soil, compared with carrier control, producing a NOEC of <31 mg/kg and an unbounded LOEC of 31 mg/kg.

Juvenile production was the more sensitive measurement endpoint for assessing NTO toxicity for *E. crypticus* compared with adult survival, based on the EC20 values determined in freshly amended treatments. The logistic hormetic model had the best fit (R^2 = 0.881) for reproduction data determined in toxicity tests with NTO freshly amended in SSL soil, due to stimulation of juvenile production at the lower treatment concentrations (Figure 7). The hormetic model yielded the EC20 and EC50 values of 197 and 310 mg/kg, respectively. The logistic Gompertz model had the best fit (R^2 = 0.951) for the reproduction data in the study with NTO weathered and aged in SSL. The EC20 and EC50 values for the production of juveniles by *E. crypticus* in weathered-and-aged treatment of SSL soil were 16 and 39 mg/kg, respectively, based on analytically determined NTO concentrations. Weathering and aging of NTO in SSL soil significantly increased (by one order of magnitude) reproduction toxicity for *E. crypticus* based on the EC20 and EC50 values and their respective 95% CIs (Table 9).

Table 9. Chronic Ecotoxicological Benchmarks for NTO Freshly Amended or Weathered and Aged in SSL Soil Determined for Production of Juveniles by *E. crypticus*

Ecotoxicological Parameter	**NTO (mg/kg)**	
	Freshly Amended	**Weathered and Aged**
NOEC	294	<31
p	0.227	ND
LOEC	388	31*
p	0.002	0.001
EC20	197	16†
CI (95%)	126–268	8–24
EC50	310	39†
CI (95%)	213–407	30–49
Model used	Hormetic	Gompertz
R^2	0.881	0.951

ND, not determined; could not be determined within the concentration range tested.
*Unbounded LOEC.
†Statistically significant (95% CI basis) increase in toxicity following weathering and aging of NTO in soil.

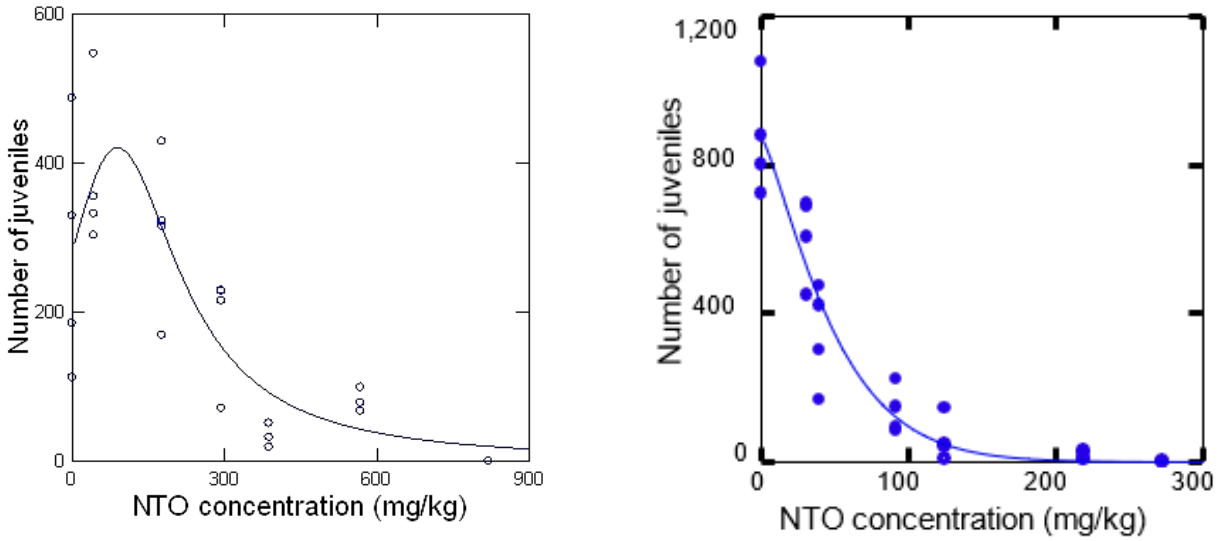

Figure 7. Effect of NTO freshly amended (left) or weathered and aged (right) in SSL soil on the production of juveniles by *E. crypticus*.

3.5.4 Ecotoxicological Effects of DNAN on the Potworm *E. crypticus* in WCL Soil

Results of toxicity tests with DNAN weathered and aged in WCL soil complied with the validity criteria defined in the ISO 16387 test guideline (ISO, 2023). The validity criteria (mean adult survival, mean number of juveniles produced, and CV) for test results from the negative control treatment were 100%, 3841, and 10%, respectively. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the DNAN treatments.

There was no effect ($p > 0.05$) on adult survival in any of the DNAN concentrations tested in WCL soil, producing an unbounded NOEC of 693 mg/kg. Juvenile production was the more sensitive measurement endpoint for assessing DNAN toxicity for *E. crypticus* compared with adult survival. These data for DNAN weathered and aged in WCL soil are summarized in Table 10. The numbers of juveniles were significantly ($p \leq 0.0001$) greater in the first positive-treatment concentration compared with carrier control, producing an unbounded LOEC value of 70 mg/kg, and the bounded NOAEC and LOAEC values of 150 and 313 mg/kg, respectively. The logistic Gompertz model had the best fit ($R^2 = 0.947$) for reproduction data (Figure 8). The EC50 and EC20 values (and corresponding 95% CIs) for the production of juveniles in WCL soil were 318 (220–415) and 182 (67–297) mg/kg, respectively, based on analytically determined DNAN concentrations.

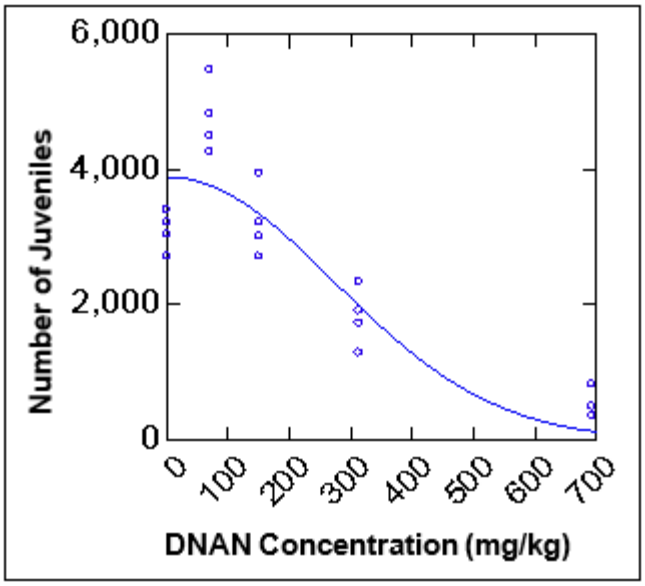

Figure 8. Effect of DNAN weathered and aged in WCL soil on the production of juveniles by *E. crypticus*.

Table 10. Chronic Ecotoxicological Benchmarks for DNAN Weathered and Aged in WCL Soil Determined for the Production of Juveniles by *E. crypticus*

Ecotoxicological Parameter	DNAN (mg/kg)
NOAEC	150
p	0.672
LOAEC	313
p	0.001
EC20	182
CI (95%)	67–297
EC50	318
CI (95%)	220–415
Model used	Gompertz
R^2	0.947

3.5.5 Ecotoxicological Effects of NTO on the Potworm *E. crypticus* in WCL Soil

Results of toxicity tests with NTO weathered and aged in WCL soil complied with the validity criteria defined in the ISO 16387 test guideline (ISO, 2023). The validity criteria (mean adult survival, mean number of juveniles produced, and CV) for test results in the negative control treatment were 100%, 2233, and 7%, respectively. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the NTO treatments.

Numbers of surviving adults were not affected by NTO up to and including the greatest concentration tested in WCL soil producing an unbounded NOEC of 201 mg/kg. Chronic ecotoxicological benchmarks for NTO weathered and aged in WCL soil are summarized in Table 11. The bounded NOEC and LOEC values were 46 and 152 mg/kg, respectively. The logistic Gompertz model had the best fit (R^2 = 0.997) for reproduction data, yielding the EC20 and EC50 values of 135 and 255 mg/kg, respectively, based on analytically determined NTO concentrations.

Table 11. Chronic Ecotoxicological Benchmarks for NTO Weathered and Aged in WCL Soil Determined for Production of Juveniles by *E. crypticus*

Ecotoxicological Parameter	**NTO (mg/kg)**
NOEC	46
p	0.981
LOEC	152
p	≤0.0001
EC20	135
CI (95%)	104–166
EC50	255
CI (95%)	209–302
Model used	Gompertz
R^2	0.997

3.6 *Folsomia* Toxicity Studies

3.6.1 Positive Control

Toxicity tests with boric acid (reference toxicant) were conducted in SSL soil to obtain the EC50 values and the corresponding 95% CLs. Nonlinear regression analyses of reproduction toxicity data established for seven testing dates produced the following EC50 values and their corresponding CLs (in parentheses) for juvenile production: 72 (68–77), 63 (53–73), 60 (53–67), 69 (61–76), 60 (51-68), 70 (59-82), and 58 (39–78) mg of H_3BO_3/kg of soil. These EC50 values were plotted on a boric acid warning chart to monitor the condition of the collembolan culture. All resulting EC50 values were within both the warning limits and the 95% CLs that were established for the *F. candida* culture in tests with boric acid (Figure 9). These charted results confirmed that the condition of the *F. candida* culture met the validity requirements of the test protocol.

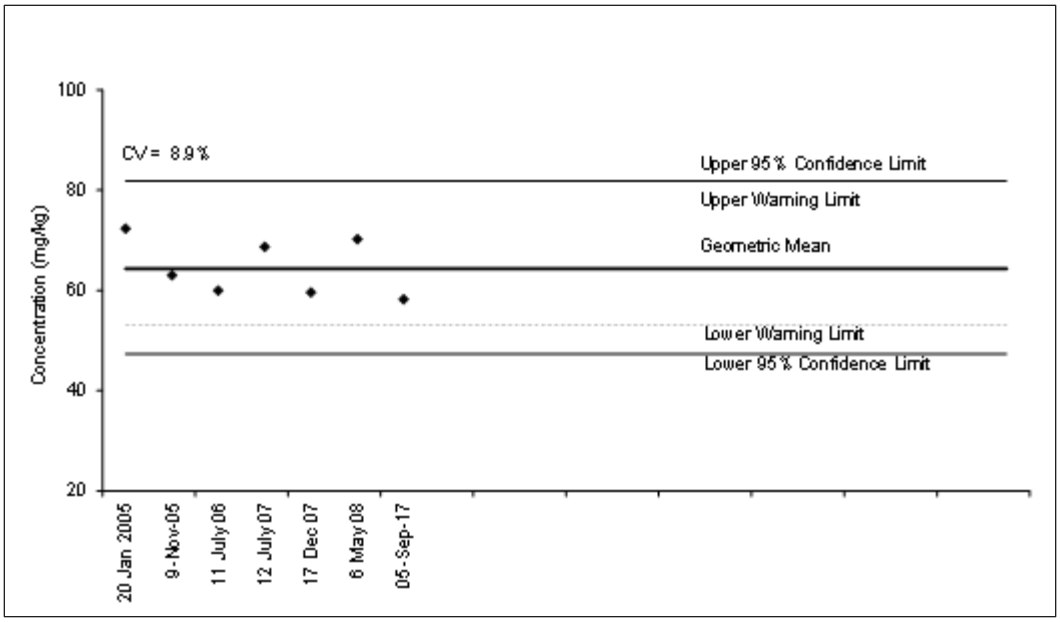

Figure 9. Warning chart for the *F. candida* culture showing the EC50 values for juvenile production established in definitive tests with the reference toxicant (boric acid) in SSL soil.

3.6.2 Ecotoxicological Effects of DNAN on Collembolan *F. candida* in SSL Soil

The definitive toxicity test with collembolan *F. candida* exposed to DNAN weathered and aged in SSL soil was conducted to assess the acute (adult mortality) and chronic (juvenile production) effects of DNAN on *F. candida* in SSL soil. Measurement endpoints included the number of surviving adults and the number of juveniles produced after 28 days. Exposure concentrations for definitive tests were selected to achieve bracketing of significant effects on reproduction endpoints (i.e., production of juveniles).

Results of the definitive toxicity test with DNAN weathered and aged in SSL soil complied with the validity criteria defined in the ISO 11267 (ISO, 2014) test guideline. The validity criteria for test results in the negative control treatment for mean adult survival, the mean number of juveniles produced, and the CV were 76%, 83, and 15%, respectively. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the DNAN treatments.

Toxicity benchmarks for DNAN weathered and aged in SSL soil are summarized in Table 12. The number of surviving adults was significantly ($p = 0.009$) lower in the lowest positive DNAN concentration compared with carrier control, producing an unbounded LOEC of 25 mg/kg. The bounded NOEC and LOEC values for DNAN effects on the production of juveniles were 25 and 34 mg/kg, respectively. The logistic Gompertz model had the best fit for both the adult survival data (acute toxicity) and the juvenile production data (chronic toxicity) in SSL soil (Figure 10). The EC20 and EC50 values were 11 and 28 mg/kg, respectively, for the survival of adults, and 27 and 32 mg/kg, respectively, for the production of juveniles.

Table 12. Ecotoxicological Benchmarks for DNAN Weathered and Aged in SSL Soil Determined for Survival of Adults and Production of Juveniles by *F. candida*

Ecotoxicological Parameter	DNAN (mg/kg)	
	Survival	Reproduction
NOEC	<25	25
p	ND	0.234
LOEC	25*	34
p	0.009	<0.0001
EC20	11	27
CI (95%)	4–19	20–33
EC50	28	32
CI (95%)	20–36	29–35
Model used	Gompertz	Gompertz
R^2	0.917	0.924

*Unbounded LOEC.
ND, not determined; could not be determined within the concentration range tested.

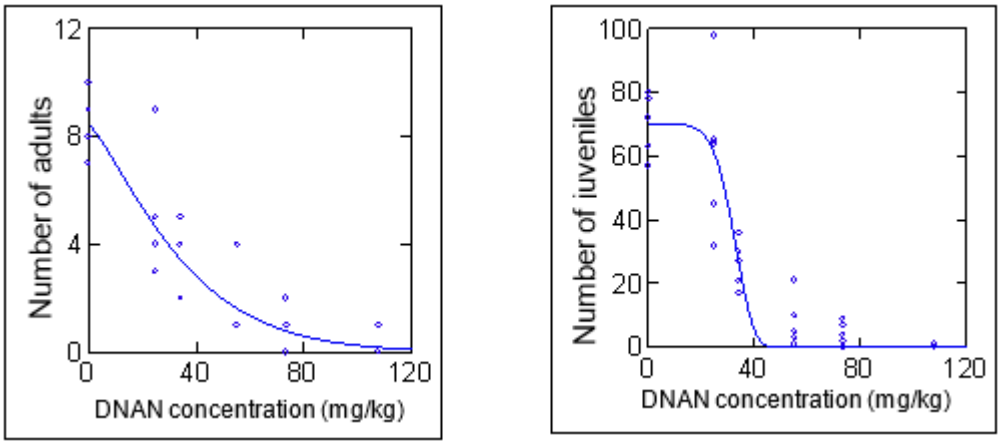

Figure 10. Effect of DNAN weathered and aged in SSL soil on survival of adults (left) and production of juveniles (right) by *F. candida*.

3.6.3 Ecotoxicological Effects of NTO on Collembolan *F. candida* in SSL Soil

Results of collembolan toxicity tests with NTO freshly amended or weathered and aged in SSL soil complied with the validity criteria defined in the ISO 11267 test guideline (ISO, 2014). The validity criteria for mean adult survival, the mean number of juveniles produced, and the CV for test results from the negative control treatment were 98%, 50, and 30%, respectively, in freshly amended treatment, and 100%, 81, and 15%, respectively, in weathered-and-aged treatment. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the NTO treatments.

The bounded NOEC and LOEC values for NTO effects on adult survival were 177 and 294 mg/kg, respectively, in freshly amended soil, and 40 and 92 mg/kg, respectively, for NTO weathered and aged in SSL soil. The logistic Gompertz model had the best fit for the adult survival data and established the EC20 and EC50 values of 141 and 306 mg/kg, respectively, for NTO freshly amended in SSL, and 72 and 138 mg/kg, respectively, for NTO weathered and aged in SSL. Weathering and aging of NTO in SSL soil significantly increased acute toxicity for *F. candida* based on the EC50 values and their respective 95% CIs (Table 13).

Table 13. Acute Ecotoxicological Benchmarks for NTO Freshly Amended or Weathered and Aged in SSL Soil Determined for Survival of Adult *F. candida*

Ecotoxicological Parameter	**NTO (mg/kg)**	
	Freshly Amended	**Weathered and Aged**
NOEC	177	40
p	0.204	0.570
LOEC	294	92
p	<0.0001	0.019
EC20	141	72
CI (95%)	73–209	40–104
EC50	306	138*
CI (95%)	237–374	108–168
Model used	Gompertz	Gompertz
R^2	0.942	0.939

*Statistically significant (95% CI basis) increase in toxicity following weathering and aging of NTO in soil.

Chronic ecotoxicological benchmarks for NTO freshly amended or weathered and aged in SSL soil are summarized in Table 14. Production of juveniles by *F. candida* was significantly decreased in the lowest concentration of NTO freshly amended in SSL, compared with carrier control, producing a NOEC of <177 mg/kg and an unbounded LOEC of 177 mg/kg. The bounded NOEC and LOEC values for NTO weathered and aged in SSL were 40 and 92 mg/kg, respectively.

The logistic Gompertz model had the best fit (R^2 = 0.920 and 0.905 for freshly amended and weathered and aged treatments, respectively) for reproduction data determined in both exposure types (Figure 11). The EC20 and EC50 values for production of juveniles by *F. candida* were 156 and 239 mg/kg for NTO freshly amended in SSL, and 63 and 111 mg/kg for NTO weathered and aged in SSL soil, based on analytically determined NTO concentrations. Weathering and aging of NTO in SSL soil significantly increased reproduction toxicity for *F. candida* based on the EC20 or EC50 values and their respective 95% CIs (Table 14).

Table 14. Chronic Ecotoxicological Benchmarks for NTO Freshly Amended or Weathered and Aged in SSL Soil Determined for Production of Juveniles by *F. candida*

Ecotoxicological Parameter	NTO (mg/kg)	
	Freshly Amended	**Weathered and Aged**
NOEC	<177	40
p	ND	0.621
LOEC	177*	92
p	0.032	0.008
EC20	156	63†
CI (95%)	103–209	32–95
EC50	239	111†
CI (95%)	199–280	85–138
Model used	Gompertz	Gompertz
R^2	0.920	0.905

*Unbounded LOEC.
†Statistically significant (95% CI basis) increase in toxicity following weathering and aging of NTO in soil.
ND, not determined; could not be determined within the concentration range tested.

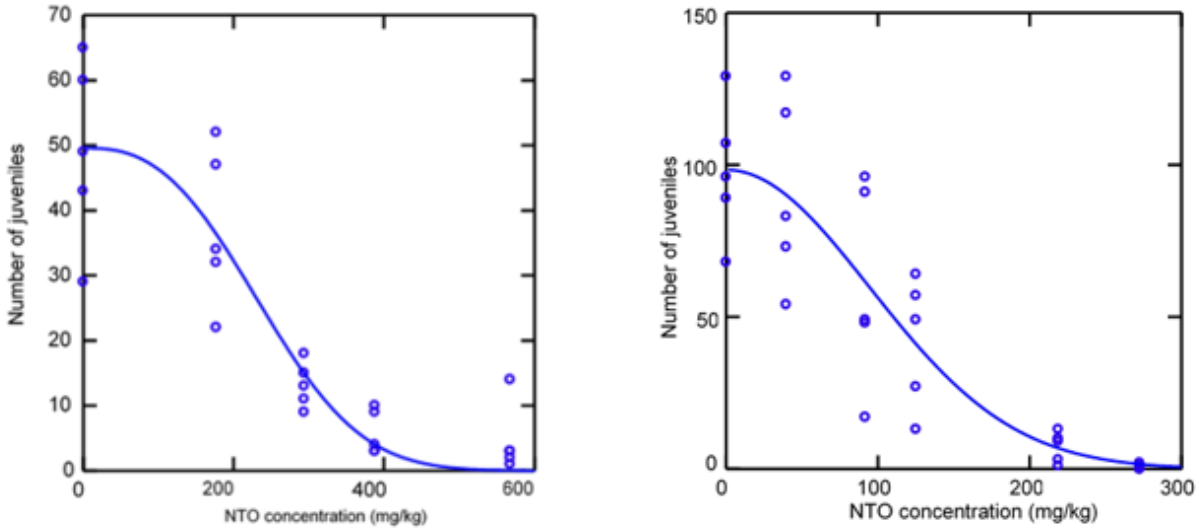

Figure 11. Effect of NTO freshly amended (left) or weathered and aged (right) in SSL soil on the production of juveniles by *Folsomia candida*.

3.6.4 Ecotoxicological Effects of DNAN on Collembolan *F. candida* in WCL Soil

The definitive toxicity test with collembolan *F. candida* exposed to DNAN weathered and aged in WCL soil was conducted to assess the acute (adult mortality) and chronic (juvenile production) effects of DNAN on *F. candida* in WCL soil. Measurement endpoints included the number of surviving adults and the number of juveniles produced after 28 days. Exposure concentrations for definitive tests were selected to achieve bracketing of significant effects on reproduction endpoints (i.e., production of juveniles).

Results of the definitive toxicity test with DNAN weathered and aged in WCL soil complied with the validity criteria defined in the ISO 11267 test guideline (ISO, 2014). The validity criteria for test results in the negative control treatment for mean adult survival, the mean number of juveniles produced, and the CV were 96%, 96, and 19%, respectively. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the DNAN treatments.

Toxicity benchmarks for DNAN weathered and aged in WCL soil are summarized in Table 15. The bounded NOEC and LOEC values for DNAN were 24 and 37 mg/kg, respectively, for the survival of adults, and 37 and 43 mg/kg, respectively, for the production of juveniles. The logistic Gompertz model had the best fit for both the adult survival data (acute toxicity) and juvenile production data (chronic toxicity) in SSL soil (Figure 12). The EC20 and EC50 values were 11 and 60 mg/kg, respectively, for the survival of adults, and 23 and 52 mg/kg, respectively, for the production of juveniles.

Table 15. Ecotoxicological Benchmarks for DNAN Weathered and Aged in WCL Soil Determined for Survival of Adults and Production of Juveniles by *F. candida*

Ecotoxicological Parameter	**DNAN (mg/kg)**	
	Survival	**Reproduction**
NOEC	24	37
p	0.619	0.155
LOEC	37	43
p	0.026	0.001
EC20	11	23
CI (95%)	4–19	7–39
EC50	60	52
CI (95%)	44–76	34–70
Model used	Gompertz	Gompertz
R^2	0.932	0.882

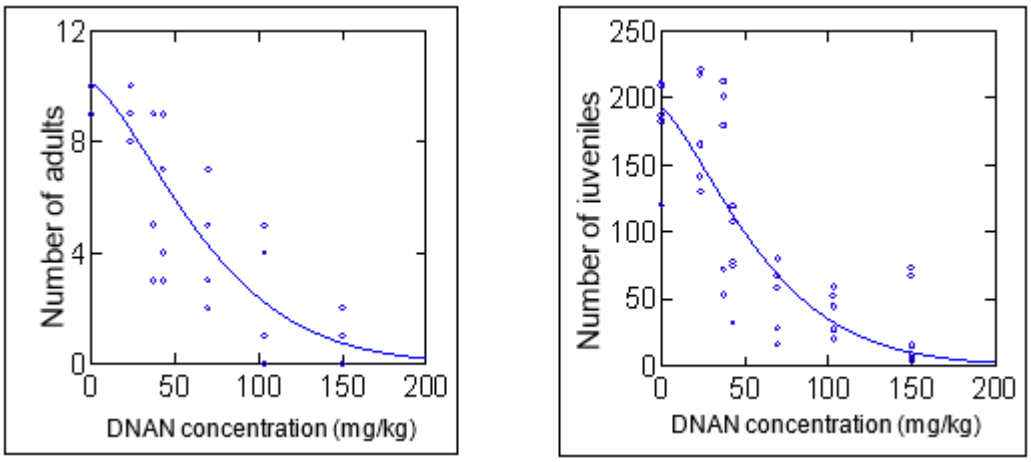

Figure 12. Effect of DNAN weathered and aged in WCL soil on survival of adults (left) and production of juveniles (right) by *F. candida*.

3.6.5 Ecotoxicological Effects of NTO on Collembolan *F. candida* in WCL Soil

The definitive toxicity test with collembolan *F. candida* exposed to NTO weathered and aged in WCL soil was conducted to assess the acute (adult mortality) and chronic (juvenile production) effects of NTO on *F. candida* in WCL soil. Measurement endpoints included the number of surviving adults and the number of juveniles produced after 28 days. Exposure concentrations for definitive tests were selected to achieve bracketing of significant effects on reproduction endpoints (i.e., production of juveniles).

Results of the definitive toxicity test with NTO weathered and aged in WCL soil complied with the validity criteria defined in the ISO 11267 test guideline (ISO, 2014). The validity criteria for test results in the negative control treatment for the mean adult survival, the mean number of juveniles produced, and the CV were 100%, 141, and 16%, respectively. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the NTO treatments.

Toxicity benchmarks for NTO weathered and aged in WCL soil are summarized in Table 16. The bounded NOEC and LOEC values for NTO were 94 and 152 mg/kg, respectively, for the survival of adults, and 72 and 152 mg/kg, respectively, for the production of juveniles. The logistic Gompertz model had the best fit for both the adult survival data (acute toxicity) and juvenile production data (chronic toxicity) in WCL soil (Figure 13). The EC20 and EC50 values were 141 and 211 mg/kg, respectively, for the survival of adults, and 104 and 145 mg/kg, respectively, for the production of juveniles.

Table 16. Ecotoxicological Benchmarks for NTO Weathered and Aged in WCL Soil Determined for Survival of Adults and Production of Juveniles by *F. candida*

Ecotoxicological Parameter	NTO (mg/kg)	
	Survival	Reproduction
NOEC	94	72
p	1.0	0.781
LOEC	152	152
p	<0.0001	<0.0001
EC20	141	104
CI (95%)	105–177	69–140
EC50	211	145
CI (95%)	185–237	122–139
Model used	Gompertz	Gompertz
R^2	0.969	0.932

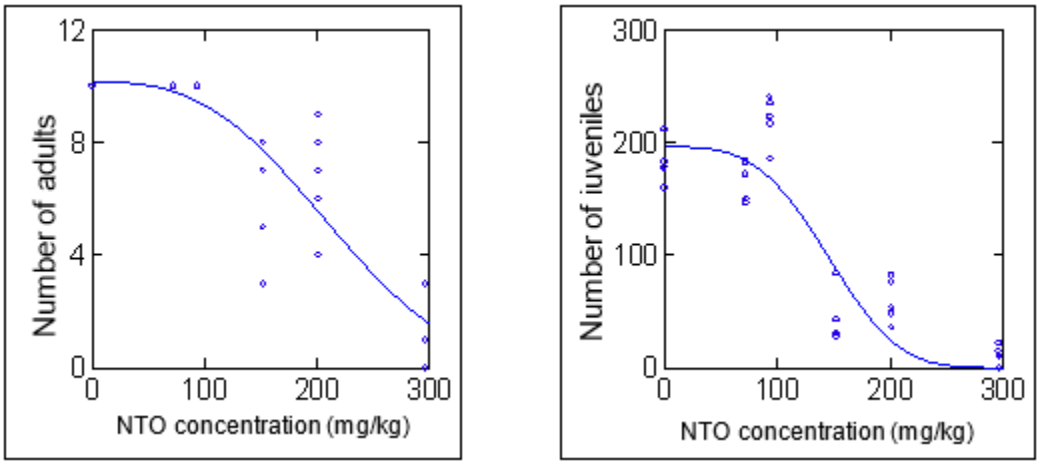

Figure 13. Effect of NTO weathered and aged in WCL soil on survival of adults (left) and production of juveniles (right) by *F. candida*.

4. DISCUSSION

Generating toxicity data to establish benchmarks that are appropriate for use when deriving the soil invertebrate-based draft Eco-SSLs for DNAN and NTO was the main objective of the present studies. Ecotoxicological testing in those studies was specifically designed to meet the criteria for Eco-SSL derivation outlined in the Eco-SSL Guideline (USEPA, 2005). The toxicity data detailed in this report were derived by using EC20 benchmark values for EM effects on soil invertebrate reproduction. These measurement endpoints were determined from standardized toxicity tests. The preferences for reproduction benchmarks and a low effect level (i.e., EC20) was justified to ensure that Eco-SSL values, when developed and approved by the USEPA, would be protective of populations of the majority of ecological receptors in soil. The Eco-SSL values would also provide confidence that IM compound concentrations posing an

unacceptable risk were not screened out early in the ERA process (i.e., the SLERA). A review of the ecotoxicological benchmarks shows that Eco-SSL requirements, including the selection and use of reproduction effects and the EC20 response level, were well justified. Reproduction measurement endpoints were more sensitive (or not statistically different, based on the EC20 values and corresponding 95% CIs) compared with adult survival in the soil invertebrate tests.

The inclusion of species from different taxonomic groups, representing a range of sensitivities, was an important consideration for selecting the test battery for Eco-SSL development because the respective sensitivities often correlated with physiologically determined modes of toxic action and can vary among taxa. The selected species were expected to represent the spectrum of diverse ecological functions that are attributed to organisms comprising different functional groups of soil invertebrates. Test species selected for the studies were representative surrogates of species that normally inhabit a wide range of site soils and geographical areas (i.e., the species are ecologically relevant). The exposure focused on ingestion of IM-contaminated soil and direct-contact exposures. These exposures were considered under conditions of very high relative bioavailability of DNAN or NTO in SSL soil. The soil invertebrate species tested are sensitive to a wide range of contaminants and represent different routes of exposure (e.g., ingestion, inhalation, and dermal absorption within the soil). Finally, selected soil invertebrate toxicity tests with representative test species have been standardized and generate reproducible, statistically valid results. This imparts greater confidence in the data and generates less uncertainty that could be associated with the decisions and recommendations that are based on the test data. Both of these are important factors for draft Eco-SSL development.

Toxicity data developed in the present research to establish benchmarks for use in the draft Eco-SSLs are intentionally conservative to provide confidence that potential contaminants that present an unacceptable risk are not screened out early in the SLERA process. The conservative nature of the benchmarks developed in this report for DNAN and NTO was achieved by:

1. utilizing natural soils with properties that support high relative bioavailability of these IMs to ecologically relevant test species;
2. using reproduction measurement endpoints for benchmark derivation; and
3. relying on a low effect level (EC20, 20% reduction from carrier control) on respective measurement endpoints.

Additional soil invertebrate toxicity studies were conducted using exposures in WCL. The QRB score for organic chemicals in natural soils was considered “medium” for WCL soil, according to the Eco-SSL criteria (USEPA, 2005); thus, WCL soil was hypothesized to pose a lower exposure risk for soil invertebrates compared with the risk in SSL soil. Results of the present studies showed that the EC50 values for either DNAN or NTO were greater (lower toxicity) in WCL in tests with potworms, whereas the toxicity of either DNAN or NTO to collembolans was not significantly different (based on EC50 values and corresponding 95% CIs)

between the two soils. Therefore, studies with WCL soil confirmed that toxicity benchmarks developed using SSL soil, which supports very high relative bioavailability of IM compounds, are sufficiently conservative for use in SLERA.

Derivation of Eco-SSL values prioritizes ecotoxicological benchmarks based on measured soil concentrations of a chemical over those based on nominal concentrations (USEPA, 2005). The exposure concentrations of DNAN and NTO in soil were analytically determined in all definitive tests from which benchmarks were determined. Furthermore, special consideration was given to the inclusion of weathering and aging of contaminant explosives in soil in the assessment of the IM effects on soil invertebrates. Consequently, ecotoxicological benchmarks for DNAN and NTO, each independently weathered and aged in SSL soil, more closely approximated the exposure conditions in the field as compared to benchmarks established in studies with freshly amended soil.

5. CONCLUSIONS

This project was undertaken specifically to develop scientifically defensible soil invertebrate-based benchmarks acceptable for deriving soil screening concentrations for DNAN and NTO. These soil screening concentrations were derived using the EC20 level toxicity benchmarks for the IM compound effects on soil invertebrate reproduction endpoints determined in standardized toxicity tests. Ecotoxicological testing was specifically designed to meet the criteria for Eco-SSL derivation outlined in the Eco-SSL Guideline (USEPA, 2005). Following the development and acceptance by the USEPA, the Eco-SSL values will allow screening of site-soil data during the SLERA to identify those IM compounds that are not of potential ecological concern and do not need to be considered in the baseline ecological risk assessment, resulting in significant cost savings during site assessments. These Eco-SSLs will also provide an indispensable tool for installation managers to gauge the ecotoxicological impacts of military operations that involve the use of DNAN and NTO and thereby ultimately promote the sustainable use of testing and training ranges.

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ACRONYMS AND ABBREVIATIONS

1,3-DNB	1,3-dinitrobenzene
2-ANAN	2-amino-4-nitroanisole
4-ANAN	4-amino-2-nitroanisole
ACN	acetonitrile
BLQ	below limit of quantification
CAS	Chemical Abstracts Service
CI	confidence interval
CL	confidence limit
CV	coefficient of variation
DAD	diode array detection
DEVCOM CBC	U.S. Army Combat Capabilities Development Command Chemical Biological Center
DNAN	2,4-dinitroanisole
1,3-DNB	1,3-dinitrobenzene
EC20	effective concentration producing a 20% effect
EC50	effective concentration producing a 50% effect (median concentration)
Eco-SSL	ecological soil screening level
ECp	effective concentration for a specified percentage effect
EM	energetic material
ERA	ecological risk assessment
FLSD	Fisher's least-significant difference
GC	gas chromatography
HPLC	high-performance liquid chromatography
IM	insensitive munitions
IMX	insensitive munition explosive
ISO	International Organization for Standardization
K_{ow}	octanol–water partition coefficient
LCS	laboratory control sample
LOAEC	lowest-observed-adverse-effect concentration
LOD	limit of detection
LOEC	lowest-observed-effect concentration
LOQ	limit of quantitation
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSp	matrix spike
MSD	matrix spike duplicate
m/z	mass-to-charge ratio
ND	not detected or not determined
NOAEC	no-observed-adverse-effect concentration
NOEC	no-observed-effect concentration
NTO	3-nitro-1,2,4-triazole-5-one
OECD	Organisation for Economic Co-operation and Development

PTFE	polytetrafluoroethylene
QRB	qualitative relative bioavailability
R^2	coefficient of determination
RDX	1,3,5-trinitro-1,3,5-triazine
SE	standard error
SLERA	screening-level ecological risk assessment
SSL	Sassafras sandy loam
TFA	trifluoroacetic acid
TNT	2,4,6-trinitrotoluene
USEPA	U.S. Environmental Protection Agency
WCL	Webster clay loam
WHC	water-holding capacity

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