ECOLOGICAL SOIL SCREENING LEVELS FOR INVERTEBRATES AT EXPLOSIVES-CONTAMINATED SITES: SUPPORTING SUSTAINABILITY OF ARMY TESTING AND TRAINING

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ABSTRACT

The Army Strategy for the Environment applies an ecosystem approach to managing natural resources on Army installations. It incorporates the principles of sustainability across the Army into all functional areas. We conducted investigations to develop critical environmental data required for successful management of Army installations in a sustainable manner, and for the knowledge-based decision making. Assessment and protection of the terrestrial environment at Army installations can be advanced by developing and applying scientifically based Ecological Soil Screening Levels (Eco-SSL) that identify concentrations of contaminant energetic materials (EM) in soil that present an acceptable ecological risk, as defined by the U.S. Environmental Protection Agency (USEPA, 2005). Without Eco-SSL, the current state of knowledge concerning the nature and extent of residual contamination with EM at U.S. Army installations is insufficient to ensure management of training and testing ranges as sustainable resources. We established the toxicity benchmark data necessary for deriving soil invertebrate-based Eco-SSL values for RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB. We determined these benchmarks using standardized toxicity bioassays. Special consideration in assessing the EM toxicity for developing ecotoxicological benchmarks was given to examining the effects of weathering and aging of EM in soil on resulting exposure effects for test species. Soil invertebrate bioassays were conducted under experimental conditions preferred for establishing toxicological benchmarks for deriving an Eco-SSL (USEPA, 2005), using a Sassafras sandy loam soil that supports relatively high bioavailability of EM. Benchmark data plus draft Eco-SSL developed in these studies will be submitted to the USEPA national Eco-SSL Work Group for use in developing soil invertebrate-based Eco-SSL for the individual EM, and will be made available for use in Ecological Risk Assessment of terrestrial habitats at the U.S. Army testing and training sites.

1. INTRODUCTION

The U.S. Army Strategy for the Environment: *Sustain the Mission – Secure the Future* established a long-range vision that enables the Army to meet its mission today and into the future (U.S. Army ASAIE, 2004). Sustainability is the foundation for this Strategy and a guiding paradigm that connects the Army's activities today to those of tomorrow with sound environmental practices. This Strategy also directs the environmental research to address both present and future Army needs to safeguard the environment and our quality of life. This Strategy aims at transitioning the Army's compliance-based environmental program to a mission-oriented approach based on the principles of sustainability through the use of innovative technologies and the principles of sustainability to enhance joint operation capability, meet current and future training and testing requirements, improve the Army's ability to operate installations, reduce costs, and minimize the environmental footprint through more sustainable practices.

Challenging the attainment of these goals is a substantially increased demand for training resources and testing facilities. Today's Soldiers, as part of the Joint Force, must be highly trained in all tasks across the spectrum of military operations. These Soldiers need demanding, highly realistic training environments to achieve full spectrum training proficiency. Increased training can lead to increased environmental impacts at training sites due, in part, to release of explosives, which may jeopardize long-term sustainability of ranges and training sites.

Manufacturing, and use of explosives during testing and training exercises, have resulted in releases of energetic materials (EM) into the environment. Consequently, soil contamination with explosives and related materials is widespread at many military installations. More than 15 million acres containing elevated levels of explosives and related materials in soil were identified in the U.S. with the estimated costs of assessments and remediation of contaminated sites ranging from $8 billion to $35 billion (U.S. General Accounting Office, 2003). Contamination at training sites due to release of explosives may pose significant risk to the Army personnel and the surrounding environment. Available data show that some EM can be persistent in the environment; however their ecotoxicological effects are incompletely unknown

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(Pennington and Brannon, 2002; Simini et al., 1995). This presents a challenge for site managers who are required to assess the environmental risks at testing and training installations in order to secure their sustainable use.

Integral to achieving sustainable use of current and future training and testing installations is the development of environmental quality criteria that can be consistently applied in order to gauge the ecotoxicological impacts of the Army operations. Assessment and protection of the terrestrial environment at the Army installations can be advanced by developing and applying scientifically based Ecological Soil Screening Levels (Eco-SSL) that identify concentrations of contaminant EM in soil that present an acceptable ecological risk. These Eco-SSL values are intended for use in Screening Level Ecological Risk Assessment (SLERA) to identify those contaminants in soil that warrant additional evaluation in a Baseline ERA (BERA), and to eliminate those that do not. Recognizing the need for establishing benchmark ecotoxicological data that are acceptable for developing the Eco-SSL values for EM in scientifically based Ecological Risk Assessment (ERA), we conducted this research.

2. TECHICAL APPROACH

2.1 Chemicals and Reagents

Crystalline EM hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX; CAS: 121-82-4; Purity: 99%), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX; CAS: 2691-41-0; Purity: 99%), 2,4,6-trinitrotoluene (TNT; CAS: 118-96-7, 99.9% purity), 2,4-dinitrotoluene (2,4-DNT; CAS: 121-14-2; Purity: 97%), 2,6-dinitrotoluene (2,6-DNT; CAS: 606-20-2; Purity: 98%), and 1,3,5-trinitrobenzene (TNB; CAS: 99-35-4; Purity: 99.7%) were obtained from the Defense Research Establishment Valcartier of the Canadian Ministry of National Defense (Val Bélair, QC, Canada). Certified standards of EM (AccuStandard, New Haven, CT, USA) were used during HPLC determinations. All other chemicals were either chromatography grade or reagent grade. Unless otherwise specified, American Society of Testing and Materials (ASTM) Type I water (ASTM, 2004) obtained using Milli-RO® 10 Plus followed by Milli-Q® PF Plus systems (Millipore®, Bedford, MA, USA) was used throughout the studies.

2.2 Test Soil Preparations

A natural soil, Sassafras Sandy Loam [SSL; Fine-loamy, siliceous, mesic Typic Hapludult] (USDA/ARS, 1999) was used in this study to assess the toxicity of RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB to soil invertebrates. The SSL soil was selected for developing ecotoxicological values protective of soil biota because it has physical and chemical characteristics that support relatively high bioavailability of EM in soil (USEPA, 2005), including low organic matter and clay contents (69% sand; 13% silt; 17% clay; 1.2% organic matter; 5.5 cmol/kg cation-exchange capacity; and pH 5.2). The SSL soil was collected from a grassland field on the property of the U.S. Army Aberdeen Proving Ground, MD. The soil was sieved through a 5-mm mesh screen, air-dried, passed through a 2-mm sieve, and then stored at room temperature before use in testing.

Treatment concentrations of individual EM in SSL soil were prepared as single batches for toxicity studies. During treatment batch preparation, each EM was mixed into soil using the organic solvent acetone as a carrier. The acetone was allowed to volatilize for a minimum of 18 h, in a dark chemical hood to prevent photolysis of EM. Amended soils were mixed for 18 h on a three-dimensional rotary soil mixer. Each soil batch representing a specific EM treatment concentration was the source of the exposure substrate for definitive tests of individual EM that were either freshly amended to SSL, or weathered-and-aged in SSL, and each soil treatment was analyzed to determine EM concentration at the time of introducing the test species.

Weathering and aging of EM in soil was conducted to simulate the EM weathering and aging process in field soils, and to more closely approximate the exposure effects on soil biota at contaminated sites. These procedures included exposing amended soils, initially hydrated to 60% of the Water Holding Capacity (WHC, 18% water on the basis of the dry SSL soil mass), in open glass containers in the greenhouse at ambient temperature to alternating wetting and drying cycles for 12-23 weeks. Each week during the weathering and aging procedure all soil treatments were weighed and readjusted to their initial mass at 60% of the WHC by adding ASTM Type I water to the soil. All soil treatments were brought to the individual test-specific moisture levels (88-100% of the WHC) 24 h prior to commencement of toxicity tests.

2.3 Extractions and Soil Analyses

Soil samples for analysis were taken after the 24-h moisture equilibration at the beginning of each definitive test. From each treatment soil batch, 2.3 g of soil was weighed in triplicate into 50-ml polypropylene centrifuge tubes, 10 ml acetonitrile was added, and the samples were vortexed for 1 min, then sonicated in the dark for 18 h at 20°C. Sonicated samples were centrifuged for 30 min at 4000 rpm. Five ml of supernatant were transferred to a glass tube, to which 5 ml of $CaCl_2$ solution (5 g/L) were added as a flocculant. Supernatant was filtered through a 0.45 μm polytetrafluoroethylene syringe cartridges. One ml of this filtered solution was transferred to a HPLC vial. The filtered samples were stored in the refrigerator at 4°C if not analyzed on the same day. The soil extracts were analyzed and EM concentrations quantified by reversed-phase HPLC (Beckman System Gold) using a modified USEPA Method 8330A (USEPA, 1998). Calibration curves were generated before each HPLC run using certified standards (AccuStandard) of each EM, in a range of concentrations appropriate for each set of determinations. The method detection limit was 0.05 mg/L corresponding to 0.5 mg/kg (dry soil mass). The acetonitrile-extractable soil concentrations of RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB (dry soil mass) were used for developing draft Eco-SSL values.

2.4 Toxicity Testing

Maintaining soil quality, fertility, and structure is essential for protecting and sustaining biodiversity and ecological integrity of terrestrial ecosystems. Central to achieving this goal is the need for a greatly improved understanding of the potential effects of EM contaminants on the sustainability of ecosystems at Army installations. Energetic soil contaminants can exert their effects directly through toxicity to soil organisms, or indirectly, by altering specific interactions and by disrupting the soil food webs. A battery of tests was required to reasonably assess the potential effects of energetic soil contaminants on populations of soil invertebrates in the field. Test species selected for our studies were representative surrogates of species that normally inhabit a wide range of site soils and geographical areas (i.e., ecologically relevant). For developing ecotoxicological benchmarks protective of soil biota, definitive tests were conducted with the soil invertebrates earthworm *Eisenia fetida* (International Standardization Organization [ISO] /11268-2, 1998), potworm *Enchytraeus crypticus* (ISO/16387, 2005), and collembola *Folsomia candida* (ISO/11267, 1999). Toxicity in soil was individually assessed for RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB, each freshly amended into SSL, and for each EM weathered-and-aged in SSL for 12-23 weeks. The effects of EM on reproduction measurement endpoints (juvenile production by each of the three test species, plus cocoon production by earthworms) were assessed using multiple treatment concentrations of each EM.

All definitive tests included negative control (no chemicals added), carrier control (acetone), and positive control. Positive control was prepared as solution of beryllium sulfate in ASTM Type I water at the test-required concentration. Each of the treatments and controls were replicated (n=4 in tests with earthworms and potworms, and n=5 in tests with collembola). The results of toxicity tests complied with validity criteria for negative conrols assessment, included in toxicity testing as part of the quality control procedures and defined in the respective ISO guidelines; plus conformed with postive controls quality assurance laboratory results. Analytically determined concentrations of EMs in soil were correlated with reproduction endpoints using regression models to determine the corresponding EC_{20} values. Draft Eco-SSL values for each EM were derived as geometric mean of EC_{20} parameters for reproduction endpoints of the three invertebrate species tested (USEPA, 2005).

2.5 Data Analysis

Linear or nonlinear regression analyses to derive ecotoxicological benchmark values were conducted on untransformed data from toxicity tests based on the concentration-response relationships for quantitative endpoint data using regression models described in Stephenson et al. (2000). Histograms of the residuals and stem-and-leaf graphs were examined to ensure that normality assumptions were met. Variances of the residuals were examined to decide whether or not to weight the data, and to select best-fit models. The 95% confidence intervals (CI) and regression coefficients (R^2) associated with the point estimates were determined. Statistical analyses were performed using SYSTAT 7.0 (SPSS Inc., 1997).

3. RESULTS

Ecotoxicological benchmarks for RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB for the 20 percent effect levels (EC_{20} values) for reproduction endpoints established in standardized definitive toxicity tests with the three soil invertebrate species in SSL soil are summarized in Table 1.

Table 1. Reproduction Toxicity Benchmarks (EC_{20} mg/kg) for Energetic Materials (EMs) Freshly Amended or Weathered-and-Aged (fresh / weathered-and-aged) in Soil Determined Using Standardized Tests for *Eisenia fetida*, *Enchytraeus crypticus*, and *Folsomia candida*.

EM	*E. fetida*		*E. crypticus*	*F. candida*
	Cocoon production	Juvenile production	Juvenile production	Juvenile production
RDX	1.2 / 19	1.6 / 5.0	3,700 / 8,800	28 / 113
HMX	2.7 / ND	0.4 / ND	NT	235 / 1,046
TNT	38 / 4*	33 / 3*	77 / 37*	17 / 53*
2,4-DNT	31 / 25	44 / 29	19 / 14	10 / 15
2,6-DNT	14 / 16	9 / 8	37 / 18*	6 / 0.96
TNB	27 / 19	21 / 13	5 / 9	4 / 48*

Table notes: All values (mg/kg, dry soil mass) are based on acetonitrile extractable concentrations in soil (USEPA

Method 8330); ND = Not Determined (no concentration-response relationship up to 600 mg/kg); NT = Not Toxic (no adverse effect up to 21,750 / 17,500 mg/kg);
* statistically significant effect of weathering and aging of energetic materials in soil on toxicity based on 95% confidence intervals.

The differential toxicity of EM to the three soil invertebrate species tested demonstrates the importance of using a battery of single-species tests for establishing ecotoxicological benchmarks for use in deriving Eco-SSL values for cyclic nitramine and nitroaromatic EM. Furthermore, our results clearly confirm that weathering and aging of EM in soil can significantly (95% CI basis) alter the toxicity to soil invertebrates (Kuperman et al., 2003; 2005; 2006; Simini et al., 2003).

The EC_{20} benchmark values for reproduction endpoints were used to derive the soil invertebrate-based draft Eco-SSL. These draft Eco-SSL values for RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB are shown in Table 2. Values were derived separately for each EM freshly amended (FA) or for EM weathered-and-aged (WA) in SSL soil. An Eco-SSL for HMX could not be developed for the weathered-and-aged treatment due to low toxicity at ≤600 mg/kg for earthworms, and at ≤17,500 mg/kg for potworms (greatest concentrations tested with respective species). We are currently conducting additional studies to establish these additional benchmark values.

Table 2. Soil Invertebrate-Based Draft Eco-SSL Values (mg/kg) for RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB Determined Using the Ecotoxicological Benchmarks Established in Individual Definitive Toxicity Tests with Sassafras Sandy Loam.

EM	FA treatment	WA treatment
RDX	21	99
HMX	6	>600
TNT	24	16
2,4-DNT	23	20
2,6-DNT	13	7
TNB	10	18

The calculated draft Eco-SSL values for EM are presented in Table 2 for comparison, and increased understanding of different outcomes based on different environmental conditions. These draft Eco-SSL are interim values because the national Eco-SSL Work Group must review experimental designs of the studies, the data produced, and their applicability, before accepting benchmarks or derivation of Eco-SSL values. Ongoing and future studies will provide additional information on the effects of HMX on soil invertebrates and will allow updating the currently reported draft Eco-SSL. Likewise, ongoing and future studies investigating the toxicity of nitroglycerin and aminonitrotoluene metabolites of nitroaromatic EM degradation in soil will establish new ecotoxicological data that will be used to expand the list of Eco-SSL.

4. DISCUSSION

The Army Strategy for the Environment represents a major advancement in the Army's appreciation of the interdependence among the mission, the community, and the environment. It builds on the four pillars of conservation, restoration, pollution prevention, and compliance, defined in the Army's Environmental Strategy published in 1992. This new Strategy applies an ecosystem approach (plus others) to managing natural resources on Army installations. It incorporates the principles of sustainability across the Army and into all functional areas. Our investigations were designed to develop critical environmental data required for successful management of Army installations in a sustainable manner, and for the knowledge-based decision making.

The main objective of this project was to generate toxicity benchmark values for soil invertebrates that can be used for developing Eco-SSL for the most common energetic soil contaminants RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB. Ecotoxicological testing was specifically designed to meet the criteria for Eco-SSL derivation outlined in the Eco-SSL Guideline (USEPA, 2005). The Eco-SSL are screening values that can be used to identify contaminants of potential concern (COPCs) in soils that require further evaluation in a BERA and to eliminate those that do not. Eco-SSL are concentrations of contaminants in soils that are protective of ecological receptors that commonly come into contact with soil or ingest biota that live in-or-on soil. Eco-SSL determined for direct soil toxicity are derived separately for two groups of ecological receptors, plants and soil invertebrates. As such, these values are expected to provide adequate protection of terrestrial ecosystems. This paper focused on developing soil invertebrate-based draft Eco-SSL values.

The draft Eco-SSL developed for these six EM, if accepted by USEPA, will be used during Step 2 of the Superfund ERA process, the screening-level risk assessment. It is expected that the Eco-SSL values will be used in the SLERA to screen site soil data to identify those EM soil contaminants that are not of potential ecological concern, so they may be eliminated from the subsequent BERA. Eco-SSL are intentionally conservative in order to provide confidence that contaminants that potentially present an unacceptable risk are not screened out early in the SLERA process. This conservation-aimed nature of Eco-SSL is achieved by using a natural soil type that has properties maximizing EM bioavailability to ecologically relevant test species, using the soil invertebrate reproduction measurement endpoints for benchmark derivation, and by relying on EC_{20} benchmark levels of the effect on measurement endpoints (20 percent reduction from controls) for Eco-SSL development.

The draft Eco-SSL apply to sites where terrestrial receptors may be exposed directly or indirectly to EM-contaminated soil. The Eco-SSL for soil invertebrates consider ingestion of soil as well as direct contact exposures. Both exposures were considered under conditions of relatively high EM bioavailability in SSL soil. By deriving soil screening values protective of this receptor group, it is assumed that the terrestrial ecosystem will be protected from possible adverse effects associated with soil contaminated with EM, when used in conjunction with Eco-SSL developed for plants, avian species, and mammalian wildlife (USEPA, 2005).

Soil physical and chemical properties affect the exposure of organisms, including terrestrial plants and soil invertebrates, to contaminants in soils. Eco-SSL are applicable to all sites where key soil parameters fall within a certain range of chemical and physical parameters (USEPA, 2005). They apply to the majority of upland aerobic soils: where the pH is greater than or equal to 4.0 and less than or equal to 8.5, and the organic matter content is less than or equal to 10%. The majority of soil toxicity tests reported in literature utilized standard artificial soil with high organic matter content (10%), which limits their usefulness for Eco-SSL derivation. In contrast, our toxicity studies were designed to specifically fill the knowledge gap regarding ecotoxicity of EM contaminants in soil, and utilized a natural soil that meets the criteria for Eco-SSL benchmark development, primarily because it has characteristics supporting relatively high bioavailability of EM. This was necessary to ensure that draft Eco-SSL values for soil invertebrates developed in this project are adequately protective for a broad range of soils within the specified boundary conditions (USEPA, 2005).

Derivation of Eco-SSL values prioritizes ecotoxicological benchmarks that are based on measured soil concentration of a chemical over those based on nominal concentrations (USEPA, 2005). In our studies, the exposure concentrations of RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB in soil were analytically determined in all definitive toxicity tests. Chemical analyses utilized the USEPA Method 8330 (USEPA, 1998) for extraction of EM from soil, and for measuring acetonitrile-extractable chemical concentrations. Comparison of results obtained using acetonitrile extraction of freshly amended soils showed good agreement between the nominal and the measured concentrations for the six energetic materials (Kuperman et al., 2003; 2005; 2006; Simini et al., 2003). This confirmed that the soil amendment procedures used in toxicity tests developing ecotoxicological benchmarks for Eco-SSL derivation were appropriate, and that the USEPA Method 8330 was efficient for determining the quantity of EMs in soil.

Among the important aspects for Eco-SSL development are selections of test methods and species for toxicity testing to generate ecotoxicological benchmarks. The USEPA preference for using standardized toxicity assays for generating benchmarks, and ecological relevance of test species in soil ecosystems was emphasized in the USEPA guidelines (USEPA, 2005). After an extensive review of existing standardized test methods, and based on the experience accumulated in the participating national and international laboratories, we selected ISO assays for toxicity testing with soil invertebrates. These assays included ISO/11268-2 Soil Quality – Effects of Pollutants on Earthworms (*Eisenia fetida*) – Part 2: Determination of Effects on Reproduction (ISO, 1998); ISO/16387 Soil Quality – Effects of pollutants on Enchytraeidae (*Enchytraeus* sp.) – Determination of Effects on Reproduction and Survival (ISO, 2005); and ISO/11267 Soil Quality – Inhibition of Reproduction of Collembola (*Folsomia candida*) by Soil Pollutants (ISO, 1999). Guidelines for these ISO assays were originally developed for use with artificial soil (OECD/USEPA Standard Artificial Soil); however research in our laboratory has shown that they can be successfully adapted for use with natural soils (Kuperman et al., 2003; 2005; 2006; Simini et al., 2003), which was necessary for Eco-SSL development. Further, the ISO/16387 assay was initially developed using the enchytraeid worm species *E. albidus*, which requires soil containing high organic matter content with soil pH 6 (±0.5) for optimal test conditions. *E. albidus* performed poorly in natural soils which have physical and chemical characteristics that support a high level of EM bioavailability (Kuperman et al., 2003; 2005; 2006). Therefore, the species of Enchytraeidae, *E. crypticus*, listed in the ISO protocol as an acceptable alternative to *E. albidus*, was selected for toxicity testing in these studies.

Energetic materials can affect populations of soil invertebrates in different ways. These include (1) direct acute toxicity, (2) chronic toxicity such as effects on growth or reproduction, (3) indirect toxicity by altering soil structure or fertility, (4) indirect toxicity adversely affecting nutrient and food supplies, or (5) by affecting predators and parasites. In addition, soil organisms may alter their environment changing the overall bioavailability of chemicals within the soil. No single test can address all these types of effects, thus a battery of tests is required to reasonably do so. Inclusion of species from different taxonomic groups representing a range of sensitivities, which often correlate with physiologically-determined modes of action and can vary among taxa, was an important consideration for selecting the test battery for Eco-SSL development. The selected species are expected to represent the spectrum of diverse ecological functions that are attributed to organisms comprising soil communities. Test species selected for our studies are representative surrogates of species that normally inhabit a wide range of Army site soils, and geographical areas (i.e., ecologically relevant). Test invertebrate species used in these investigations actively move through soil, thus ensuring contact with contaminants. The soil invertebrate species tested are sensitive to a wide range of contaminants, and reflect different routes of exposure (e.g., ingestion, dermal absorption). It was important for Eco-SSL development, that selected test invertebrate species were amenable to life-cycle tests to identify vulnerable developmental stages of the test organisms (e.g., adult survival, cocoon or juvenile production). Finally, selected soil invertebrate toxicity tests with representative test species, have been standardized and generate reproducible, statistically-valid results, which imparts a greater confidence in the data and generates less uncertainty associated with the decisions and recommendations that are based on the test data, including Eco-SSL development.

A draft Eco-SSL for an EM-receptor pairing (e.g., RDX-invertebrates) was calculated as the geometric mean of EC_{20} benchmark toxicity values determined from the individual studies. Three toxicity data values generated under specified conditions were the minimum required to calculate an Eco-SSL (USEPA, 2005). Separate draft Eco-SSL values were derived for freshly amended, and for weathered-and-aged EM in soils. Reproduction measurement endpoints in tests with soil invertebrates were more sensitive compared with adult survival (Kuperman et al., 2003; 2005; 2006; Simini et al., 2003). This supported the Eco-SSL requirement of using of reproduction endpoints for the soil invertebrate-based benchmark development (USEPA, 2005). Consequently, reproduction measurement endpoints were used for derivation of draft Eco-SSL values for soil invertebrates. These endpoints included cocoon production and juvenile production for earthworms, and juvenile production for potworms and collembola.

Draft Eco-SSL for soil invertebrates were developed for RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB freshly amended in SSL soil, and for RDX, TNT, 2,4-DNT, 2,6-DNT, and TNB weathered-and-aged in soil. No Eco-SSL for soil invertebrates could be developed for HMX weathered-and-aged in amended soil because EC_{20} value was established only for one test species, *F. candida*. HMX did not adversely affect reproduction of either earthworms or potworms in weathered-and-aged treatments at concentrations tested.

Review of ecotoxicological benchmark values, herein used for developing draft Eco-SSL values under different stipulations, shows that, although majority of values were fairly uniform, there were instances of high variability among EC_{20} estimates determined in toxicity bioassays. Greatest differences were found for earthworm and potworm juvenile production benchmark values for RDX, which ranged from 1.6 mg/kg for earthworm to 3,715 mg/kg for potworm in studies with freshly amended soil; and from 5 to 8,800 mg/kg, respectively, in studies with RDX weathered-and-aged in soil. Large differences were also found for benchmark estimates of juvenile production by earthworm and collembola exposed to HMX in soil, which ranged from 0.4 mg/kg for earthworm to 235 mg/kg for collembola in studies with freshly amended soil, while no adverse effect was evident for potworm juvenile production up to 21,750 mg/kg, the greatest HMX concentration tested. These examples of species-specific variability in toxicity endpoint values provide clear evidence in support of the USEPA requirement for use of multiple species for generating ecotoxicological benchmarks for Eco-SSL development, and for having selection rules for determining which data are most appropriate for developing Eco-SSL.

The experimental designs of our ecotoxicological investigations complied with all screening criteria used by the national Eco-SSL Workgroup for selecting soil invertebrate benchmarks for Eco-SSL development. The toxicity benchmark data developed in this project will be provided to the Eco-SSL National Taskgroup for quality control review of the experimental designs of the studies, the data produced, and applicability, before entering the benchmarks into the Eco-SSL database for deriving national Eco-SSL for RDX, HMX, TNT 2,4-DNT, 2,6-DNT, and TNB.

5. CONCLUSIONS

The Army Strategy for the Environment incorporates the principles of sustainability across the Army and into all functional areas. Our investigations established critical environmental data required for successful management of Army installations in a sustainable manner and for the knowledge-based decision making. Assessment and protection of the terrestrial environment at the Army installations will be advanced by applying scientifically based Eco-SSL developed on the basis of toxicity benchmark data for soil invertebrates established in our definitive studies with RDX, HMX, TNT, 2,4-DNT, 2,6-DNT, and TNB. These Eco-SSL values will allow screening of site soil data to identify those EM contaminants that are not of potential ecological concern and do not need to be considered in the BERA, resulting in significant cost-savings during site assessments and remedial investigations. Furthermore, these Eco-SSL will provide an indispensable tool for the Army installation managers to gauge the ecotoxicological impacts of the Army operations that involve the use of explosives, thus ultimately promoting the sustainable use of testing and training ranges by today's and future Warfighters.

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