EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1273

TOXICITY DETERMINATIONS FOR FIVE ENERGETIC MATERIALS, WEATHERED AND AGED IN SOIL, TO THE COLLEMBOLAN *FOLSOMIA CANDIDA*

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

RESEARCH AND TECHNOLOGY DIRECTORATE

Geoffrey I. Sunahara

NATIONAL RESEARCH COUNCIL OF CANADA
Montréal, QC, Canada

Jalal Hawari

DEPARTMENT OF CIVIL, GEOLOGICAL, AND MINING ENGINEERING, POLYTECHNIQUE MONTRÉAL
Montréal, QC, Canada

March 2015

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-03-2015	Final	Apr 2002 – Oct 2006

4. TITLE AND SUBTITLE
Toxicity Determinations for Five Energetic Materials, Weathered and Aged in Soil, to the Collembolan *Folsomia candida*

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Phillips, Carlton T.; Checkai, Ronald T.; Kuperman, Roman G.; Simini, Michael (ECBC); Sunahara, Geoffrey I. (NRC); and Hawari, Jalal (Polytechnique Montréal)

5d. PROJECT NUMBER
SERDP ER-1416

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRT, APG, MD 21010-5424
National Research Council of Canada, 6100 Royalmount Avenue, Montréal QC, Canada H4P 2R2
Department of Civil, Geological, and Mining Engineering, Polytechnique Montréal, P.O. Box 6079, Station Centreville, Montréal QC, Canada H3C 3A7

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1273

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Development Program
4800 Mark Center Drive, Suite 17D08
Alexandria, VA 22350-3605

10. SPONSOR/MONITOR'S ACRONYM(S)
SERDP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT-LIMIT 200 WORDS

We investigated the toxicity of the following chemicals to the soil invertebrate Collembola *Folsomia candida*: 2,4-dinitrotoluene (2,4-DNT); 2-amino-4,6-dinitrotoluene (2-ADNT); 4-amino-2,6-dinitrotoluene (4-ADNT); octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX); and nitroglycerine (NG). Each chemical was separately and independently weathered and aged in soil. Four soil types that differed in organic matter, clay content, and pH were utilized: Sassafras sandy loam, Teller sandy loam, Kirkland loam, and Webster clay loam. Adult *F. candida* survival and juvenile production were assessed using replicated multiple treatment concentrations and appropriate controls in a standardized Folsomia reproduction test format. Concentrations in soil were analytically determined from acetonitrile extraction using U.S. Environmental Protection Agency (USEPA) method 8330A. The data sets for adult survival and juvenile production were analyzed using nonlinear regression models to determine EC_{20} and EC_{50} values (20 and 50% effect concentrations, respectively). The present studies established toxicity data for 2,4-DNT, 2-ADNT, 4-ADNT, HMX, and NG that meet the USEPA criteria for inclusion in the development of scientifically based ecological soil-screening levels, which can be used in ecological risk assessment at sites that are contaminated with energetic materials.

15. SUBJECT TERMS
2,4-Dinitrotoluene (2,4-DNT) *Folsomia candida* Natural soil 2-Amino-4,6-dinitrotoluene (2-ADNT)
4-Amino-2,6-dinitrotoluene (4-ADNT) Nitroglycerine (NG) Toxicity Ecological soil-screening level (Eco-SSL)
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Renu B. Rastogi
U	U	U	UU	44	19b. TELEPHONE NUMBER *(include area code)* 410-436-7545

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Blank

PREFACE

The work described in this report was authorized under project no. SERDP ER-1416. The work was started in April 2002 and completed in October 2006.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

Acknowledgments

This project was completed in cooperation with and funded by the Strategic Environmental Research and Development Program (SERDP).

Blank

CONTENTS

FIGURES

TABLES

Blank

TOXICITY DETERMINATIONS FOR FIVE ENERGETIC MATERIALS, WEATHERED AND AGED IN SOIL, TO THE COLLEMBOLAN *FOLSOMIA CANDIDA*

1. INTRODUCTION

The substantially increased demand for training resources is usually associated with an increase in environmental impacts at testing and training ranges, which are due, in part, to the release of energetic materials (EMs). Consequently, soil contamination with explosives, propellants, and related materials at many U.S. military installations is widespread. By some accounts, more than 15 million acres of land have been contaminated with EMs (U.S. GAO, 2003). Among the common energetic residues found in soil are: 2,4-dinitrotoluene (2,4-DNT); octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX for high-melting explosive); and nitroglycerin (NG). 2,4-DNT does not mineralize either aerobically or anaerobically when exposed to the environment, but it can be environmentally transformed into a variety of nitroaromatic species (Jenkins, 2007; Monteil-Rivera et al., 2009). HMX does not degrade aerobically to any extent and is persistent in surface soils (Jenkins, 2007; Monteil-Rivera et al., 2009). Consequently, concentrations of these EMs in soil have been reported to exceed 117 mg/kg for 2,4-DNT and 3000 mg/kg for HMX (Phillips et al., 1994; Simini et al., 1995). Partially reduced degradation products of 2,4,6-trinitrotoluene (TNT) and dinitrotoluenes (DNTs) and 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) frequently occur simultaneously in soil that has been contaminated with nitroaromatic EMs (Kuperman et al., 2009a).

NG can be released into the environment from the nitrocellulose matrix of solid propellants that is used in rockets and artillery ammunitions. NG is mobile in soil due to its moderate aqueous solubility of 1.8 g/L at 20 °C (Verscheuren, 1983; Pal and Ryon, 1986) and its low-partition coefficient values, such as log K_{ow} (octanol–water partition coefficient) of 1.62 (Sunahara et al., 2009) and log K_{oc} (organic carbon partition coefficient) of 2.77 (Spanggord et al., 1980). Environmental assessments that were conducted at 23 military firing ranges in the United States and Canada identified NG as a soil contaminant at anti-tank rocket ranges, with concentrations in the soil as high as 4700 mg of NG/kg of soil (Jenkins et al., 2006). Notwithstanding the persistence of these EMs in soil, their effects on soil invertebrates have not been sufficiently investigated (Kuperman et al., 2009a). As a result, scientifically defensible screening values, which could be used in ecological risk assessments (ERAs), are not currently available for 2,4-DNT; 2-ADNT; 4-ADNT; HMX; and NG in soil.

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) for EMs released into upland aerobic soil environments (USEPA, 2005). The U.S Environmental Protection Agency (USEPA) describes Eco-SSLs as follows: "The Eco-SSLs are concentrations of contaminants in soil that are protective of ecological receptors that commonly come into contact with soil or ingest biota that live in or on such soils" (USEPA, 2005). These concentration values can be used in the screening-level ERA (SLERA) to identify those contaminants that are not of potential ecological concern in soils, and therefore, do not require further evaluation in the baseline ecological risk assessment (BERA). Eco-SSLs are consistent

national screening values that can be applied, potentially resulting in cost savings during ecologically based site assessments and remedial investigations. Use of Eco-SSL values can help site managers distinguish sites that do not pose significant environmental risks from those that do, prioritize contaminated sites by the level of potential risk, quantify the relative risks at each site, and decide whether further investigation in the form of a BERA is merited so that the appropriate remedial actions can be determined.

Eco-SSLs are derived using published data that have been generated from laboratory toxicity tests with different test species that are relevant to soil ecosystems. An extensive literature review (Kuperman et al., 2009a) showed that, despite considerable attention to ecotoxicity assessments of EMs, the available data for 2,4-DNT, 2-ADNT, 4-ADNT, HMX, and NG were insufficient to generate Eco-SSL values for soil invertebrates. To fill the existing data gaps, we conducted definitive studies that were designed to specifically meet the USEPA criteria (USEPA, 2005) for the derivation of toxicity benchmarks acceptable for Eco-SSL development and to expand the ecotoxicological data set. These toxicity benchmarks, in conjunction with the development of corresponding Eco-SSL values, can aid site managers in the knowledge-based, decision-making process needed to secure the sustainable use of testing and training installations.

2. MATERIALS AND METHODS

2.1 Test Soils

Field-collected soils were used to determine the effects of soil characteristics on the concentration-related response to the EMs by *F. candida*. The field-collected soils selected for use in these studies were: Teller sandy loam (TSL [fine-loamy, mixed, active, thermic Udic Argiustolls]), Sassafras sandy loam (SSL [fine-loamy, siliceous, semiactive, mesic Typic Hapludults]), Kirkland loam (KL [fine, mixed, superactive, thermic Udertic Paleustolls]), and Webster clay loam (WCL [fine-loamy, mixed, superactive, mesic Typic Endoaquolls]). These soils differ in pH, organic matter (OM), and clay content. During collection, vegetation and the OM horizon were removed to just below the root zone, and the top 6 in. of the A horizon were then collected. Each soil was sieved through a 5 mm^2 mesh screen to remove roots, rocks, and other debris. The soil was spread out on benches in the greenhouse to air-dry for at least 72 h and mixed periodically to ensure uniform drying. Each soil was then passed through a 2 mm sieve and stored at room temperature before use in testing. Soils were analyzed for physical and chemical characteristics by the Agricultural Analytical Services Laboratory, Penn State University (University Park, PA). Results of the soil analyses are provided in Table 1.

Table 1. Physical and Chemical Characteristics of Test Soils

Soil Parameter	TSL	SSL	KL	WCL
Sand (%)	65	70	39	33
Silt (%)	22	16	42	39
Clay (%)	13	14	19	28
Texture	sandy loam	sandy loam	loam	clay loam
CEC (meq/100 g)	4.3	9.6	13	21
OM (%)	1.4	2	1.5	5.3
pH	4.4	4.4	5.7	5.9

CEC: cation exchange capacity

2.2 Test Chemicals

We obtained 2,4-DNT (Chemical Abstracts Service [CAS] no. 121-14-2; purity 97%) from Sigma-Aldrich, Inc. (St. Louis, MO). The EMs 2-ADNT (CAS no. 35572-78-2; purity 99%), 4-ADNT (CAS no. 19406-51-0; purity 99%), NG (CAS no. 55-63-0; purity 99%), and the nitramine HMX (CAS no. 2691-41-0; purity 99%) were all obtained from Defence Research and Development Canada–Valcartier (Quebec City, QC, Canada). Boric acid (H_3BO_3; CAS no. 10043-35-3; purity 99.99%; metals basis) was obtained from Alfa Aesar (Ward Hill, MA) and used as the positive control in these studies. High-performance liquid chromatography (HPLC)-grade acetone (CAS no. 67-64-1) was used to prepare individual EM solutions before amending soils. Acetonitrile (ACN; CAS no. 75-05-8; HPLC grade), methanol (CAS no. 67-56-1; chromatography grade; purity 99.9%), and calcium chloride ($CaCl_2$; CAS no. 10043-52-4; reagent grade) were used for the soil extractions and in the analytical HPLC determinations. Certified standards of EMs (AccuStandard, Inc.; New Haven, CT) were used in the HPLC determinations. ASTM type I water (18 MΩ cm at 25 °C; ASTM, 2004) was used throughout the toxicity studies. It was obtained using Milli-RO 10 Plus followed by Milli-Q PF Plus systems (EMD Millipore; Billerica, MA). The same grade of water was used throughout the analytical determinations. Glassware was washed with phosphate-free detergent and sequentially rinsed with the following: tap water, ASTM type II water (>5 MΩ cm at 25 °C; ASTM, 2004), analytical reagent-grade nitric acid 1% (v/v), and then ASTM type I water.

2.3 Soil Amendment Procedures

To determine toxicity benchmark values for explosive-contaminated soils, studies were performed separately and independently for EM that was weathered and aged in soil at each treatment level. Treatment levels were prepared as single batches for toxicity tests. Each treatment level was analyzed to determine the concentration of EM when the test species was introduced. During the amendment procedure, each EM was amended into separate aliquots of soil using an organic solvent (acetone) as a carrier. This was necessary to distribute the EMs evenly and uniformly to a large soil surface area. Carrier control soils were amended with acetone only. Soil was spread to a thickness of 2.5 cm. Individual EMs were dissolved in acetone and placed in glass volumetric flasks, then the solution was removed and pipetted across the soil surface. The volume of solution added to the soil at any one time was not allowed to

exceed 15% (v/w) of the soil dry mass. After the solution was removed from the flask and placed on the soil, the volumetric flask was rinsed twice with a known volume of acetone, and the acetone rinsate was also pipetted onto the soil. If the total volume of solution needed to amend the soil exceeded 15% (v/w), the solution was added to the soil in successive stages. The acetone was allowed to evaporate between these additions for a minimum of 2 h within a darkened chemical hood. The same total EM–acetone solution volume, at different EM concentrations, was added to every amendment treatment to equal the volume required to dissolve EM at the greatest dissolved concentration. To prevent photolysis of the EM, amended soil was air-dried overnight (minimum of 18 h) in a darkened chemical hood. Each soil treatment sample was then transferred into a fluorocarbon-coated, high-density, polyethylene container and mixed for 18 h on a three-dimensional rotary soil mixer. After three-dimensional mixing, samples of soil were collected from each soil treatment batch and sent overnight to the National Research Council of Canada (NRC; Montréal QC, Canada) for analytical determinations of the initial EM concentrations using USEPA method 8330A (USEPA, 2007).

To provide the appropriate benchmark data for Eco-SSL development, special consideration was given to the weathering-and-aging of EMs in soil and to assessing their toxicities to the Collembola *Folsomia candida*. The necessity for weathering-and-aging chemicals in soil (e.g., cycles of moistening and air-drying soil containing amendments of the chemicals of interest), rather than fresh amendment or aging alone, was initially recognized during preliminary investigations in support of studies on the fate of EMs in soil (Checkai et al., 1993a, 1993b, 1993c, 1993d). Standardized methods for weathering and aging of EMs in soil were not available. However, we developed and applied protocols for toxicity testing (Phillips et al., 2002) that simulated, at least partially, the weathering-and-aging process in soil and more closely approximated the exposure effects on soil biota in the field.

The soil treatment steps were as follows: (1) Before beginning toxicity testing, samples of each freshly amended soil were initially hydrated with ASTM type I water to 60% of the respective water-holding capacity (WHC). This hydration step initiated the weathering-and-aging of EMs in soil in open glass containers. (2) Soil was then subjected to alternating hydrating and air-drying cycles at ambient temperatures in a greenhouse. (3) All soil treatments were weighed and readjusted to their initial mass by adding ASTM type I water each week. (4) Any soil surface crusting that formed during the week was broken with a spatula before water was added. (5) After completion of the EM weathering-and-aging procedures, all soil treatments were brought to 88% of the WHC of each soil 24 h before commencement of toxicity tests. (6) After the weathering-and-aging procedure, which corresponded to the beginning of the definitive toxicity tests, soil samples collected from each treatment were sent overnight to the NRC for analytical determinations of EM concentrations.

2.4 Treatment Concentrations

2.4.1 Range-Finding Tests

Range-finding tests were conducted to estimate the soil treatment concentrations of EMs for definitive tests. Nominal concentrations, used in the range-finding tests with respective EMs in SSL soil, fell within the range of 1–5000 mg of EM/kg of soil, on a soil

dry-mass basis. Carrier (acetone) control soil was treated with acetone only, as described in Section 2.3.

2.4.1.1 Range-Finding Test with NG

A range-finding toxicity test was conducted with NG that was freshly amended into SSL to determine treatment concentrations for the definitive test. All other procedures for this test were the same as those used for the definitive tests. Nominal concentrations selected for this test with NG were 0 (acetone control), 1, 10, 100, 1000, and 5000 mg of EM/kg of dry SSL soil. Corresponding analytically determined concentrations of NG in SSL soil from ACN-extraction are shown in Section 3.1.1.

2.4.1.2 Range-Finding Test with 2-ADNT

A range-finding toxicity test was performed using *F. candida* with 2-ADNT that was weathered and aged for 3 months in SSL soil to determine the 2-ADNT treatment concentrations for the definitive toxicity test. Procedures for this test were the same as those for the definitive tests. Nominal concentrations of 2-ADNT used in this study were 0 (negative control), 0′ (acetone control), 50, 100, 200, 400, and 800 mg/kg. The results of this range-finding test were also used to select the concentrations needed for the definitive toxicity test with 4-ADNT.

2.4.2 Definitive Tests

Definitive tests included negative controls, carrier (acetone) controls, and positive controls. Positive-control tests were conducted in conjunction with the definitive tests. A stock solution of the positive control was prepared by first mixing boric acid in ASTM type I water. This solution was then mixed with an additional amount of ASTM type I water in sufficient quantity to produce nominal concentrations of 0, 30, 50, 80, 100, and 200 mg of H_3BO_3/kg of air-dried SSL soil. All ecotoxicological parameters in the definitive tests were established using measured concentrations of EMs in conjunction with the ecotoxicological test results.

2.5 Chemical Extractions and Analyses

Concentrations of EMs at the beginning of each definitive test were analytically determined in triplicate for all control and treated soils using ACN-extraction and USEPA method 8330A (USEPA, 2007). After the addition of ASTM type I water (60% of the WHC of SSL soil), soil samples were equilibrated in the dark for 24 h at room temperature. For extraction, 2 g each of treatment and control samples were collected from each soil batch. The respective samples were then placed into 50 mL polypropylene centrifuge tubes, and 10 mL of ACN was added to each tube. Internal standards were then added (100 µL) to each tube to evaluate the extraction efficiency. Internal standards were 1,3-dinitrobenzene (1,3-DNB) for 2,4-DNT, 2-ADNT, and 4-ADNT; 2,4-DNT for HMX; and HMX for NG. Glass tubes were vortexed for 1 min and then sonicated in darkness for 18 ± 2 h at 20 °C. Five milliliters of sonicated sample were transferred to a new tube, to which 5 mL of 5 g/L $CaCl_2$ solution were added. Supernatant was filtered through 0.45 µm Millex-HV cartridges (EMD Millipore). Soil extracts

were analyzed and quantified using HPLC. Extractions were repeated if the 1,3-DNB internal standard recovery was less than 90%.

Soil extracts were analyzed, and EM concentrations were quantified using a Waters Corporation (Milford, MA) HPLC system composed of a model 600 pump, a model 717 Plus injector, a model 2996 photodiode-array, and a temperature-control module. Calibration curves were generated before each HPLC analysis using certified standards of each EM (AccuStandard Inc.; New Haven, CT or Cerilliant Corporation; Round Rock, TX) in a range of concentrations that were appropriate for each set of determinations. The limits of detection were 0.01, 0.005, 0.005, 0.034, and 0.05 mg/L for 2,4-DNT; 2-ADNT; 4-ADNT; HMX; and NG, respectively, corresponding to 0.1, 0.05, 0.05, 0.34, and 0.5 mg of EM/kg of soil (dry soil mass), respectively. All chemical concentrations in soil were expressed on the basis of dry mass. Results of analytical determinations were reported in Kuperman et al. (2013). Nominal and analytically determined ACN-extracted (measured) concentrations used in the definitive tests are shown in Section 3.

2.6 Toxicity Assessments

The Folsomia reproduction test, which is a chronic bioassay, was used to assess the effects of EM on the reproduction of the *F. candida*. The test is an adaptation of an International Organization for Standardization (ISO) bioassay ISO 11267 (ISO, 1999). The ISO guideline for this assay was originally developed for use with standard artificial soil (SAS). Research in our laboratory has shown that this test can also be conducted using natural soils (Phillips et al., 2002). The measurement endpoints for the test included the production of juveniles and the survival of *F. candida* as adults.

2.6.1 Principle of the Test

F. candida are exposed to a range of concentrations of the test substance, which has been mixed into soil. The total number of juvenile *F. candida* produced (i.e., indicator of effective reproduction) and the number that survive as adult *F. candida* are determined by counting the live organisms after the 28 day test duration. The effective reproduction and the survival of adult *F. candida* exposed to the test substance are compared with that of the control treatments to quantify ecotoxicological parameters. These parameters include the no-observed-effect concentration (NOEC), the lowest-observed-effect concentration (LOEC), the effective concentration that causes a *p* percentage reduction (EC_p) in the production of juveniles compared with those in the carrier controls (e.g., EC_{20} or EC_{50}; 20 or 50% effect concentration, respectively), and the number of *F. candida* surviving as adults on Day 28.

2.6.2 Test Validity Criteria

Validity criteria are part of quality control procedures. Adaptation of the Folsomia reproduction test for use with natural soils included the following performance parameters for the negative controls:

(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 coefficient of variation for reproduction should not exceed 30% at the end of the test.

2.6.3 Culturing Conditions

The U.S. Army Edgewood Chemical Biological Center (ECBC) laboratory culture of *F. candida* was established in 2001 from a stock culture obtained from the Soil Fauna and Ecotoxicology Research Unit, Department of Terrestrial Ecology, National Environmental Research Institute (Silkeborg, Denmark). The ECBC *F. candida* culture was maintained in darkness at 20 °C on a mixture of charcoal and plaster of Paris in culture jars. The *F. candida* were fed baker's yeast and kept moist by routine misting with ASTM Type I water approximately twice per week. Synchronized *F. candida* cultures were established for the experiments by removing egg clusters from the stock cultures and placing them into new jars. The eggs were monitored daily to determine the onset of hatching. Once hatching began, the process was allowed to proceed for 2 days, after which juvenile *F. candida* were transferred to new jars. These synchronized juveniles were then held for 10 days, thus providing the 10–12 day old juveniles that were used in these tests.

2.6.4 Test Performance

Glass test containers (42 mm i.d.; 45 mm height) were rinsed successively with acetone, tap water, and ASTM type I water before the test. To prepare five replicates of each treatment, 100 g of each air-dried treatment soil was hydrated to 88% of WHC. Then one-fifth by 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 surface of the soil. Ten 10–12 day old *F. candida* juveniles were placed in each test container and lightly misted with ASTM type I water. A piece of plastic food wrap was placed on each container and held in place with a rubber band. The mass of each container was then recorded to monitor soil moisture loss during the test. Five replicates were used for each treatment concentration and for the control treatments.

The test containers were randomly placed in an incubator at 20 ± 0.5 °C with a relative humidity of 88 ± 5%. During the course of the study, the containers were misted weekly to maintain soil moisture level.

To terminate a test, approximately 15 mL of tap water was added to a test container and allowed to equilibrate for several minutes to fully hydrate the soil. After the soil was gently mixed with a spatula, an additional 10 mL of water was added. The contents of the test container were mixed a final time 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.

Measurement endpoints were the number of surviving *F. candida* adults and juveniles produced after 28 days. All ecotoxicological parameters were estimated using ACN-extractable concentrations of each explosive for each treatment concentration.

2.7 Data Analyses

F. candida juvenile production data were analyzed using nonlinear regression models described in Kuperman et al. (2004) and in an Environment Canada guidance document (EC, 2005). 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: (1) the regression lines generated by the models were closest to the data points; (2) the regression coefficients for point estimates were the greatest; (3) the residuals were homoscedastic (i.e., had most random scattering); and (4) the means, standard errors (SEs), and variances of the residuals were the smallest. The logistic model (Gompertz; eq. 1) had the best fit for data in toxicity tests using 2,4-DNT; 4-ADNT; HMX; and NG. The logistic Hormetic model (eq. 2) best fit the data for 2-ADNT. These models were:

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

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

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 percent effect (e.g., 0.50 for a 50% decrease from the control response; EC_{50});
- C is the exposure concentration in test soil;
- EC_p 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 and high-dose inhibition (Calabrese, 2008), were fitted to the hormetic model. The EC_p parameters used in this study included the concentrations producing 20% (EC_{20}) or 50% (EC_{50}) reductions in the measurement endpoints. The asymptotic SE and 95% confidence intervals (CIs) associated with the point estimates were determined. The EC_{20} parameter, based on a reproduction endpoint, is the preferred parameter for deriving soil invertebrate Eco-SSL benchmarks. The $EC_{50,}$ a commonly reported value, was included to enable comparisons of the results produced in this study with the results reported previously by other researchers.

Analysis of variance (ANOVA) was used to determine the bounded-NOEC and bounded-LOEC values for adult *F. candida* survival or juvenile production data. ANOVA analyses and adult *F. candida* survival data were included to enable comparisons of the results produced in this study with the results previously reported by other researchers. Mean separations were determined using Fisher's least-significant difference (FLSD) pairwise comparison tests. A Pearson's correlation analysis was performed using the soil properties %OM, % clay, and soil pH and the NOEC, LOEC, EC_{20}, and EC_{50} values for adult *F. candida* survival and juvenile production. All analyses were performed using untransformed data and analytically determined EM concentrations. A significance level of P (probability) ≤ 0.05 (95% CI) was accepted for all statistical tests. Statistical analyses were performed using Systat version 11.0 (Systat Software, Inc., Chicago, IL).

3. RESULTS

3.1 Range-Finding Tests

3.1.1 Toxicity of NG in SSL Soil

Carrier (acetone) control results from the NG range-finding study yielded a mean adult *F. candida* survival of 90%, a mean number of juveniles equal to 127, and a coefficient of variation of 17%. These results met the validity criteria specified in the ISO 11267 method (ISO, 2005). The numbers of surviving *F. candida* adults decreased by 13, 20, and 96% at NG concentrations of 0.84, 4.9, and 85 mg of EM/kg of soil, respectively, compared with the mean number in carrier (acetone) control. No adult *F. candida* survived in the 898 and 4558 mg of EM/kg of soil treatments (Table 2). The production of juvenile *F. candida* decreased by 21 and 48% in the 0.84 and 4.9 mg of EM/kg of soil treatments, respectively, compared with the acetone control. No juvenile *F. candida* were produced in 85, 898, and 4558 mg of EM/kg of soil treatments. These results (Table 2) were used to determine the range of NG concentrations for the definitive tests with *F. candida*.

Table 2. Effects of NG on Adult *F. candida* Survival and Juvenile Production Determined in a Range-Finding Test with Freshly Amended SSL Soil

Concentration of NG in SSL[1] (mg of EM/kg of dry soil)	**Surviving Adults[2]**	**Juveniles Produced[2]**
0.00 (control)[3]	9 ± 0.5	127 ± 10
0.84 ± 0.02	8 ± 0.4	101 ± 10
4.9 ± 0.2	7 ± 0.4	66 ± 11
85 ± 3	0.4 ± 0.4	0 ± 0
898 ± 97	0 ± 0	0 ± 0
4558 ± 119	0 ± 0	0 ± 0

Notes:
[1] Analytically determined concentrations of NG from ACN-extractions of soil treatments (means and SEs, $n = 3$)
[2] Values are means and SEs ($n = 5$)
[3] Carrier (acetone) control

3.1.2 Toxicity of 2-ADNT, Weathered and Aged in SSL Soil, in a Range-Finding Test

A range-finding toxicity test was performed on *F. candida* with 2-ADNT, weathered and aged for 3 months in SSL soil, to determine the range of 2-ADNT concentrations for the definitive toxicity test. Nominal concentrations of 2-ADNT used in this study were 0 (negative control), 0′ (acetone control), 50, 100, 200, 400, and 800 mg of EM/kg of soil. Corresponding analytically determined concentrations of 2-ADNT at the start of *F. candida* exposures in SSL soil were 0, 0′, 25, 51, 121, 293, and 670 mg of EM/kg of soil.

Survival of *F. candida* adults was affected by exposure to 2-ADNT that was weathered and aged in SSL soil within the concentration range tested. The bounded NOEC and LOEC values were 25 and 51 mg of EM/kg of soil, respectively (Table 3). The Gompertz model had the best fit for adult *F. candida* survival data (Figure 1), resulting in EC_{20} and EC_{50} values of 38 and 65 mg of EM/kg of soil, respectively (Table 3). The bounded NOEC and LOEC values for juvenile *F. candida* production were 25 and 51 mg of EM/kg of soil, respectively (Table 3). The Gompertz model had the best fit for the juvenile *F. candida* production data (Figure 1), resulting in EC_{20} and EC_{50} values of 45 and 51 mg of EM/kg of soil, respectively (Table 3).

Table 3. Summary of Toxicological Parameters
for 2-ADNT, Weathered and Aged in SSL Soil

Ecotoxicological Parameter	**Toxicity of 2-ADNT (mg of EM/kg of soil)**	
	Adult Survival	Production of Juveniles
NOEC	25	25
P	0.859	0.986
LOEC	51	51
P	0.009	<0.0001
EC_{20}	38	45
CI (95%)	19–56	0–154
EC_{50}	65	51
CI (95%)	47–84	45–58
Model used	Gompertz	Gompertz
R^2	0.938	0.968

Notes:
R^2: coefficient of determination
Values were determined from the range-finding toxicity tests with *F. candida*.
Toxicity benchmarks are based on analytically determined chemical concentrations in soil from ACN-extraction (USEPA, 1998).
NOEC and LOEC values were derived from ANOVA and FLSD pairwise means comparison test.

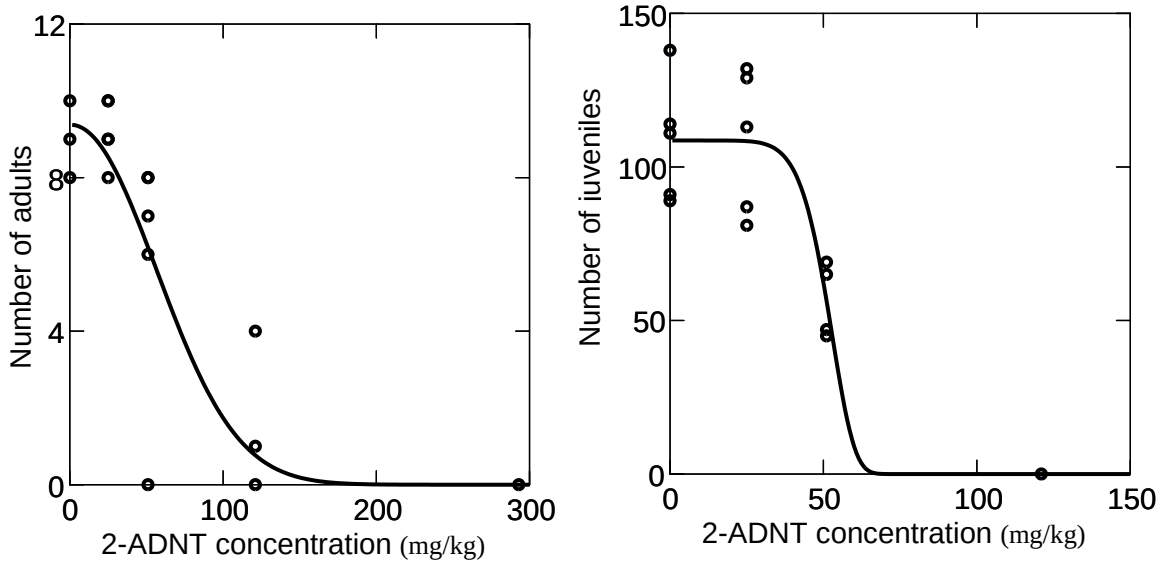

Figure 1. Effects of 2-ADNT, weathered and aged in SSL soil, on adult *F. candida* survival and juvenile production in the range-finding study.

3.2. Definitive Test Results

3.2.1 Toxicity of Boric Acid in SSL Soil

A positive chemical control is required for toxicity testing with the soil invertebrate *F. candida* to validate the condition of the test species and the reliability and precision of the results. We conducted toxicity tests to generate a data set and establish a baseline for a new reference toxicant that would be used as a positive control. Tests with a reference toxicant, boric acid, were conducted to monitor the condition of the *F. candida* cultures used in definitive studies. The ISO 11267 protocol (ISO, 1999) was modified for toxicity testing in SSL soil, which has physical and chemical properties that support the high relative bioavailability of many organic and inorganic chemicals (USEPA, 2005).

Toxicity tests with boric acid were conducted in SSL soil to obtain EC_{50} values and the corresponding 95% confidence limits (CL). Nonlinear regression analyses of toxicity data, which were established for six dates throughout the testing period of these independent studies, produced the following EC_{50} values and their corresponding CLs (in parentheses) for juvenile production: 72 (68–77), 63 (53–73), 60 (53–67), 67 (61–76), 60 (51–68) and 70 (59–82) mg of H_3BO_3/kg of soil. These EC_{50} values were plotted on a Boric Acid Warning Chart to monitor the condition of the collembolan culture (Figure 2). All resulting EC_{50} values were within both the Warning Limits of plus or minus two standard deviations (SDs) and the 95% CL that was established for the *F. candida* culture in tests with boric acid (Figure 2). These charted results confirmed that the condition of the *F. candida* culture met the validity requirements of the test protocol.

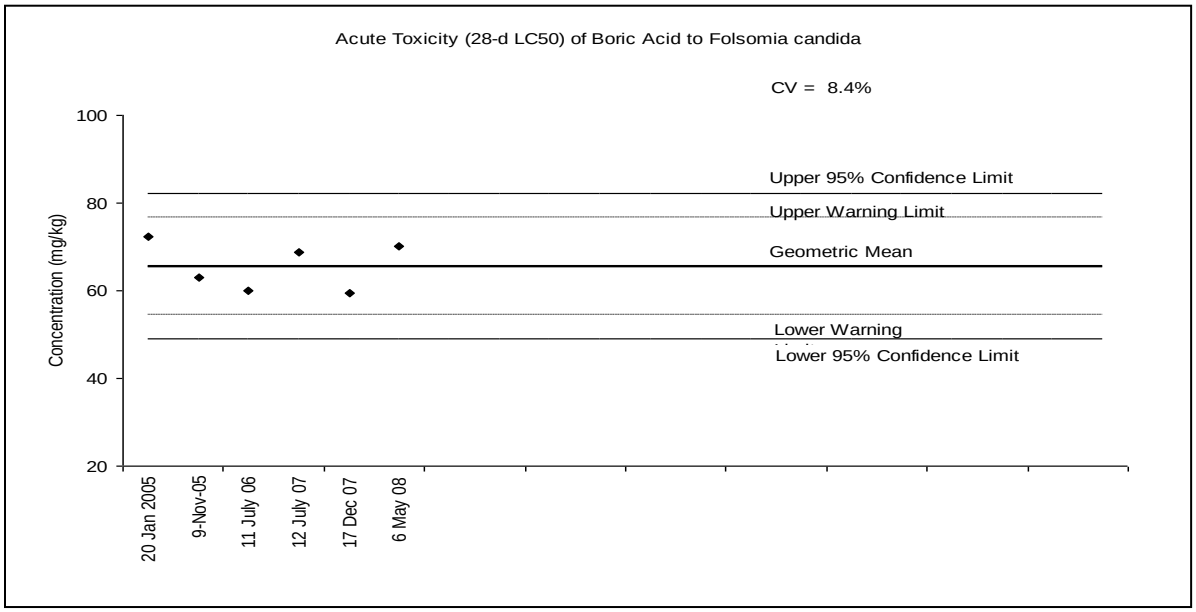

Figure 2. Warning chart for the *F. candida* culture showing the EC_{50} values for juvenile production established in definitive tests with the reference toxicant (boric acid) in SSL soil.

3.2.2 Toxicity of 2,4-DNT, Weathered and Aged in TSL, KL, and WCL Soils

Test results using TSL, KL, and WCL soils met the validity criteria for the carrier control defined in the ISO 11267 test guideline (ISO, 1999). The means for adult *F. candida* survival were 88, 92, and 86% in TSL, KL, and WCL, respectively. The means for number of *F. candida* juveniles were 176, 133, and 152 in TSL, KL, and WCL, respectively. The coefficients of variation were 7.7, 12.4 and 6.9% in TSL, KL, and WCL, respectively.

The measurement endpoints of this toxicity test included adult *F. candida* survival and juvenile production. Separate toxicity tests were performed using each of the three soil types. Treatment concentrations of 2,4-DNT, as the EM used in the definitive tests, were selected on the basis of data established in the earlier studies (Kuperman, 2003). Analytically determined treatment concentrations (Table 4) of 2,4-DNT used in the definitive studies were: 0, 0′, 2, 4, 8, 15, 29, 44, 63, 127, 160, 346, and 556 mg of EM/kg of TSL soil; 0, 0′,1, 3, 5, 9, 18, 29, 36, 87, 115, and 239 mg of EM/kg of KL soil; and 0, 0′, 2, 4, 8, 14, 28, 39, 54, 97, 115, 260, and 447 mg of EM/kg of WCL soil.

Survival of adult *F. candida* was affected by exposure to 2,4-DNT within the concentrations tested and weathered and aged in TSL, KL, and WCL soils. The bounded NOEC and LOEC values were 14.5 and 29 mg of EM/kg of TSL soil, 2.5 and 4.6 mg of EM/kg of KL soil, and 14.4 and 28 mg of EM/kg of WCL soil, respectively. The Logistic (Gompertz) model had the best fit for adult *F. candida* survival data in the three soil types tested. The analysis produced the following EC_{20} and EC_{50} values, with their corresponding CIs (in parentheses): 32 (28–35) and 38 (36–40) mg of EM/kg of TSL soil, 6 (4–9) and 14 (11–17) mg of EM/kg of KL soil, and 41 (35–46) and 60 (54–65) mg of EM/kg of WCL soil, respectively (Figures 3–6).

Table 4. Results of Soil Analyses for 2,4-DNT, Freshly Amended and after Weathering and Aging in TSL, KL, and WCL Soils

Nominal Concentration[1] (mg/kg)	TSL			KL			WCL		
	Initial Analysis[2] (mg/kg)	Final Analysis[3] (mg/kg)	% Remaining ACN-Extractable (Final/Initial)	Initial Analysis[2] (mg/kg)	Final Analysis[3] (mg/kg)	% Remaining ACN-Extractable (Final/Initial)	Initial Analysis[2] (mg/kg)	Final Analysis[3] (mg/kg)	% Remaining ACN-Extractable (Final/Initial)
2	2.27	1.7	75	2	0.8	40	2.4	1.8	75
5	5.29	3.7	70	5.6	2.5	45	5	3.8	76
10	10.5	7.5	71	10.7	4.6	43	9.8	7.5	77
20	20.9	14.5	69	20	8.7	44	21	14.4	69
40	42	29	69	42	18	43	40	28	70
60	63.6	44	69	63	29	46	64	39	61
80	84	63	75	82	36	44	80	54	68
160	170	127	75	160	87	54	175	97	55
200	212	160	75	212	115	54	219	115	53
400	425	346	81	391	239	61	440	260	59
600	624	556	89				677	447	66

[1] Target concentration for soil that received EM amendments
[2] Analytically determined concentration from ACN-extraction of soil at the start of weathering and aging
[3] Analytically determined concentration from ACN-extraction of soil at the end of weathering and aging

The bounded NOEC and LOEC values for juvenile production by *F. candida* were 3.7 and 7.5 mg of EM/kg of TSL soil, 4.6 and 8.7 mg of EM/kg of KL soil, and 14.4 and 28 mg of EM/kg of WCL soil, respectively. The Logistic (Gompertz) model had the best fit for the juvenile *F. candida* production data (Figures 3–6). The model produced the following EC_{20} and EC_{50} values, with their corresponding CIs (in parentheses): 24 (20–28) and 30 (28–33) mg of EM/kg of TSL soil, 3 (1–5) and 10 (7–12) mg of EM/kg of KL soil, and 27 (22–32) and 47 (43–51) mg of EM/kg of WCL soil, respectively. All ecotoxicological parameters for 2,4-DNT, determined in weathered-and-aged amended soils, are given in Table 5. Results from a previous toxicity test of 2,4-DNT in SSL soil are provided for comparison to the other soils.

Table 5. Summary of Toxicological Parameters
for 2,4-DNT, Weathered and Aged in SSL, TSL, KL, and WCL Soils

Ecotoxicological Parameter	**Toxicity of EM (mg of EM/kg of soil)**			
	SSL*	TSL	KL	WCL
Adult Survival				
NOEC	5.2	14.5	2.5	14.4
P	0.325	0.212	0.237	0.139
LOEC	11.5	29	4.6	28
P	<0.0001	0.033	0.022	0.029
EC_{20}	12	32	6	41
CI (95%)	4–20	28–35	4–9	35–46
EC_{50}	38	38	14	60
CI (95%)	27–48	36–40	11–17	54–65
Model used	Gompertz			
R^2	0.983	0.986	0.969	0.987
Production of Juveniles				
NOEC	3	3.7	4.6	14.4
P	0.084	0.199	0.057	0.333
LOEC	5.2	7.5	8.7	28
P	0.001	0.039	<0.0001	<0.0001
EC_{20}	15	24	3	27
CI (95%)	11–19	20–28	1–5	22–32
EC_{50}	23	30	10	47
CI (95%)	20–26	28–33	7–12	43–51
Model used	Gompertz			
R^2	0.980	0.978	0.954	0.987

Notes:

Values were determined from definitive tests with *F. candida*.

Values are soil concentration means determined using USEPA method 8330A (USEPA, 2007).

NOEC and LOEC values were derived from ANOVA procedures and FLSD pairwise means comparison test.

*Results for 2,4-DNT, weathered and aged in SSL soil, are from the Strategic Environmental Research and Development Program (SERDP) CU-1221 investigations (Kuperman, 2003).

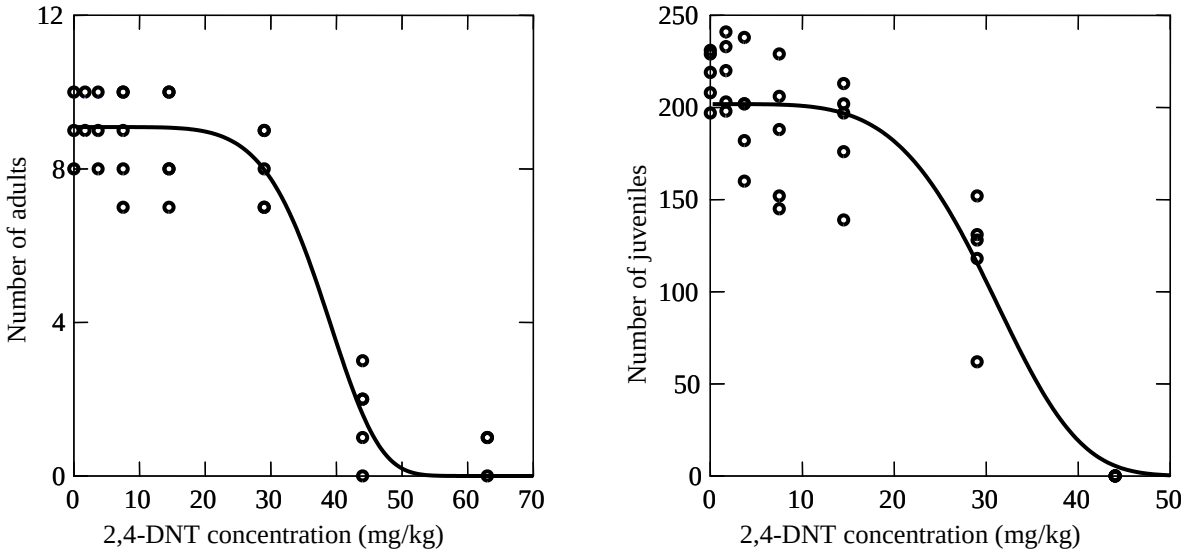

Figure 3. Effects of 2,4-DNT, weathered and aged in TSL soil, on adult *F. candida* survival and juvenile production.

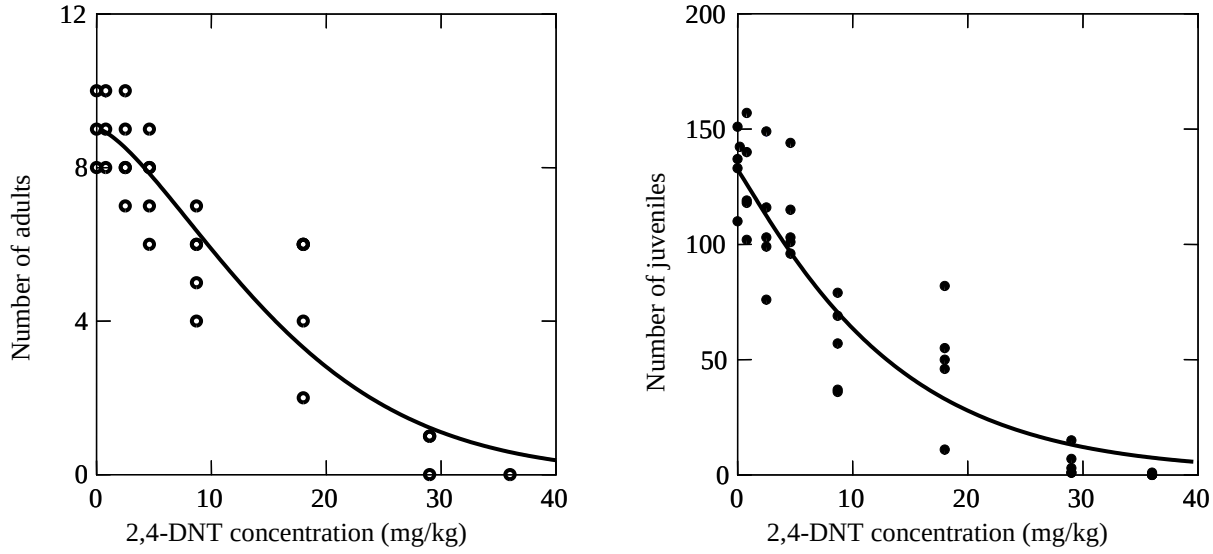

Figure 4. Effects of 2,4-DNT, weathered and aged in KL soil, on adult *F. candida* survival and juvenile production.

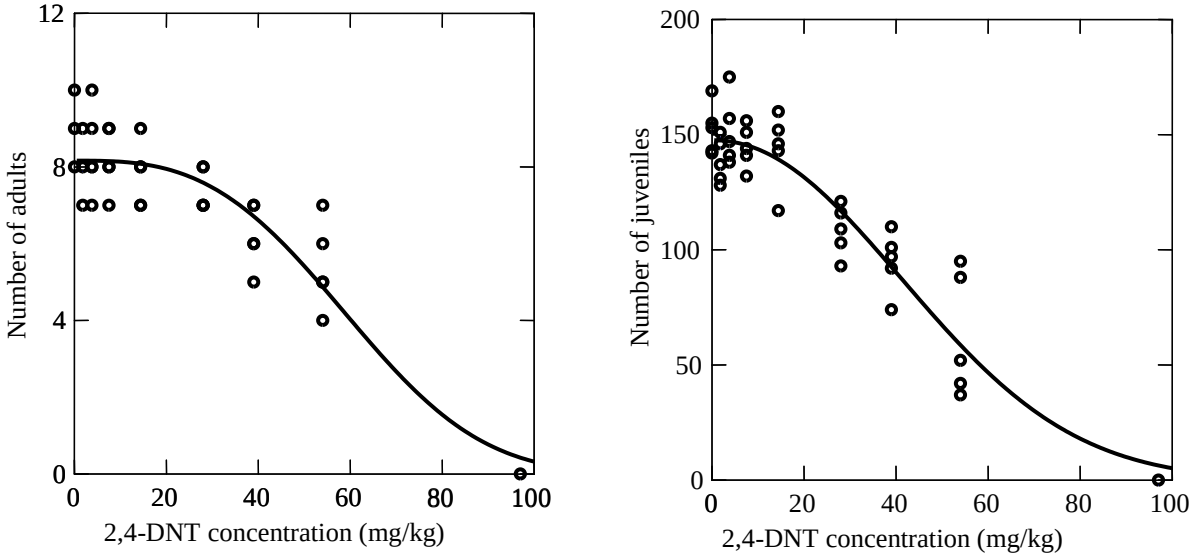

Figure 5. Effects of 2,4-DNT, weathered and aged in WCL soil, on adult *F. candida* survival and juvenile production.

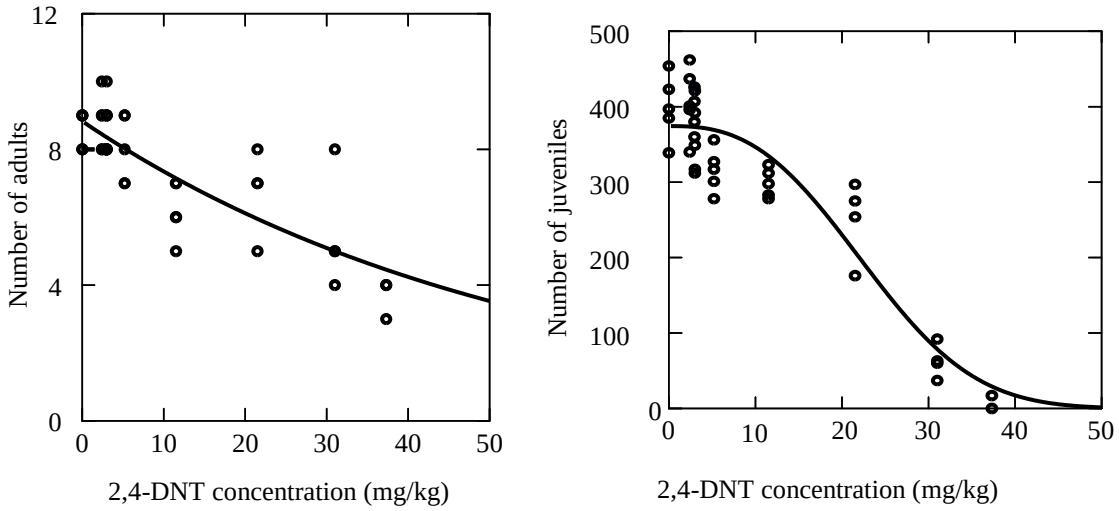

Figure 6. Effects of 2,4-DNT, weathered and aged in SSL soil, on adult *F. candida* survival and juvenile production (from SERDP CU-1221 investigations; Kuperman, 2003).

3.2.3 Toxicity of 2-ADNT, Weathered and Aged in SSL Soil

The Folsomia Toxicity Test was used to investigate the effects of 2-ADNT as the EM, weathered and aged in SSL soil, on the adult *F. candida* survival and juvenile production. Analytically determined concentrations of 2-ADNT in amended SSL soil, based on ACN-extraction, were 0, 0′, 10, 21, 34, 37, 45, 57, 90, 129, and 241 mg of EM/kg of dry soil mass.

Exposure to the 2-ADNT soil treatments significantly ($P < 0.05$) reduced adult *F. candida* survival and juvenile production compared with the respective numbers of *F. candida* in the carrier (acetone) control. The bounded NOEC and LOEC values were 21 and 34 mg of EM/kg of soil, respectively for both adult survival and production of juveniles. Nonlinear regression analyses of toxicity data (Figure 7) produced the following EC_{20} and EC_{50} values and their corresponding CIs (in parentheses): 37 (33–41) and 55 (52–58) mg of EM/kg of soil, respectively, for adult survival (Gompertz model) and 30 (26–34) and 42 (39–46) mg of EM/kg of soil, respectively, for juvenile production (Hormetic model). These results will undergo a data quality review by the USEPA before the EC_{20} concentration for juvenile *F. candida* production is included as a toxicity benchmark within a derivation of a soil invertebrate-based Eco-SSL value for 2-ADNT. All ecotoxicological parameters determined for the toxicity of 2-ADNT, weathered and aged in SSL soil, to *F. candida* are given in Table 6.

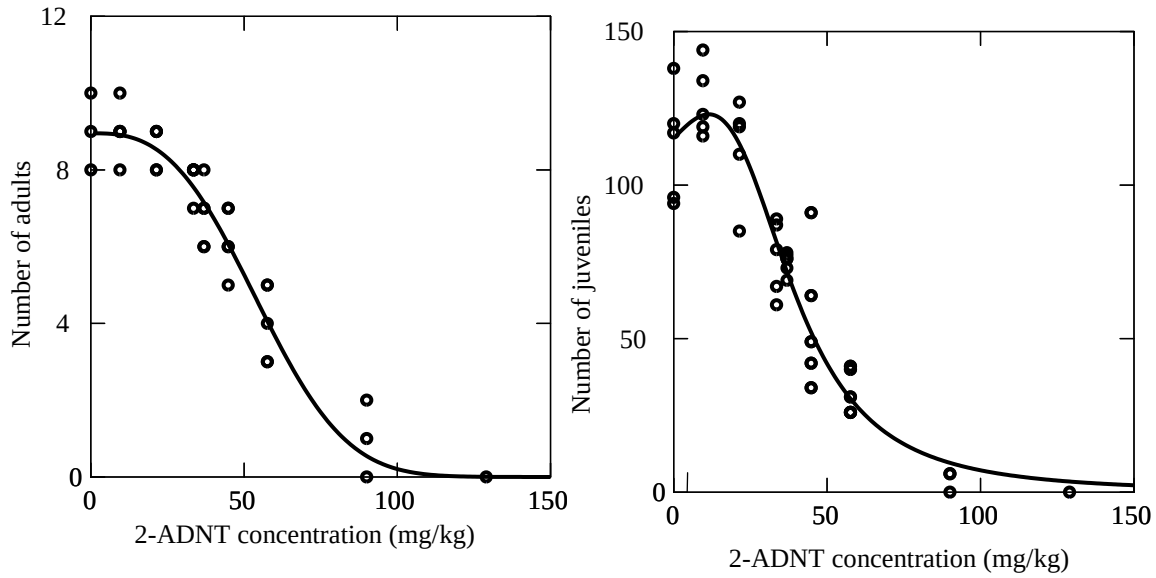

Figure 7. Effects of 2-ADNT, weathered and aged in SSL soil, on adult *F. Candida* survival and juvenile production.

Table 6. Summary of Toxicological Parameters
for 2-ADNT, 4-ADNT, and NG, Weathered and Aged in SSL Soil

Ecotoxicological Parameter	Toxicity of EM (mg of EM/kg of soil)		
	2-ADNT	4-ADNT	NG
Adult Survival			
NOEC	21	22	0.2
P	0.671	1.000	0.215
LOEC	34	28	0.6
P	0.039	0.004	0.012
EC_{20}	37	22	2
CI (95%)	33–41	11–32	0.9–3.0
EC_{50}	55	55	9
CI (95%)	52–58	42–67	6–12
Model used	Gompertz		
R^2	0.991	0.970	0.990
Production of Juveniles			
NOEC	21	22	0.2
P	0.922	0.785	0.26
LOEC	34	28	0.6
P	<0.0001	<0.0001	0.04
EC_{20}	30	26	1
CI (95%)	26–34	19–33	0.5–2
EC_{50}	42	47	6
CI (95%)	39–46	41–53	3-9
Model used	Hormetic	Gompertz	
R^2	0.977	0.981	0.980

Notes:
Values were determined from definitive tests with *F. candida*.
EMs were separately and independently weathered and aged in SSL soil.
Values are means of soil concentration determined using USEPA method 8330A (USEPA, 2007).
NOEC and LOEC values were derived from ANOVA procedures and FLSD pairwise means comparison test.

3.2.4 Toxicity of 4-ADNT Weathered and Aged in SSL Soil

We investigated the effects of 4-ADNT as the EM, weathered and aged in SSL soil, on adult *F. candida* survival and juvenile production. For these definitive toxicity tests, analytically determined concentrations of 4-ADNT in the amended SSL soil, which were based on ACN-extraction, were: 0, 0′, 3, 8, 13, 12, 22, 28, 59, 75, and 150 mg of EM/kg of soil. Exposure to the 4-ADNT soil treatments significantly ($P < 0.05$) reduced both adult *F. candida*

survival and juvenile production, compared with the respective numbers of *F. candida* in the carrier (acetone) control. The bounded NOEC and LOEC values were 22 and 28 mg of EM/kg of soil, respectively, for both adult *F. candida* survival and production of juveniles. Nonlinear regression analyses of toxicity data (Figure 8) produced the following interim EC_{20} and EC_{50} values and their corresponding CIs (in parentheses): of 22 (11–32) and 55 (42–67) mg of EM/kg of soil, respectively, for adult survival (Gompertz model) and 26 (19–33) and 47 (41–53) mg of EM/kg of soil, respectively, for production of juveniles (Gompertz model). All ecotoxicological parameters determined for the toxicity of 4-ADNT, weathered and aged in SSL soil, to *F. candida* are given in Table 6.

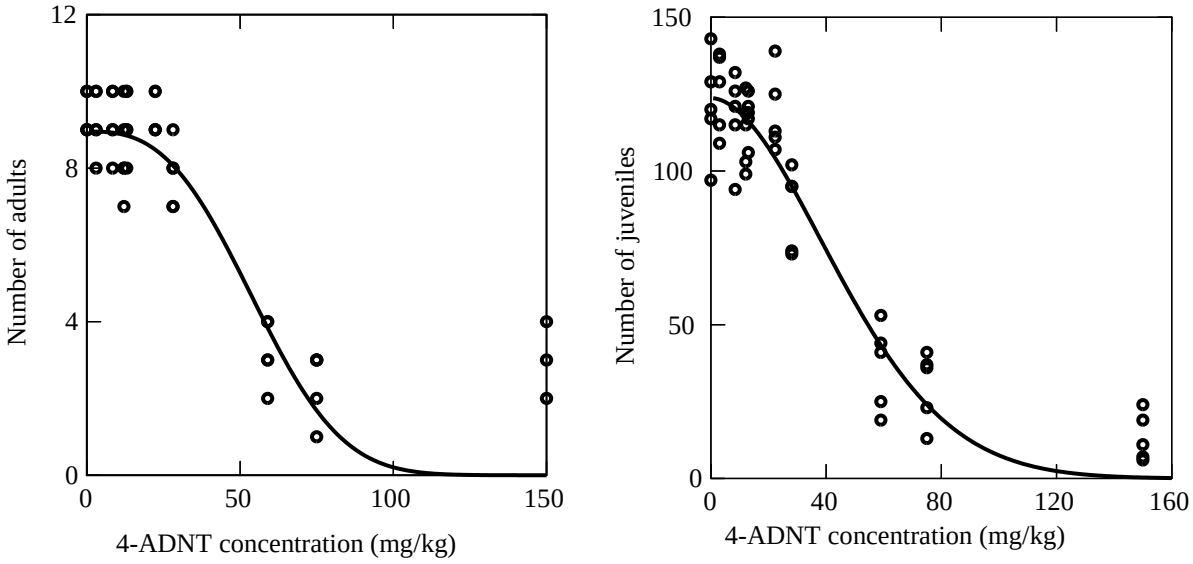

Figure 8. Effects of 4-ADNT, weathered and aged in SSL soil, on adult *F. candida* survival and juvenile production.

3.2.5 Toxicity of HMX, Weathered and Aged in TSL Soil

The effects of HMX, weathered and aged in TSL soil, on adult *F. candida* survival and juvenile production were determined using the Folsomia Toxicity Test. On the basis of data established in the earlier studies (Kuperman, 2003), nominal HMX concentrations, which were selected for toxicity tests with TSL soil, were as follows: 0 (negative control); 0′ (carrier acetone control); 100; 1,000; 5,000; and 10,000 mg of EM/kg of soil. Analytically determined HMX concentrations, based on ACN-extraction after 3 months of weathering and aging in TSL, were 0; 0′; 72; 913; 4,888; and 10,208 mg of EM/kg of soil at test commencement. Based on the results of soil invertebrate testing in SSL soil (from SERDP CU-1221 investigations; Kuperman, 2003), a composite toxicity test design, with variable replication, was used in this investigation of HMX toxicity in TSL soil to *F. candida*. This design combined range-finding and definitive-limit (Organisation for Economic Co-operation and Development [OECD], 2009) test components. The range-finding test component included the selection of a limited number of treatment concentrations and a reduced number of replicates for the intermediate treatments. The definitive-limit test component included increased replication in the carrier (acetone) control and

in the greatest treatment concentration (nominal 10,000 mg of EM/kg of soil, which was the greatest nominal test concentration used in limit tests for soil invertebrates; OECD, 2009). The limit test is a variant of the definitive test and can be performed when a statistical analysis of sufficient range-finding test data shows no significant effect at all treatment levels. Such composite toxicity test design provides the information necessary to meet definitive-test requirements with multiple treatment levels, if the effect of exposure on measurement endpoints is statistically significant. This test design also expedites the assessment of test material effects, if no statistically significant ($P > 0.05$) adverse effects are found. The results of the present study met the validity criteria for the carrier control treatment specified in the ISO 11267 method (ISO, 1999). Survival of *F. candida* adults was 88%, the mean number of juveniles produced was 109, and the coefficient of variation for number of juveniles was 17%.

F. candida adults and juveniles were counted after 28 days of exposure to HMX. Adult *F. candida* survival in all HMX treatments was not significantly different ($P > 0.05$) compared with the carrier (acetone) control. Adult *F. candida* survival rates were 90, 88, 86, and 88% for HMX treatment concentrations of 0′; 72; 913; 4,888; and 10,208, respectively. Juvenile *F. candida* production was not significantly ($P > 0.05$) affected by exposure to HMX that was weathered and aged in TSL soil when compared with that of the carrier (acetone) control. The average numbers of *F. candida* juveniles were: 106, 110, 101, 110, and 117 in HMX treatments of 0′; 72; 913; 4,888; and 10,208 mg of EM/kg of soil, respectively. These results showed that exposure to HMX that was weathered and aged in TSL for 3 months did not affect either the survival of *F. candida* adults or the production of juveniles up to and including 10,208 mg of EM/kg of soil (unbounded NOEC).

3.2.6 Toxicity of NG, Weathered and Aged in SSL Soil

The Folsomia Toxicity Test was used to investigate the effects of NG as the EM, weathered and aged in SSL soil, on adult *F. candida* survival and juvenile production. Analytically determined exposure concentrations of NG for these definitive toxicity tests in SSL soil, were 0, 0’, 0.2, 1.3, 0.6, 1.8, and 36 mg/kg. Exposure to the NG soil treatments significantly ($P < 0.05$) reduced both adult survival and juvenile production, compared with respective numbers of *F. candida* in the carrier (acetone) control. The respective bounded NOEC and LOEC values were 0.2 and 0.6 mg/kg for both adult survival and production of juveniles. Nonlinear regression analyses of toxicity data (Figure 9) yielded the following EC_{20} and EC_{50} values and their corresponding CIs (in parentheses): 2 (0.9–3) and 9 (6–12) mg of EM/kg of soil, respectively, for adult *F. candida* survival (Gompertz model) and 1 (0.5–2) and 6 (3–9) mg of EM/kg of soil, respectively, for juvenile *F. candida* production (Gompertz model). These results will undergo a data quality review by the USEPA before the EC_{20} concentration for juvenile *F. candida* production is included as a toxicity benchmark within a derivation of a soil invertebrate-based Eco-SSL value for NG. All ecotoxicological parameters determined for the toxicity of NG, weathered and aged in SSL soil, to *F. candida* are given in Table 6.

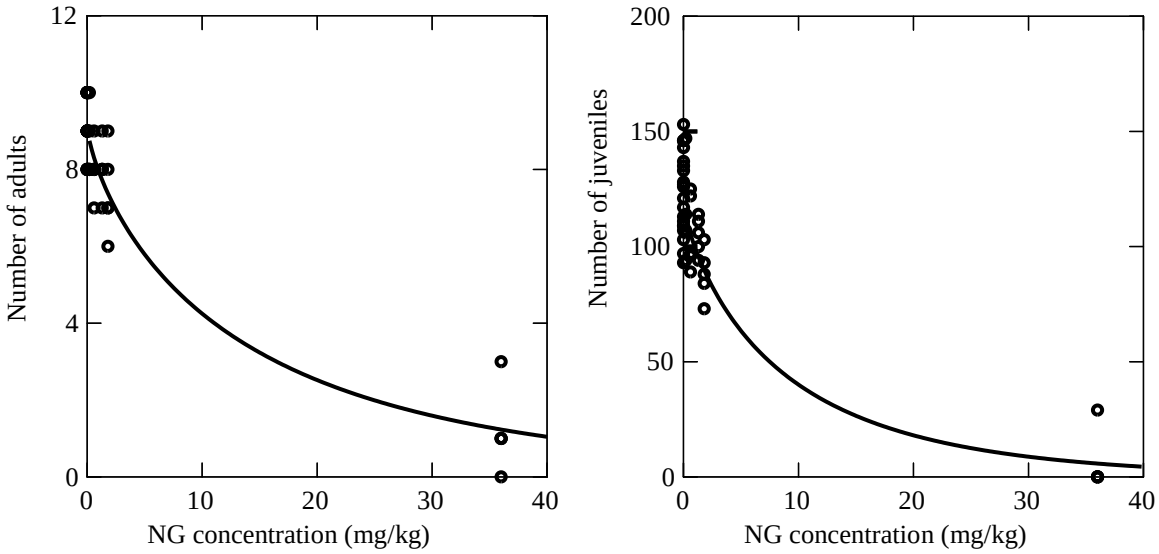

Figure 9. Effects of NG, weathered and aged in SSL soil, on adult *F. candida* survival and juvenile production.

4. DISCUSSION

Development of screening-level benchmarks for ERAs of contaminated soils has become a critical need in recent years (Kuperman et al., 2009b). An extensive review of literature (USEPA, 2005) led to the determination that there was insufficient information regarding EM soil contaminants to generate benchmarks for soil invertebrates. Our toxicity studies included the use of natural soils that meet the criteria for benchmark development (USEPA, 2005), which requires soil characteristics that can support relatively high bioavailability of the contaminant of interest, in this case EMs. Investigation of EM toxicities to soil invertebrates for benchmark development utilized EM amendments that were weathered and aged in soils to more closely simulate the effects of exposure in the field (Kuperman et al., 2003; Kuperman et al., 2004a; Kuperman et al., 2005; Simini et al., 2003). Weathering and aging of chemicals in soil may reduce the exposure of soil invertebrates to EMs as a result of photodecomposition, hydrolysis, reaction with OM, sorption or fixation, precipitation, immobilization, occlusion, microbial transformation, and other fate processes that commonly occur at contaminated sites. These fate processes can reduce the amount of a chemical that is bioavailable or may reveal increased toxicity due to the presence of more-toxic transformation products.

Ecotoxicological benchmarks are based on measured concentrations of a chemical in soil rather than nominal concentrations (USEPA, 2005). In our studies, the exposure concentrations of EMs in soil were analytically determined for all definitive-toxicity tests. Chemical analysis utilized USEPA method 8330A (USEPA, 1998) based on ACN-extraction of EM from soil. Results from ACN-extraction of EM, weathered and aged in soils, showed good correlation between nominal and measured concentrations, which confirmed that the soil

amendment procedure used in the toxicity tests was appropriate and that the USEPA method 8330A was efficient for quantifying the amount of EM in soil.

We conducted toxicity tests to generate a data set that was used to establish a baseline for a new positive control reference toxicant that replaced the beryllium sulfate ($BeSO_4$) used in earlier studies. Boric acid is less harmful for the environment than is beryllium (the reference toxicant used in our previous studies). The ISO 11267 protocol (ISO, 1999) was modified for toxicity testing with natural soils, including SSL and TSL sandy loam soils, that each support the relatively high bioavailability of many organic and inorganic chemicals. Toxicity tests with boric acid were conducted using SSL soil, and the results were plotted on a Boric Acid Warning Chart to monitor the condition of the *F. candida* culture (Figure 2). These values established the warning limits and the 95% CL for *F. candida* culture used in the definitive tests. These results also confirmed that boric acid was a suitable replacement for beryllium as a positive control for toxicity tests with *F. candida* and met the validity requirements of the test protocol.

Benchmark screening concentrations for *F. candida* toxicity were determined for 2,4-DNT; 2-ADNT; 4-ADNT; HMX; and NG, weathered and aged in field soils, using accepted toxicity-testing protocols. These studies incorporated reproduction endpoints and lethal endpoints. Results showed that the *F. candida* reproduction endpoints were the more-sensitive indicators of toxicity as compared with adult survival.

The relative bioavailability scores (USEPA, 2005) for nonionic organic compounds in these soils were "high" for TSL and SSL soils and "medium" for KL and WCL soils. The results of the toxicity testing of 2,4-DNT, weathered and aged in TSL, KL, and WCL soils, were compared with the results of 2,4-DNT toxicity testing in SSL soil from a previous study (from SERDP CU-1221 investigations; Kuperman, 2003). Comparison of toxicity values showed that 2,4-DNT was somewhat more toxic in KL, which has a "medium" relative bioavailability score, as compared with either SSL or TSL, which have "high" relative bioavailability scores (USEPA, 2005). This result indicated that additional factors, beyond soil clay and OM contents and soil pH, may enable greater-than-anticipated toxicity to *F. candida* from 2,4-DNT, weathered and aged in KL soil. Based on these results for *F. candida*, the order of toxicity for 2,4-DNT that was weathered and aged in these soils was (from greatest to least): KL > SSL > TSL > WCL.

Toxicities of the EMs studied were the least in WCL soil, which has the greatest percentage of OM (5%) as compared with TSL (1.4%) and KL (1.5%). However, the variance in toxicities of 2,4-DNT in different soils to *F. candida* was not explained by the results obtained for any soil parameter investigated. No discrepancies were found for toxicity results from the sandy loam SSL and TSL soils, each of which has characteristics that support high relative bioavailability of nonionic organic compounds, such as the EMs that were tested. However, an undetermined additional soil characteristic may allow greater-than-anticipated toxicity of 2,4-DNT in soils that are similar to KL. Alternatively, the specific microenvironment of ecological niches in soil occupied by *F. candida* (i.e., air-filled soil pores) may minimize their direct contact with 2,4-DNT in soil pore water or solid-phase soil, when compared with the soil annelids earthworm or potworm, which are directly exposed to both soil pore water and solid-

phase soil. Data for toxicity of 2,4-DNT, weathered and aged in soil, to earthworms and potworms showed stronger correlations between the toxicity endpoints and soil constituents (Kuperman, 2003) than does the present data for *F. candida*.

Toxicity testing of 2-ADNT and 4-ADNT, weathered and aged in SSL soil, yielded EC_{20} values (with their corresponding 95% CIs in parentheses) for production of *F. candida* juveniles of 30 (26–34) and 26 (19–33) mg of EM/kg of soil, respectively. These results showed that ADNTs are significantly more toxic to *F. candida* than the parent material TNT with an EC_{20} and CI of 53 (44–63) mg of EM/kg of soil (Kuperman et al., 2006).

Results of toxicity testing of HMX, weathered and aged for 3 months in TSL soil, showed that exposure did not affect either the survival of adult *F. candida* or the juvenile production, up to and including 10,208 mg of EM/kg of soil (unbounded NOEC). In a previous study with *F. candida*, results for HMX, weathered and aged in SSL soil, produced a bounded NOEC of 130 mg of EM/kg of soil and a LOEC of 280 mg of EM/kg of soil. Exposure of *F. candida* to HMX in freshly amended SSL soil produced a significant effect on the production of juveniles, resulting in an EC_{20} value of 235 mg of EM/kg of soil (Kuperman, 2003). Correspondingly, the EC_{20} value using HMX increased to 1046 mg of EM/kg of soil in weathered and aged soil treatment; however, the LOEC for the production of *F. candida* juveniles decreased under the same conditions (Kuperman, 2003). These results indicated that different soils with differing soil properties can yield different results even though the same species and same test compound are utilized in testing. In the literature we can find discrepancies regarding the toxicity of the same chemical to different organisms. For the earthworm *E. andrei*, exposed to the EM hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition explosive; RDX) in SAS soil, Robidoux et al. (2000) found that the LOEC for reproduction was 95 mg EM/kg of soil. However, no effects were found on the mortality and reproduction of two other terrestrial invertebrates, the enchytraeid worm *Enchytraeus crypticus* and the collembolan *F. candida* in soils that were amended with up to 1000 mg of EM/kg of soil using either RDX or HMX as the EM (Schafer and Achazi, 1999). Those studies were conducted either in SAS (Robidoux et al., 2000) or in soil with relatively high (2.5–3.0% organic carbon) OM content (Schafer and Achazi, 1999), which limits their usefulness for describing natural systems or development of Eco-SSLs.

Toxicity testing of NG, weathered and aged in SSL soil, yielded EC_{20} and EC_{50} values and their corresponding 95% CIs (in parentheses) for juvenile *F. candida* production of 1 (0.5–2) and 6 (3–9) mg of EM/kg of soil, respectively. These results showed that NG was the most toxic of the EMs tested in this study. Therefore, the toxicity to juvenile *F. candida* production of the present EM compounds, weathered and aged in SSL soil, followed this order (from least to greatest toxicity on the basis of EC_{20} values): HMX << 2-ADNT ≈ 4-ADNT < 2,4-DNT < NG.

5. CONCLUSIONS

The studies were designed to develop scientifically defensible toxicity data for application in the derivation of Eco-SSL values (USEPA, 2005). These Eco-SSL values will be used as tools for the successful management of defense testing and training ranges, in a sustainable manner, and for knowledge-based decision making. Primary among the main

objectives of this project was to generate toxicity data that could be used to establish benchmarks for deriving the soil invertebrate-based Eco-SSLs for 2,4-DNT; 2-ADNT; 4-ADNT; HMX; and NG. Ecotoxicological testing was specifically designed to include experimental designs and formats to produce studies that will successfully meet the criteria for Eco-SSL derivation, outlined in the Eco-SSL Guideline (USEPA, 2005). The natural soils, TSL and SSL, which were used in the toxicity tests herein, had low OM and clay contents and low soil pH. This fulfilled the USEPA requirement to use soil with characteristics that support high relative bioavailability of organic contaminants for developing realistic, yet conservative, Eco-SSL values (USEPA, 2005).

Definitive studies, using *F. candida* exposures in the upland aerobic sandy loam soils, SSL and TSL, enabled the establishment of new ecotoxicological data for the effects of 2,4-DNT; 2-ADNT; 4-ADNT; HMX; and NG on soil invertebrates under conditions of very high relative bioavailability for organic chemicals in soil (as defined in USEPA, 2005). The preferences for reproduction benchmarks and for low-effective concentration level (i.e., EC_{20}), were justified to ensure that Eco-SSL values would be protective of the majority of ecological-receptor populations in soil and provide confidence that EM concentrations posing an unacceptable risk are not screened out early in the ERA process (i.e., during SLERA).

Using USEPA method 8330A, the exposure concentrations of 2,4-DNT; 2-ADNT; 4-ADNT; HMX; and NG in soil were analytically determined on the basis of ACN-extraction at the beginning of each definitive toxicity test (USEPA, 2007). Consequently, the ecotoxicological benchmarks were determined using measured EM concentrations. This complied with the USEPA's preference for derivation of Eco-SSL values on the basis of measured concentrations of a chemical in soil, over those based on nominal concentrations (USEPA, 2005). Analyses of the freshly amended soils using USEPA method 8330A showed good correlation between the nominal and measured ACN-extracted concentrations, which confirmed that the soil amendment procedures used in the definitive toxicity tests were appropriate and that this method was efficient for quantifying the amounts of 2,4-DNT; 2-ADNT; 4-ADNT; HMX; and NG in soil. Overall, the definitive studies using *F. candida* exposures in TSL or SSL soils supported the development of ecotoxicological benchmarks for EMs in compliance with Eco-SSL test-acceptance criteria (USEPA, 2005). All ecotoxicological benchmarks determined in these studies will be provided to the USEPA Eco-SSL Work Group for quality-control review before inclusion in the Eco-SSL database and for subsequent use in the development of individual soil invertebrate-based Eco-SSL values for 2,4-DNT; 2-ADNT; 4-ADNT; HMX; and NG.

Toxicity testing was also conducted with additional natural soils to extend the range of investigation of soil physicochemical characteristics that may affect the 2,4-DNT toxicity to soil invertebrates. Soil-related differences were evident in both acute (adult survival) and chronic (juvenile production) toxicity benchmarks that were established in this study with *F. candida* exposed to 2,4-DNT that was weathered and aged in each of the natural soils tested.

This study included the weathering and aging of EMs in soil in the experimental procedures to produce a soil microenvironment that was similar to field conditions. This would more closely approximate the exposure effects found in actual contaminated sites. Results of analyses showed that exposure conditions of *F. candida* to EMs weathered and aged in SSL soil

differed from those freshly amended in soil. Toxicity alterations after weathering and aging of 2,4-DNT or NG in soil were soil- and endpoint-specific. Overall, the results of this study showed that giving special consideration to the effects of weathering and aging of EM in soil for assessing toxicity was well-justified. Toxicity benchmarks generated herein will contribute to development of Eco-SSL values that better represent the exposure conditions of soil invertebrates at contaminated sites.

Additional studies are required to resolve the current uncertainties in our understanding of the mechanisms contributing to the change in toxicities of EMs following weathering and aging in soil. These studies should be conducted with soil types that are selected for a wide range of properties, particularly clay type and content and OM, which affect the fate and bioavailability of EMs. This will contribute to a better understanding of the complex interactions among the physical, chemical, and biological components that affect the outcome of ecotoxicity testing.

LITERATURE CITED

ASTM International. *Standard Specification for Reagent Water* [Online]; ASTM D1193-99. ASTM International: West Conshohocken, PA, 2004; UNCLASSIFIED Specification. http://www.astm.org/DATABASE.CART/HISTORICAL/D1193-99.htm (accessed December 2014).

Checkai, R.T.; Major, M.A.; Nwanguma, R.O.; Amos, J.C.; Phillips, C.T.; Wentsel, R.S.; Sadusky, M.C. *Transport and Fate of Nitroaromatic and Nitramine Explosives in Soils from Open Burning/Open Detonation Operations: Radford Army Ammunition Plant (RAAP)*; ERDEC-TR-133; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1993a; UNCLASSIFIED Report (ADA278465).

Checkai, R.T.; Major, M.A.; Nwanguma, R.O.; Amos, J.C.; Phillips, C.T.; Wentsel, R.S.; Sadusky, M.C. *Transport and Fate of Nitroaromatic and Nitramine Explosives in Soils from Open Burning/Open Detonation Operations: Anniston Army Depot (AAD)*; ERDEC-TR-135; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1993b; UNCLASSIFIED Report (ADA278547).

Checkai, R.T.; Major, M.A.; Nwanguma, R.O.; Amos, J.C.; Phillips, C.T.; Wentsel, R.S.; Sadusky, M.C. *Transport and Fate of Nitroaromatic and Nitramine Explosives in Soil from Open Burning/Open Detonation Operations: Milan Army Ammunition Plant (MAAP)*; ERDEC-TR-136; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1993c; UNCLASSIFIED Report (ADA279145).

Checkai, R.T.; Wentsel, R.S.; Phillips, C.T.; Major, M.A.; Nwanguma, R.O.; Amos, J.C. Environmental Fate of RDX, HMX, TNT, 2,4-DNT, and 2,6-DNT in Clarksville-Fullerton Cherty Loam Soil. In *Proceedings of the 17th Annual Army Environmental R&D Symposium*, 22–24 June 1993; U.S. Army Corps of Engineers Toxic and Hazardous Materials Agency: Aberdeen Proving Ground, MD, 1993d.

Environment Canada (EC). *Guidance Document on Statistical Methods for Environmental Toxicity Tests;* EPS 1/RM/46; Environment Canada: Quebec, Canada, 2005.

Calabrese, E.J. Hormesis: Why It Is Important to Toxicology and Toxicologists. *Environ. Toxicol. Chem.* **2008**, *27*, 1451–1474.

International Organization for Standardization (ISO). *Soil Quality – Inhibition of Reproduction of Collembola (*Folsomia candida*) by Soil Pollutants*; ISO 11267; Geneva, Switzerland, 1999.

Jenkins, T.F. Energetic Munitions Constituents on DoD Training Ranges: Deposition, Accumulation, and Appropriate Characterization Technology. Final Report. In *Strategic Environmental Research and Development Program (SERDP) and Environmental Security Technology Certification Program (ESTCP) Technical Exchange Meeting on DoD Operational Range Assessment and Management Approaches*, October 2007; pp B-3–B-24; http://serdp-estcp.org/content/download/8237/101218/file/RAWorkshopRDTENeedsRpt.pdf (accessed December 2014).

Jenkins, T.F.; Hewitt, A.D.; Grant, C.L.; Thiboutot, S.; Ampleman, G.; Walsh, M.E.; Ranney, T.A.; Ramsey, C.A.; Palazzo, A.; Pennington, J.C. Identity and Distribution of Residues of Energetic Compounds at Army Live-Fire Training Ranges. *Chemosphere* **2006**, *63*, 1280–1290.

Kuperman, R. *Development of Ecological Toxicity and Biomagnification Data for Explosives Contaminants in Soil, Final Technical Report* [Online]; SERDP CU-1221; U.S. Army Edgewood Chemical Biological Center: Aberdeen, MD, 2003. https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Risk-Assessment/ER-1221/ER-1221/(language)/eng-US (accessed December 2014).

Kuperman, R.G.; Checkai, R.T.; Simini, M.; Phillips, C.T. Manganese Toxicity in Soil for *Eisenia fetida*, *Enchytraeus crypticus* (Oligochaeta), and *Folsomia candida* (Collembola). *Ecotoxicol. Environ. Saf.* **2004**, *57*(1), 48–53.

Kuperman, R.G.; Checkai, R.T.; Simini, M.; Phillips, C.T.; Kolakowski, J.E.; Chester, N.A. Ecological Soil Screening Levels for Invertebrates at Explosives-Contaminated Sites: Supporting Sustainability of Army Testing and Training. In *Proceedings of 25th Army Science Conference*, 27–30 November 2006; Orlando, FL, 2006.

Kuperman, R.G.; Simini, M.; Siciliano, S.; Gong, P. Effects of Energetic Materials on Soil Organisms. In *Ecotoxicology of Explosives*; Sunahara, G.I., Lotufo, G., Kuperman, R.G., Hawari, J., Eds; CRC Press: Boca Raton, FL, 2009a, pp 35–76.

Kuperman, R.G.; Checkai, R.T.; Johnson, M.; Robidoux, P.Y.; Lachance, B.; Thiboutot, S.; Ampleman, G. Ecological Risk Assessment of Soil Contamination with Munition Constituents in North America. In *Ecotoxicology of Explosives*; Sunahara, G.I., Lotufo, G., Kuperman, R.G., Hawari, J., Eds; CRC Press: Boca Raton, FL, 2009b, pp 277–307.

Kuperman, R.G.; Checkai, R.T.; Simini, M.; Phillips, C.T.; Kolakowski, J.E.; Kurnas, C.W. Weathering-and-Aging of 2,4,6-Trinitrotoluene in Soil Increases Toxicity to Potworm *Enchytraeus crypticus*. *Environ. Toxicol. Chem.* **2005**, *24*, 2509–2518.

Kuperman, R.G.; Checkai, R.T.; Phillips, C.T.; Simini, M.; Kolakowski, J.E.; Kurnas, C.W.; Sunahara, G.I. *Toxicity of Nitro-Heterocyclic and Nitroaromatic Energetic Materials to Enchytraeid Worm* Enchytraeus crypticus *in a Natural Sandy Loam Soil*; ECBC-TR-344; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2004a; UNCLASSIFIED Report.

Kuperman, R.G.; Checkai, R.T.; Simini, M.; Phillips, C.T.; Sunahara, G.I.; Hawari, J.; Rocheleau, S.; Paquet, L. *Development of Toxicity Benchmarks for Nitrogen-based Energetic Materials for the Enchytraeid Worm,* Enchytraeus crypticus; ECBC-TR-1134; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2013; UNCLASSIFIED Report.

Kuperman, R.G.; Simini, M.; Phillips, C.T.; Checkai, R.T.; Kolakowski, J.E.; Kurnas, C.W.; Sunahara, G.I. Survival and Reproduction of *Enchytraeus crypticus* (Oligochaeta, Enchytraeidae) in a Natural Sandy Loam Soil Amended with the Nitro-Heterocyclic Explosives RDX and HMX. *Pedobiologia* **2003**, *47*, 651–656.

Monteil-Rivera, F.; Halasz, A.; Groom, C.; Zhao, J.S.; Thiboutot, S.; Ampleman, G. Fate and Transport of Explosives in the Environment: A Chemist's View. In *Ecotoxicology of Explosives;* Sunahara, G.I., Lotufo, G., Kuperman, R.G., Hawari, J., Eds; CRC Press: Boca Raton, FL, 2009, pp 5–33.

Organisation for Economic Co-operation and Development (OECD). OECD Guidelines for Testing Chemicals: Collembolan Reproduction Test in Soil, Test No. 232; 2009. http://www.oecd-ilibrary.org/docserver/download/9723201e.pdf?expires=1425670 (accessed December 2014)

Pal, B.C.; Ryon, M.G. *Database Assessment of Pollution Control in the Military Explosives and Propellants Production Industry*; ORNL-6202, NTIS # DE86007934; Oak Ridge National Laboratory: Oak Ridge, TN, 1986; UNCLASSIFIED Report (ADA165853)

Phillips, C.T.; Checkai, R.T.; Chester, N.A.; Wentsel, R.S.; Major, M.A.; Amos, J.C.; Simini, M. T*oxicity Testing of Soil Samples from Joliet Army Ammunition Plant, IL*; ERDEC-TR-137; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1994; UNCLASSIFIED Report (ADA279091).

Phillips, C.T.; Kuperman, R.; Checkai, R.T. Toxicity of Chemical Warfare Agent HD to *Folsomia candida* in Different Soil Types. *Eur. J. Soil Biol.* **2002**, *38*, 281–285.

Robidoux, P.Y.; Svendsen, C.; Caumartin, J.; Hawari, J.; Ampleman, G.; Thiboutot, S.; Weeks, J.M.; Sunahara, G.I. Chronic Toxicity of Energetic Compounds in Soil Determined Using the Earthworm (*Eisenia andrei*) Reproduction Test. *Environ. Toxicol. Chem.* **2000**, *19*, 1764–1773.

Schäfer, R.; Achazi, R.K. The Toxicity of Soil Samples Containing TNT and Other Ammunition Derived Compounds in the *Enchytraeid* and *Collembola* Biotest. *Environ. Sci. Pollut. Res.* **1999**, *6*, 213–219.

Simini, M.; Wentsel, R.S.; Checkai, R.T.; Phillips, C.T.; Chester, N.A.; Major, M. A. Evaluation of Soil Toxicity at Joliet Army Ammunition Plant. *Environ. Toxicol. Chem.* **1995**, *14*, 623–630.

Simini, M.; Checkai, R.T.; Kuperman, R.G.; Phillips, C.T.; Kolakowski, J.E.; Kurnas C.W.; Sunahara, G.I. Reproduction and Survival of *Eisenia fetida* in a Sandy Loam Soil Amended with the Nitro-Heterocyclic Explosives RDX and HMX. *Pedobiologia* **2003**, *47*, 657–662.

Spanggord, R.J.; Mill, T.; Chou, T.W.; Smith, J.H.; Lee, S. *Environmental Fate Studies on Certain Munitions Wastewater Constituents. Final Report, Phase II — Laboratory Studies*; LSU-7934; SRI International: Menlo Park, CA; 1980; UNCLASSIFIED Report (ADA099256).

Sunahara, G.I.; Lotufo, G.L.; Kuperman, R.G.; Hawari, J. *Ecotoxicology of Explosives*; CRC Press: Boca Raton, FL, 2009.

U.S. Environmental Protection Agency (USEPA). *Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC) - Method 8330A. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*; SW-846 update III, part 4:1 (B); USEPA Office of Solid Waste: Washington, DC, 1998.

USEPA. *Guidance for Developing Ecological Soil Screening Levels*; OSWER Directive 9285.7-55; U.S. Environmental Protection Agency: Washington, DC, 2005. http://www.epa.gov/ecotox/ecossl/pdf/ecossl_guidance_chapters.pdf (accessed December 2014).

USEPA. Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC). Method 8330A, Revision 1, February, 2007. In *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods,Third Edition, Final Update IV*; SW-846; Office of Solid Waste, U.S. Environmental Protection Agency: Washington, DC, 2007. http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/8330a.pdf (accessed December 2014).

U.S. General Accountability Office (GAO). *Military Munitions: DOD Needs To Develop a Comprehensive Approach for Cleaning Up Contaminated Sites*; GAO-04-147; Report to the Honorable John D. Dingell, Ranking Minority Member, Committee on Energy and Commerce, House of Representatives; U.S. GAO: Washington, DC, 2003. http://www.gao.gov/new.items/d04147.pdf (accessed December 2014).

Verscheuren, K. *Handbook of Environmental Data on Organic Chemicals*,2nd ed; Van Nostrand Reinhold: New York, 1983; p 1310.

ACRONYMS AND ABBREVIATIONS

2-ADNT	2-amino-4,6-dinitrotoluene
4-ADNT	4-amino-2,6-dinitrotoluene
1,3-DNB	1,3-dinitrobenzene
2,4-DNT	2,4-dinitrotoluene
ACN	acetonitrile
ANOVA	analysis of variance
BERA	baseline environmental risk assessment
CAS	Chemical Abstracts Service
CEC	cation exchange capacity
CI	confidence interval
CL	confidence limits
DNT	ditrotoluene
EC	Environment Canada
EC_{20}	20% effect concentration
EC_{50}	50% effect concentration
ECBC	U.S. Army Edgewood Chemical Biological Center
Eco-SSL	Ecological Soil-Screening Level
EC*p*	effective concentration for a specified percent effect
EM	energetic material
ERA	ecological risk assessment
FLSD	Fisher's least-significant difference
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (high-melting explosive)
HPLC	high-performance liquid chromatography
ISO	International Organization for Standardization
KL	Kirkland loam
K_{oc}	organic carbon water partition coefficient
K_{ow}	octanol–water partition coefficient
LOEC	lowest-observed-effect concentration
NG	nitroglycerin
NOEC	no-observed-effect concentration
NRC	National Research Council of Canada
OECD	Organisation for Economic Co-operation and Development
OM	organic matter
P	probability value
R^2	coefficient of determination
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition explosive)
SAS	standard artificial soil
SD	standard deviation
SE	standard error
SERDP	Strategic Environmental Research and Development Program
SLERA	screening-level ecological risk assessment
SSL	Sassafras sandy loam
TNT	2,4,6-trinitrotoluene

TSL	Teller sandy loam
USEPA	U.S. Environmental Protection Agency
WCL	Webster clay loam
WHC	water-holding capacity

DISTRIBUTION LIST

The following organizations received one Adobe portable distribution format (pdf) electronic version of this report:

U.S. Army Edgewood Chemical Biological Center
RDCB-DRT-E
Attn: Roman Kuperman, Research Biological Scientist (roman.g.kuperman.civ@mail.mil)
E5641
5183 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5424

U.S. Army Edgewood Chemical Biological Center
RDCB-DRT
Attn: Christopher Whalley (christopher.e.whalley.civ@mail.mil)
E3150
5183 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5424

Defense Threat Reduction Agency
Attn: Thomas Ward, DTRA/RD-CBD T, Rm 3226 (thomas.ward@dtra.mil)
Eric L. Moore, Chief, Advanced & Emerging Threats Division (J9-CBS), MSC 6201 (eric.moore@dtra.mil)
8725 John J. Kingman Road
Fort Belvoir, VA 22060-6201

Department of Homeland Security
DHS ORD CSAC
Attn: George Famini (george.famini@scitech.dhs.gov)
E3401
5183 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5424

G-3 History Office
U.S. Army RDECOM
Attn: Jeffrey K. Smart, Command Historian (jeffrey.k.smart.civ@mail.mil)
E5027
5183 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5424

ECBC Technical Library
RDCB-DRB-BL
Attn: Janett A. Stein, Hendall Inc. (janett.a.stein.ctr@mail.mil)
E3330
5183 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5424

Office of the Chief Counsel
AMSRD-CC
Attn: Vicki A. Upchurch, Paralegal Specialist (vicki.a.upchurch.civ@mail.mil)
Building 6001
Combat Drive
Aberdeen Proving Ground, MD 21005-1846

Defense Technical Information Center
Attn: DTIC OA
8725 John J. Kingman Road, Suite 0944
Fort Belvoir, VA 22060-6218

ECBC Rock Island
RDCB-DE
Attn: Nannette M. Ramsey, Associate Director of Engineering (nannette.m.ramsey.civ@mail.mil)
1 Rock Island Arsenal
Building 62/1/SW
Rock Island, IL 61299-7390


EDGEWOOD CHEMICAL BIOLOGICAL CENTER
A U.S. Army RDECOM Laboratory