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DERIVATION OF DRAFT ECOLOGICAL SOIL SCREENING LEVELS FOR TNT AND RDX UTILIZING TERRESTRIAL PLANT AND SOIL INVERTEBRATE TOXICITY BENCHMARKS

Ronald T. Checkai
Roman G. Kuperman
Michael Simini
Carlton T. Phillips

RESEARCH AND TECHNOLOGY DIRECTORATE

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14. ABSTRACT

The ecotoxicological benchmarks developed for terrestrial plants and soil invertebrates were compiled, reviewed, and used in this report to develop scientifically defensible terrestrial plant-based and soil invertebrate-based draft ecological soil screening levels (Eco-SSLs) for 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The draft Eco-SSL values were derived using the EC_{20} (the 20% negative) level toxicity benchmarks for the effects of each explosive compound as a contaminant in soil and on plant growth or soil invertebrate reproduction endpoints, which were determined in standardized toxicity tests. Ecotoxicological testing was specifically designed and reviewed for these studies to meet the U.S. Environmental Protection Agency (USEPA) criteria for Eco-SSL derivation. Following acceptance by the USEPA, these draft Eco-SSL values will be used to screen site-soil data to identify the contaminants that are not of potential ecological concern and do not need to be considered in the baseline ecological risk assessment, which will result 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 explosives, thus ultimately promoting the sustainable use of testing and training ranges.

15. SUBJECT TERMS

Ecological soil screening level	TNT	Terrestrial plants	Weathering-and-aging
Toxicity assessment	RDX	Soil invertebrates	Natural soils

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PREFACE

The work described in this report was authorized under Project No. SERDP CU-1210. The work was started in April 2002 and completed in November 2011.

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1. INTRODUCTION

The manufacturing and use of explosives during testing and training exercises, have resulted in the release of energetic materials (EM) into the environment. Consequently, soil contamination with explosives, propellants, and related materials at many U.S. military installations is widespread. The extent of land that has become contaminated exceeds 15 million acres by some accounts (U.S. Government Accounting Office [USGAO], 2003). Soil contaminated with EM may pose significant risks to military personnel, the surrounding environment, and off-site human and ecological receptors, thereby jeopardizing the long-term sustainability of military ranges and training sites. Although available data shows that some EM compounds can be persistent and highly mobile in the environment, their affects on terrestrial ecological receptors have not been sufficiently investigated. Among the most prevalent EM residues found in soil at testing and training ranges are 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Therefore, development of ecotoxicological benchmarks for these explosive compounds within soil has become a critical need in the United States.

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 to gauge the ecotoxicological impacts of military operations. Assessment and protection of the terrestrial environment at defense installations can be advanced by developing and applying scientifically based ecological soil screening levels (Eco-SSLs; http://www.epa.gov/ecotox/ecossl/)* for EM compounds released into soil (U.S. Environmental Protection Agency [USEPA], 2005). Eco-SSL values are concentrations of contaminants in upland aerobic soils that, when not exceeded, are deemed protective of ecological receptors that commonly come into contact with soil or ingest biota that live in or on such soils (USEPA, 2005). These values can be used in the screening-level ecological risk assessment (SLERA) to identify the contaminants that are not of potential ecological concern in soils, and thus do not require further evaluation in the baseline ecological risk assessment (BERA), which will result in cost savings during ecologically based site assessments.

Despite the considerable attention given to assessing ecotoxicities of various energetic compounds, an extensive literature review (Kuperman et al., 2009a) showed that the available data for TNT and RDX were insufficient to develop Eco-SSL values for terrestrial plants and soil invertebrates. Several definitive multiyear ecotoxicity studies were conducted to fill the existing data gaps (Kuperman et al., 2003; 2004; 2005; 2006a,b; 2012 in press; Phillips et al., 2012 in press; Rocheleau et al., 2003; 2006; Simini et al., 2003; 2006a; 2012a,b in press). These studies were designed to specifically meet the USEPA criteria (USEPA, 2005) for derivation of acceptable toxicity benchmarks for Eco-SSL development, and to expand ecotoxicological data that can aid site managers in the knowledge-based decision-making

* Last accessed 9 August 2012.

process for securing the sustainable use of testing and training installations. The ecotoxicological benchmarks developed in these studies for terrestrial plants and soil invertebrates were compiled, reviewed, and utilized in this report for derivation of draft Eco-SSLs for TNT and RDX. The general concepts of USEPA guidelines (USEPA, 2005) for Eco-SSL development are summarized in this report to assist users in reviewing and interpreting its findings.

2. TECHNICAL APPROACH

Natural light-textured upland aerobic soils, Teller sandy loam (TSL [fine-loamy, mixed, active, thermic Udic Argiustoll]) or Sassafras sandy loam (SSL [fine-loamy, siliceous, semiactive, mesic Typic Hapludult]) were utilized in the definitive toxicity studies (referenced above) to develop ecotoxicological benchmarks for utilization in deriving draft Eco-SSL values. These soils had low organic matter and clay contents, which fulfilled the USEPA requirement for utilizing upland aerobic soil with characteristics that support high relative bioavailability of organic contaminants, for developing realistic yet conservative Eco-SSL values (USEPA, 2005). The physical and chemical characteristics of these TSL and SSL soils are presented in Table 1.

Table 1. Physical and Chemical Characteristics of Soils Utilized in Toxicity Testing

Soil Parameter	TSL	SSL
Sand %	65	70
Silt %	22	13
Clay %	13	17
Texture	sandy loam	sandy loam
CEC cmol kg^{-1}	4.3	5.5
Organic Matter %	1.4	1.2
pH	4.4	5.2

Selection of appropriate test methods and test species for toxicity testing to generate appropriate ecotoxicological benchmarks is among the important aspects in the process of developing benchmarks and deriving a draft Eco-SSL. The USEPA preference for using standardized toxicity assays for generating benchmarks and the importance of ecological relevance of test species within the soil ecosystem were emphasized in the draft guideline (USEPA, 2005).

Several terrestrial toxicity tests have been developed, or improved by standardization, by different agencies and organizations. Leading among them are the International Organization for Standardization (ISO), the American Society for Testing and Materials (ASTM), Environment Canada (EC), Organization for Economic Co-operation and Development (OECD), and USEPA.

After an extensive review of existing standardized test methods and based on the experience accumulated in the previous ecotoxicity studies, the ASTM standard guide for conducting terrestrial plant toxicity tests (ASTM, 2002) and the USEPA early seedling growth toxicity test (USEPA, 1996) were selected for assessing TNT or RDX effects on terrestrial plants. The toxicity studies were conducted using the following plant species:

- Dicotyledonous symbiotic species alfalfa (*Medicago sativa* L.)
- Monocotyledonous species barnyard grass (also referred to as Japanese millet in some publications) (*Echinochloa crusgalli* [L]. Beauv.)
- Monocotyledonous species Perennial ryegrass (*Lolium perenne* L.)

These three plant species were sensitive to the EM compounds tested and had performance parameters in SSL or TSL soil that were sufficient to meet the validity criteria that were required for these standardized definitive toxicity tests.

Toxicity testing with soil invertebrates was conducted using the ISO assays for earthworms (*Eisenia fetida*), potworms (*Enchytraeus crypticus*), and Collembola (springtails [*Folsomia candida*]). The specific assays were

- Earthworm: ISO/11268-2:1998 *Soil Quality—Effects of Pollutants on Earthworms* (Savigny, 1826)*—Part 2: Determination of Effects on Reproduction* (ISO, 1998a)
- Potworm: ISO/16387 *Soil quality—Effects of pollutants on Enchytraeidae* (*Enchytraeus sp.*)*—Determination of effects on reproduction and survival* (ISO, 2004), with the potworm species *Enchytraeus crypticus* (Westheide and Graefe, 1992) selected as the species for establishing benchmarks for draft Eco-SSL development
- Springtails: ISO/11267 *Soil quality—Inhibition of Reproduction of Collembola* (Willem, 1902) *by Soil Pollutants* (ISO, 1998b)

Guidelines for these ISO assays were originally developed for use with OECD artificial soil (OECD, 1984). Similar artificial soil formulation was later adapted for USEPA standard artificial soil (SAS [USEPA, 1996]) and for ASTM artificial soil (ASTM E1676-04, 2004). However, research studies, including those with EM compounds, have demonstrated that these ISO assays can be successfully adapted for use with natural soils (Amorim et al., 2009; 2005a,b; Dodard et al., 2005; Kuperman et al., 1999; 2003; 2004; 2005; 2006a–d; 2009a,b; 2012; Robidoux et al., 2002; 2004; Simini et al., 2003; 2006b; 2012b), which was necessary for establishing benchmarks for draft Eco-SSLs development.

Toxicological benchmarks utilized in the derivation of respective draft Eco-SSLs for TNT and RDX were determined in definitive studies on the basis of concentration-response relationships, using regression models selected from among those described in EC Guidance Document (EC, 2005). All the benchmarks were established using acetonitrile extractable concentrations of TNT or RDX in soil, using USEPA Method 8330A (USEPA, 2007). A draft Eco-SSL for an EM-receptor pairing (e.g., TNT-invertebrates) was calculated as the geometric mean of the EC_{20} (i.e., the 20% negative effect levels) toxicity benchmarks determined in individual toxicity studies. Growth measurement endpoints, including fresh and dry shoot mass, were used for developing toxicity benchmarks for terrestrial plants. Reproduction measurement endpoints, including cocoon production and juvenile production for the earthworms and juvenile production for the potworms and Collembola, were used to develop toxicity benchmarks for soil invertebrates. Selection of these measurement endpoints complied with USEPA requirement of using growth or reproduction endpoints for developing toxicity benchmarks in the derivation of Eco-SSLs for terrestrial plants and soil invertebrates, respectively (USEPA, 2005). The derivation process for the draft Eco-SSL values was completed separately for terrestrial plants and soil invertebrates for each EM weathered-and-aged in soil. The minimum number of benchmarks required by USEPA to derive an Eco-SSL are three independent toxicity benchmark values, generated under specific conditions detailed within Eco-SSL Guidance (USEPA, 2005). The research conditions met specified the USEPA conditions and benchmarks exceeded USEPA requirements for deriving an Eco-SSL.

3. DERIVATION OF DRAFT ECO-SSL VALUES FOR TNT AND RDX

Phytotoxicity benchmarks (EC_{20} values for growth inhibition endpoints), utilized for the derivation of the terrestrial plant-based draft Eco-SSL for TNT weathered-and-aged in soil, are presented in Table 2. These benchmarks were established in studies with TSL or SSL soil reported by Simini et al. (2006a; 2012a) and in separate studies with SSL soil reported by Rocheleau et al. (2003; 2006). A total of 18 phytotoxicity benchmarks developed in these studies were utilized to derive a draft Eco-SSL for TNT, yielding an Eco-SSL value of 8 mg kg^{-1} (soil dry mass basis; Table 2).

Table 2. Derivation of Terrestrial Plant-Based Draft Eco-SSL Value for TNT Weathered-and-Aged in SSL or TSL Soils, Utilizing Growth Benchmarks for Alfalfa (*Medicago sativa*), Barnyard Grass (*Echinochloa crusgalli*), and Perennial Ryegrass (*Lolium perenne*)

Receptor Group	Soil	EC_{20} (mg kg^{-1})	95% Confidence Internals (mg kg^{-1})	**Draft Eco-SSL (mg kg^{-1})**
Alfalfa				**8**
Fresh mass	SSL	7[A]	4–11	
	SSL	3[B]	1–4	
Dry mass	SSL	10[A]	4–16	
	SSL	1.4[B]	0.5v2.3	
Fresh mass	TSL	12[C]	1–22	
Dry mass	TSL	18[C]	3v33	
Barnyard grass				
Fresh mass	SSL	5[A]	4–6	
	SSL	6[B]	3–10	
Dry mass	SSL	6[A]	5–7	
	SSL	11[B]	1–21	
Fresh mass	TSL	21[C]	7–34	
Dry mass	TSL	28[C]	6–50	
Ryegrass				
Fresh mass	SSL	7[A]	5–8	
	SSL	15[B]	6–23	
Dry mass	SSL	7[A]	5–8	
	SSL	13[B]	1–24	
Fresh mass	TSL	8[C]	4–12	
Dry mass	TSL	5[C]	0–10	

Notes: All values are based on acetonitrile extractable concentrations (USEPA Method 8330A; USEPA, 2007). Phytotoxicity data for TNT were established for each test species in two separate studies with SSL soil ([A] Simini et al., 2006a and [B] Rocheleau et al., 2006), and for the same species in studies with TSL soil ([C] Simini et al., 2012a).

Phytotoxicity benchmarks (EC_{20} values for growth inhibition endpoints), utilized for the derivation of the terrestrial plant-based draft Eco-SSL for RDX weathered-and-aged in soil, are presented in Table 3. These benchmarks were established in studies with TSL or SSL soils reported by Simini et al. (2012a). There was no significant ($p > 0.05$) inhibition of alfalfa growth (compared with growth in carrier control soil; acetone) in the respective limit tests at 9929 mg kg^{-1} in SSL soil and at 8320 mg kg^{-1} in TSL soil (Simini et al., 2012a). Therefore, data for alfalfa were not used in the derivation of draft Eco-SSL for RDX. The Limit Test, a variant of a definitive test, was performed when statistical analysis of the range-finding test data showed no significant inhibition in a measurement endpoint (compared with carrier control soil; acetone) at the highest concentration tested (nominal 10,000 mg kg^{-1} in studies with RDX). Seven phytotoxicity benchmarks, developed in studies by Simini et al. (2012a), were utilized to derive a draft Eco-SSL for RDX, yielding an Eco-SSL value of 71 mg kg^{-1} (soil dry mass basis; Table 3).

Table 3. Derivation of Terrestrial Plant-Based Draft Eco-SSL Value for RDX Weathered-and-Aged in SSL or TSL Soils, Utilizing Growth Benchmarks for Alfalfa (*Medicago sativa*), Barnyard Grass (*Echinochloa crusgalli*), and Perennial Ryegrass (*Lolium perenne*)

Receptor Group	Soil	EC_{20} (mg kg^{-1})	95% Confidence Internals (mg kg^{-1})	**Draft Eco-SSL (mg kg^{-1})**
Barnyard grass				**71**
Fresh mass	SSL	ND*	ND	
Dry mass	SSL	33	10–57	
Fresh mass	TSL	100	2–197	
Dry mass	TSL	73	30–115	
Ryegrass				
Fresh mass	SSL	51	4–98	
Dry mass	SSL	78	0–157	
Fresh mass	TSL	91	0–204	
Dry mass	TSL	104	0–237	

Notes: All values are based on acetonitrile-extractable concentrations in soil (USEPA Method 8330A; USEPA, 2007). Phytotoxicity data for RDX were established for each test species in separate studies with SSL or TSL soil (Simini et al., 2012a). *ND (Not Determined): could not be determined within the concentration range tested in the study.

Soil invertebral toxicity benchmarks (EC_{20} values for reproduction endpoints), utilized for the derivation of the soil invertebrate-based draft Eco-SSL for TNT weathered-and-aged in soil, are presented in Table 4. These benchmarks were established in studies with TSL or SSL soil reported by Kuperman et al. (2005; 2006a; 2012), Phillips et al. (2012), and Simini et al. (2012b). Eight invertebral toxicity benchmarks developed in those studies were utilized to derive a draft Eco-SSL for TNT, yielding an Eco-SSL value of 15 mg kg^{-1} (Table 4).

Table 4. Derivation of Soil Invertebrate-Based Draft Eco-SSL Value for TNT Weathered-and-Aged in SSL or TSL Soils, Utilizing Reproduction Benchmarks for Earthworm (*Eisenia fetida*), Potworm (*Enchytraeus crypticus*), and Collembolan (*Folsomia candida*)

Receptor Group	Soil	EC_{20} (mg kg^{-1})	95% Confidence Internals (mg kg^{-1})	**Draft Eco-SSL (mg kg^{-1})**
Earthworm				
Cocoon production	SSL	4[A]	1–7	
Juvenile production	SSL	3[A]	0.5–5	
Cocoon production	TSL	26[B]	15–38	
Juvenile production	TSL	6[B]	3–10	
Potworm				**15**
Juvenile production	SSL	37[C]	29–44	
Juvenile production	TSL	26[D]	19–32	
Collembola				
Juvenile production	SSL	53[A]	44–63	
Juvenile production	TSL	21[E]	7–35	

Notes: All values are based on acetonitrile extractable concentrations in soil (USEPA Method 8330A; USEPA, 2007). Toxicity data for TNT were reported in the following sources: [A] Kuperman et al., 2006a; [B] Simini et al., 2012b; [C] Kuperman et al., 2005; [D] Kuperman et al., 2012; [E] Phillips et al., 2012.

Soil invertebral toxicity benchmarks (EC_{20} values for reproduction endpoints), utilized for the derivation of the soil invertebrate-based draft Eco-SSL for RDX weathered and aged in soil are presented in Table 5. These benchmarks were established in studies with TSL or SSL soil reported by Kuperman et al. (2003; 2006a; 2012), Phillips et al. (2012), and Simini et al. (2003; 2012b). Eight invertebral-toxicity benchmarks developed in those studies were used to derive draft Eco-SSL for RDX, yielding an Eco-SSL value of 72 mg kg^{-1} (Table 5).

Table 5. Derivation of Soil Invertebrate-Based Draft Eco-SSL Value for RDX Weathered-and-Aged in SSL or TSL Soils, Utilizing Reproduction Benchmarks for Earthworm (*Eisenia fetida*), Potworm (*Enchytraeus crypticus*), and Collembolan (*Folsomia candida*)

Receptor Group	Soil	EC_{20} (mg kg^{-1})	95% Confidence Internals (mg kg^{-1})	Draft Eco-SSL (mg kg^{-1})
Earthworm				
Cocoon production	SSL	19[A]	0–39	
Juvenile production	SSL	5[A]	0.2–9	
Cocoon production	TSL	9[B]	1–16	
Juvenile production	TSL	4[B]	0.3–7	
Potworm				72
Juvenile production	SSL	8,800[C]	761–16,834	
Juvenile production	TSL	13,000[D]	10,000–16,300	
Collembola				
Juvenile production	SSL	113[E]	29–197	
Juvenile production	TSL	16[F]	4–28	

<u>Table notes:</u> All values are based on acetonitrile extractable concentrations in soil (USEPA Method 8330A; USEPA, 2007). Toxicity data for RDX were reported in the following sources: [A] Simini et al., 2003; [B] Simini et al., 2012b; [C] Kuperman et al., 2003; [D] Kuperman et al., 2012; [E] Kuperman et al., 2006a; [F] Phillips et al., 2012

4. DISCUSSION

Generating toxicity data to establish benchmarks that are appropriate for use when deriving the terrestrial plant-based and the soil invertebrate-based draft Eco-SSLs for the most common energetic soil contaminants, TNT and RDX, was among the main objectives of the studies reviewed in this report. 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 draft Eco-SSL values detailed in this report were derived utilizing EC_{20} benchmark values for EM effects on plant growth or soil invertebrate reproduction. These measurement endpoints were determined from standardized toxicity tests. The preference for growth (plant) and reproduction (soil invertebrate) benchmarks and for low effect level (i.e., EC_{20}) was justified to ensure that Eco-SSL values would be protective of populations of the majority of ecological receptors in soil. The Eco-SSL values would also provide confidence that EM concentrations posing an unacceptable risk were not screened out early in the ecological risk assessment (ERA) process (i.e., SLERA). Review of the ecotoxicological benchmarks shows that Eco-SSL requirements, including the selection and use of growth/reproduction effects and of the EC_{20} response level, are well justified. Growth measurement endpoints were more sensitive indicators of EM effects on terrestrial plants compared with seedling emergence. Reproduction measurement endpoints were more sensitive (or not statistically different, based on the EC_{20} values and corresponding 95% CI) 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 soil communities: primary producers and 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). For Eco-SSLs of terrestrial plants, the exposure focused on direct contact with EMs in soils. For soil invertebrates, the exposure focused on ingestion of EM-contaminated soil and direct-contact exposures. These exposures were considered under conditions of high relative bioavailability of EM in SSL or TSL soil. The terrestrial plant and soil invertebrate species tested are sensitive to a wide range of contaminants, and represent different routes of exposure (e.g., ingestion, inhalation, dermal absorption within soil for soil invertebrates, and uptake from soil solution for plants). Finally, selected terrestrial 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.

A review of ecotoxicological benchmarks used to derive draft Eco-SSLs shows that although the majority of values were fairly uniform, there were instances of substantial variability among the EC_{20} estimates determined in toxicity tests. The greatest differences were found for RDX effects on the soil invertebrate reproduction benchmarks, which ranged from 4 mg kg^{-1} for the earthworms to 13,000 mg kg^{-1} for the potworms (Table 5). This example of species-specific variability in toxicity provides clear evidence in support of the USEPA requirement for using multiple species to generate ecotoxicological benchmarks for Eco-SSL development, and for having selection criteria to determine which data are most appropriate for developing Eco-SSLs.

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 Eco-SSLs developed in this report for TNT and RDX was achieved by

1. utilizing natural soils with properties that supported high relative bioavailability of these EMs to ecologically relevant test species

2. using growth (for terrestrial plants) or reproduction (for soil invertebrates) measurement endpoints for benchmark derivation

3. relying on a low effect level (EC_{20}; 20% reduction from carrier control) on respective measurement endpoints

4. using the geometric mean of the respective benchmarks to establish an Eco-SSL value (i.e., more conservative than an arithmetic mean)

Conservative yet realistic soil-screening values protective of these receptor groups were derived. It was assumed that when these respective TNT and RDX Eco-SSL values for soil invertebrates and plants are used in conjunction with corresponding values developed for avian and mammalian wildlife, the terrestrial ecosystem will be protected from unacceptable adverse effects associated with upland aerobic soil that is contaminated with TNT or RDX.

These draft Eco-SSLs are applicable to all sites where key soil parameters fall within a certain range of chemical and physical parameters (USEPA, 2005). They apply to upland aerobic soils where the pH is ≥4.0 and ≤8.5 and the organic matter content is ≤10%. The majority of soil toxicity tests that were reported in literature utilized SAS with high organic matter content (10%), which limited their usefulness for Eco-SSL derivation. In contrast, ecotoxicological benchmarks, utilized in this report for developing draft Eco-SSLs for TNT and RDX, were established in toxicity studies. These studies were performed utilizing natural soils that met the criteria for Eco-SSL development, in large part, because they are aerobic upland soils that have characteristics (low organic matter and clay content; Table 1) supporting the high relative bioavailability of EMs. This was necessary to ensure that the draft Eco-SSLs for terrestrial plants and soil invertebrates developed in this project were adequately conservative 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 concentrations of a chemical over those based on nominal concentrations (USEPA, 2005). The exposure concentrations of TNT or RDX in soil were analytically determined in all definitive tests from which benchmarks were determined, reported, and utilized in the derivation of draft Eco-SSL values included in this report. Analytical determinations were performed using the USEPA Method 8330A (USEPA, 2007), both for extraction of EMs from soil and for measuring acetonitrile-extractable chemical concentrations. Furthermore, for draft Eco-SSL development, special consideration was given to the inclusion of weathering-and-aging of contaminant explosives in soil in the assessment of the EM effects on terrestrial receptors. Consequently, ecotoxicological benchmarks for TNT and RDX, each independently weathered-and-aged in TSL or SSL soil, more closely approximate the exposure conditions in the field, compared to benchmarks established in studies with freshly amended soil. These benchmarks are more relevant for ERA because Eco-SSL development by USEPA was specifically undertaken for use at Superfund sites, locations where contaminants have long been present.

To ensure that draft Eco-SSL values developed in this report comply with all criteria and would obtain the highest score in each selection criteria category, experimental designs of toxicity tests used to establish the respective benchmarks for TNT and RDX were evaluated using the literature evaluation criteria accepted by the Eco-SSL Workgroup (USEPA, 2005), summarized in Table 6. Such review will expedite the transition of the results of these investigations and the derivations of the respective TNT and RDX draft Eco-SSL values to the USEPA Eco-SSL workgroup. This workgroup will also apply rules of selection to determine the most appropriate benchmarks for establishing the respective Eco-SSL values for TNT and RDX.

Table 6. Summary of Literature Evaluation Process for Plant and Soil Invertebrate Eco-SSLs (modified from USEPA, 2005)

Criteria	Rationale
1: Testing is performed under conditions of high bioavailability.	Bioavailability of metals and polar organic compounds is influenced by pH and soil organic matter, cationic exchange capacity, and clay content. The scoring is intended to favor relatively high bioavailability.
2 (A) Laboratory and **(B)** field: Experimental designs for studies are documented and appropriate.	Experimental design can significantly influence the quality of a study. Higher quality studies will use an experimental design that is sufficiently robust to allow analysis of the test variables and discriminate nontreatment effects.
3: Concentration of test substance in soil is reported.	The concentration of the contaminant tested must be reported unambiguously.
4: Control responses are acceptable**.**	Negative controls are critical to distinguish treatment effects from nontreatment effects.
5: Chronic or life cycle test was used**.**	Chronic toxicity tests assessing long-term adverse sublethal impacts on the life-cycle phases of an organism are considered superior to acute toxicity tests.
6: Contaminant dosing procedure is reported and appropriate for contaminant and test.	Contaminant dosing procedure may affect the outcome of a test. Dosing procedure should include: (A) The form of the contaminant; (B) The carrier or vehicle (e.g., solvent, water, etc.); (C) How the carrier was dealt with following dosing (i.e., allowed to volatilize, controls, etc.); and (D) procedure for mixing of soil with contaminant (homogeneity).
7: A dose-response relationship is reported or can be established from reported data.	Two methodologies that can be used to identify this benchmark concentration exist. The first method generates a no-observed effect concentration (NOEC) and a lowest-observed effect concentration (LOEC). The second method uses a statistical model to calculate a dose-response curve and estimate an effect concentration for some percentage of the population (EC_x), usually between an EC_5 and an EC_{50}.
8: The statistical tests used to calculate the benchmark and the levels of significance are described.	Statistical tests and results reported in the study should be sufficient to determine the significance of the results.
9: The origin of the test organisms is described.	The results of a toxicity test can be influenced by the condition of the test organisms. Culture conditions should be maintained so that the organisms are healthy and have had no exposure above background to contamination prior to testing (invertebrates) or detailed information is provided about the seed stock (plants).

Information relevant for each criterion of the evaluation processes is summarized as follows:

1. Natural soils, TSL (fine-loamy, mixed, active, thermic Udic Argiustoll) or SSL (fine-loamy, siliceous, mesic Typic Hapludult) were utilized in the studies to assess the EM toxicity for the chosen test species. These soils were selected for developing ecotoxicological values protective of soil biota because they are upland aerobic soils that have physical and chemical characteristics supporting the high relative bioavailability of TNT and RDX (USEPA, 2005).

2. Toxicity assays were conducted to determine the effects of TNT or RDX on terrestrial plants and soil invertebrates. Testing was designed to specifically meet the criteria required for Eco-SSL development. All the methods used were documented within the cited publications and included detailed accounts of individual studies. All assays included range-finding tests to bracket a TNT or RDX concentration range for each test species and definitive tests to determine ecotoxicological benchmarks required for development of draft Eco-SSL values.

3. Nominal concentrations were analytically verified in all definitive test treatments. All ecotoxicological parameters were determined using measured chemical concentrations of each treatment level.

4. Each toxicity test was appropriately replicated and included negative (no chemicals added), positive (reference chemical), and carrier (acetone) controls. Test validity criteria were met in all the definitive assays. Validity criteria in definitive toxicity tests with terrestrial plants specified minimal percent germination in negative controls for each species tested and the quality control limit for EC_{50} values in a positive control (boric acid). Validity criteria for negative controls in the definitive toxicity tests with soil invertebrates specified the minimal percent adult survival, the minimal number of juveniles produced, and the boundaries for a coefficient of variation for reproduction. A positive control was prepared as a solution of beryllium sulfate in ASTM type I water at the test-required concentration.

5. Toxicity tests were based on the assessments of EM effects on growth (for plants) and reproduction (for soil invertebrates). Although not utilized in the derivation of Eco-SSL values, the additional endpoints for seedling emergence and adult survival, respectively, were determined for comparison to the historic database of acute measurement endpoints.

6. Soil amendment procedures were documented to include the form of the EMs used, analytical purity of each EM, procedures for the preparation of treatment concentrations using an acetone carrier, time allowed to volatilize acetone in the chemical hood, and duration of three-dimensional mixing to ensure the homogeneity of EM incorporation in test soil.

7. Toxicity data were analyzed using appropriate regression models to establish concentration–response relationships for each EM–test species measurement endpoint pairing. SYSTAT software version 11 (Systat Inc., Chicago, IL) was used to determine the EC_{20} (and EC_{50} values) for seedling emergence and growth endpoints in the phytotoxicity assays and cocoon/juvenile production in the soil invertebrate assays. The EC_{20} benchmark is preferred for deriving Eco-SSL values. The EC_{50}, a commonly reported benchmark, was included to enable comparisons of the results produced in this study with results reported by other researchers.

8. The statistical tests included regression analyses and analysis of variance (ANOVA). Regression analyses were performed using SYSTAT software, version 11. 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 appropriate mathematical models. The asymptotic standard error (a.s.e.) and 95% confidence intervals (CI) associated with the point estimates were determined. Mathematically modeled concentration–response relationships are preferred for establishing benchmarks for use in Eco-SSL derivation (USEPA, 2005) and were utilized to derive the draft Eco-SSL values in this report. ANOVA was used to determine the bounded NOEC and LOEC values. Mean separations were done using Fisher's-least-significant-difference (FLSD) pairwise comparison tests. A significance level of $p \leq 0.05$ was accepted for determining the NOEC and LOEC values. Student's *t*-Test (two-tailed), with the significance level set at $p \leq 0.05$, was used in the limit tests with plants and invertebrates exposed to RDX using Microsoft Excel 2007 software (Microsoft Corporation, Redmond, WA).

9. Sources of seed stocks and soil invertebrates included:

    - Alfalfa

        - Studies by Biotechnology Research Institute (BRI)used variety Canada no. 1; catalog no. 550, lot packed and tested 2000. Supplier: Williams Dam Seeds Ltd. (Box 8400, Dundas Ontario, Canada, L9H 6M1).

        - Studies performed by the Environmental Toxicology Branch Laboratory, U.S. Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD 21010-5424used variety Canada no. 1; catalog no. 550, lot packed and tested 2000. Supplier: Williams Dam Seeds Ltd.

- Nitrogen-fixing bacteria for alfalfa

    - ➢ Studies by BRI used Nitragin Gold; catalog no. 309-9, lot no. NGA33. Supplier: Labon Inc. (1350 Newton, Boucherville, Quebec, Canada, J4B 5H2).

    - ➢ Studies by ECBC used southern states alfalfa-clover nitrogen-fixing bacteria; catalog no. 111-08000, lot no.3092002. Supplier: Southern States Cooperative, Inc. (6606 W. Broad St., Richmond, VA 23230).

- Barnyard grass/Japanese millet

    - ➢ Studies by BRI used Barnyard grass variety common no. 1; catalog no. 300-380, lot no. 9-6. Supplier: Labon Inc.

    - ➢ Studies by ECBC used Barnyard grass variety common no. 1; catalog no. 300-380, lot no. 9-6. Supplier: Labon Inc.

- Perennial ryegrass

    - ➢ Studies by BRI used variety express; catalog no. 1269. Suppliers: Pickseed Canada Inc. (St-Hyacinthe, Quebec, Canada) and Labon Inc.

    - ➢ Studies by ECBC used variety express; catalog no. 1269. Suppliers: Pickseed Canada Inc. and Labon Inc.

- Soil invertebrates

    - ➢ Test species used in toxicity assays came from cultures maintained by ECBC.

A review of the information provided for each criterion showed that the experimental design of the ecotoxicological investigations complied with all the screening criteria used by the Eco-SSL workgroup during the literature evaluation processes for selecting or developing terrestrial plant and soil invertebrate benchmarks for deriving Eco-SSL values. Benchmark data and draft Eco-SSL values developed in these studies will be submitted to the USEPA Eco-SSL workgroup for quality control review, determination of benchmarks to include in the Eco-SSL database, and for use in deriving terrestrial plant-based and soil invertebrate-based Eco-SSLs for TNT and RDX, respectively. Following acceptance by the USEPA, these Eco-SSL values will be made available for use in ecological risk assessment of terrestrial habitats at military testing and training sites.

5. CONCLUSIONS

This project was undertaken specifically to develop scientifically defensible terrestrial plant-based and soil invertebrate-based benchmarks acceptable for deriving draft Eco-SSL values for TNT and RDX. These draft Eco-SSL values were derived using the EC_{20} level toxicity benchmarks for the EM effects on plant growth or soil invertebrate reproduction endpoints determined utilizing 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 acceptance by the USEPA, these Eco-SSL values will allow screening of site-soil data during the SLERA to identify those EMs 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. These Eco-SSLs will also provide an indispensable tool for the installation managers to gauge the ecotoxicological impacts of military operations that involve the use of explosives, thus ultimately promoting the sustainable use of testing and training ranges.

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