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TOXICITIES OF RDX OR TNT FRESHLY AMENDED OR WEATHERED-AND-AGED IN FIVE NATURAL SOILS TO THE COLLEMBOLAN *FOLSOMIA CANDIDA*

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14. ABSTRACT The toxicities of 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to <i>Folsomia candida</i> (Collembola) were investigated using an ISO 11267:1998 test. Studies were designed to identify and characterize soil physicochemical parameters that may affect the acute (adult mortality) and chronic (reproduction) toxicities of TNT and RDX to <i>F. candida</i> and also to generate ecotoxicological benchmarks for development of ecological soil screening levels for risk assessments of contaminated soils. Soils studied included Teller and Sassafras sandy loams (TSL and SSL, respectively) and Richfield, Kirkland, and Webster clay loams (RCL, KCL, and WCL, respectively). Based on the effective concentrations for 50% of the population (EC ₅₀ values), for TNT weathered-and-aged in soil, chronic toxicity to <i>F. candida</i> was in the order KCL > RCL > TSL > WCL > SSL, and for RDX weathered-and-aged in soil, the order was RCL > WCL > TSL > KCL > SSL. Organic matter was the dominant soil property that mitigated TNT toxicity for adult survival in freshly amended soil; soil pH correlated strongly with acute toxicity benchmarks (the effective concentrations for 20% of the population [EC ₂₀ values] for adult survival) for RDX freshly amended in soil. These correlations were not sustained after weathering-and-aging of TNT or RDX in soil.					
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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 December 2011.

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TOXICITIES OF RDX OR TNT FRESHLY AMENDED OR WEATHERED-AND-AGED IN FIVE NATURAL SOILS TO THE COLLEMBOLAN *FOLSOMIA CANDIDA*

1. INTRODUCTION

Many sites associated with military operations that involve munition manufacturing, disposal, testing, and training have been contaminated with elevated levels of explosives and related materials in soil. Concentrations of explosives in soil have been reported to exceed 87,000 mg kg⁻¹ for 2,4,6-trinitrotoluene (TNT) (Simini et al., 1995) and 74,000 mg kg⁻¹ for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Best et al., 2006). Although these energetic materials (EMs) can be persistent in the environment, their effects on soil biota have not been sufficiently investigated (Kuperman et al., 2009a) because scientifically defensible screening values, which could be used in ecological risk assessment (ERA), were not available for these explosives in soil (Kuperman et al., 2009b).

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 (U.S. Environmental Protection Agency [U.S. EPA], 2005). Eco-SSLs represent 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. These values can be used in the screening level ecological risk assessment (SLERA) to identify those contaminants that are not of potential ecological concern in soils and thus do not require further evaluation in the baseline ecological risk assessment (BERA). This would potentially result in cost savings during ecologically based site assessments. To address the existing data needs, we conducted definitive studies that were specifically designed to meet the U.S. EPA criteria (U.S. EPA, 2005) for derivation of toxicity benchmarks acceptable for Eco-SSL development. Our intention was to increase the availability of ecotoxicological data to assist site managers in the knowledge-based decision-making process of securing the sustainable use of testing and training installations. The main objectives of the present studies were (1) to establish toxicity data that are appropriate ecotoxicological benchmarks for TNT or RDX in natural soils with properties that support conditions of high relative bioavailability of each EM for use in the development of Eco-SSLs, and (2) to learn which relationships can be defined among predominant soil physicochemical parameters and the TNT or RDX toxicities for survival and reproduction of the soil invertebrate *Folsomia candida*.

2. MATERIALS AND METHODS

2.1 Test Soils

Toxicity testing was conducted using soils with a relatively wide range of physicochemical characteristics. These soils included the following:

- Teller sandy loam (TSL), a fine-loamy, mixed, active, thermic Udic Argiustoll collected from agricultural land of the Oklahoma State University Perkins Experiment Station, Payne County, OK;

- Sassafras sandy loam (SSL), a fine-loamy, siliceous, semiactive, mesic Typic Hapludult collected from an open grassland field in the coastal plain on the property of the U.S. Army Aberdeen Proving Ground, Harford County, MD;
- Kirkland clay loam (KCL), a fine, mixed, superactive, thermic Udertic Paleustoll collected from Payne County, OK;
- Richfield clay loam (RCL), a fine, smectitic, mesic Aridic Argiustoll collected from Texas County, OK; and
- Webster clay loam (WCL), a fine-loamy, mixed, superactive, mesic Typic Endoaquoll collected from Story County, IA.

The qualitative relative bioavailability (QRB) scores for organic chemicals in natural soils were considered “very high” for TSL and SSL, “medium” for KCL and WCL, and “low” for RCL according to the Eco-SSL criteria (U.S. EPA, 2005). During soil collection in the field, vegetation and the organic horizon were removed, and the top 12 cm of the A-horizon were then collected. Soil was sieved through a 5 mm mesh screen, air-dried for at least 72 h, mixed periodically to ensure uniform drying, passed through a 2 mm sieve, and stored at room temperature. The respective soils were then analyzed for physical and chemical characteristics. Results of these analyses are presented in Table 1.

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

Soil Parameter	TSL	SSL	KCL	RCL	WCL
Sand (%)	65	70	37	30	33
Silt (%)	22	13	34	43	39
Clay (%)	13	17	28	28	28
Texture	Sandy loam	Sandy loam	Clay loam	Clay loam	Clay loam
Cation exchange capacity (cmol kg ⁻¹)	4.3	5.5	10.3	27.6	20.8
Organic matter (%)	1.4	1.2	2.6	3.3	5.3
pH	4.4	5.2	6.4	7.4	5.9
Water-holding capacity (%)	13	18	20	21	23
QRB ^a	Very high	Very high	Medium	Low	Medium

Note: Analyses were performed by the Cooperative Extension Service, University of Maryland Soil Testing Laboratory, College Park, MD.

^a Based on QRB scores for nonionizing organic contaminants in natural soils (U.S. EPA, 2005).

2.2 Chemicals and Reagents

EMs used in these studies included TNT (Chemical Abstracts Service [CAS] no. 118-96-7; purity, 99.9%) and RDX (CAS no. 121-82-4; purity, 99%). These EMs were obtained from Defence Research and Development Canada-Valcartier (Quebec City, Quebec, Canada). Beryllium sulfate ($\text{BeSO}_4 \cdot 4\text{H}_2\text{O}$; CAS no. 7787-56-6; purity, 99.99%) was used as the positive control in all toxicity tests. High-performance liquid chromatography (HPLC)-grade acetone (CAS no. 67-64-1) was used to prepare individual EM solutions for soil amendments. 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 the HPLC analytical determinations. Certified standards of TNT and RDX (AccuStandard; New Haven, CT) were used in HPLC determinations. ASTM Type I water (18 M Ω cm at 25 °C; ASTM, 2004a) was used throughout the toxicity studies. It was obtained using a Milli-RO 10 Plus water purification system followed processing with Milli-Q PF Plus systems (Millipore; Bedford, MA). The same grade of water was used throughout the analytical determinations. Glassware was washed with phosphate-free detergent and sequentially rinsed with tap water, ASTM Type II water (>5 M Ω cm at 25 °C), 1% (v/v) nitric acid (analytical reagent-grade), and ASTM Type I water.

2.3 Soil Amendment Procedures

Studies were performed separately and independently for TNT or RDX in freshly amended (FA) and weathered-and-aged (W-A) soil to determine toxicity benchmark values for TNT or RDX in each exposure type. During the soil amendment procedure, TNT or RDX was amended into separate aliquots of soil using an organic solvent (acetone) as a carrier. This was necessary to distribute the TNT or RDX evenly and uniformly to a large soil surface area, which would have been difficult to achieve if solid chemical crystals had been added to soil. Carrier control soils were amended with acetone only. Soil was spread to a thickness of 2.5 cm. Individual EMs were dissolved in acetone in glass volumetric flasks then pipetted across the soil surface to ensure that the volume of solution added at any one time did not exceed 15% (v/w) of the soil dry mass. After the solution was added, the volumetric flask was rinsed twice with a known volume of acetone, which was also pipetted onto the soil. If the total volume of solution required to amend the soil exceeded 15% (v/w), the solution was added in successive stages. Between additions, the acetone was allowed to evaporate 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 treatment to equal the volume required to dissolve the EM at the greatest dissolved concentration amended. Amended soil was air-dried overnight (minimum of 18 h) in a darkened chemical hood to prevent photolysis of the EM. 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 mixing, soil containing the FA EM was hydrated with ASTM Type I water to 88% of the respective water-holding capacity (WHC) of each soil. The soil was allowed to moisture-equilibrate for 24 h before the test species, *F. candida*, was added for commencement of toxicity testing. Additionally, samples of each FA soil were hydrated with ASTM Type I water to 60% of the respective WHC to initiate weathering-and-aging of the EM in each soil prior to additional toxicity testing. Positive controls were prepared as a solution of beryllium sulfate in ASTM Type I water using the nominal concentration of 50 mg of beryllium per kilogram of soil (dry weight) in all tests.

2.4 Weathering-and-Aging Explosives in Soil

At many contaminated sites, explosives and other EMs in soils have been exposed to weathering-and-aging processes for many years. Therefore, to provide appropriate benchmark data for Eco-SSL development, special consideration was given to weathering-and-aging of these EMs in soil to assess the TNT or RDX toxicities for *F. candida*. Standardized methods for weathering-and-aging of explosives in soil are not available. We have developed procedures that simulate, at least in part, the weathering-and-aging processes for chemicals in soil. These procedures allowed us to more accurately approximate the exposure conditions for soil biota in the field, compared with tests conducted with FA chemicals or tests conducted after a short equilibration period (e.g., 24 h) (Kuperman et al., 2003, 2005, 2006a–2006e; Simini et al., 2003, 2006). Air-dried soil batches were amended with several concentrations of TNT or RDX and were initially hydrated in open glass containers with ASTM Type I water to 60% of the WHC of each soil. Soil was then subjected to alternating cycles of hydration and air-drying at ambient temperatures in a greenhouse. Each soil treatment was weighed and readjusted to its initial mass by weekly addition of ASTM Type I water. Any soil surface crust that formed during the week was broken with a spatula before water was added. After the conclusion of EM weathering-and-aging procedures, all soil treatments were brought to 88% of the WHC of each soil 24 h before toxicity tests were started. The effects of weathering-and-aging TNT or RDX in soil on the toxicity to *F. candida* were investigated by comparing test results for EMs W-A in soils with results obtained for EMs FA in soils.

Soil treatments with TNT concentrations representing low, intermediate, and high levels were monitored periodically during the weathering-and-aging process to determine the times when TNT concentrations were effectively stabilized or had declined to $\leq 5\%$ of the initial concentration in FA soil treatments with the highest rates of decrease. Nominal TNT concentrations selected for monitoring in these studies were: 20, 100, 200, and 300 mg kg⁻¹ in TSL; 50, 100, 200, and 400 mg kg⁻¹ in SSL or KCL; 5, 25, 100, and 500 mg kg⁻¹ in RCL; and 40, 100, 200, and 400 mg kg⁻¹ in WCL. The respective times determined for each TNT–soil pairing were then designated for termination of the weathering-and-aging procedures for that respective soil and commencement of the corresponding definitive toxicity tests.

Previous studies have shown that RDX did not significantly degrade under aerobic conditions, and that toxicity to soil invertebrates did not change significantly ($p \leq 0.05$) when RDX was subjected to the weathering-and-aging process in soil (Dodard et al., 2005; Kuperman et al., 2003, 2004a; Simini et al., 2003). Therefore, soil RDX concentrations were not monitored during the 3 month weathering-and-aging process. RDX concentrations were analytically determined in each soil treatment immediately before toxicity testing was started.

2.5 ACN Extraction of TNT and RDX from Soil

Concentrations of TNT and RDX were analytically determined in all control and treated soils, in triplicate, at the beginning of each definitive test using ACN extraction and U.S. EPA Method 8330A (U.S. EPA, 2007). In accordance with Soil Amendment Procedures (Section 2.3), soil subsamples for analytical determinations were hydrated for 24 h before extraction. The soil dry fraction (dry weight/wet weight) was determined in triplicate from

subsamples of each treatment concentration. For extraction, 2 g soil samples were collected from each soil batch treatments and controls and placed into respective 50 mL polypropylene centrifuge tubes, and 10 mL of ACN was added to each tube. Samples were vortexed with the ACN for 1 min and then sonicated in darkness for 18 h at 20 °C. After the sonicated samples were allowed to settle for 1 h at room temperature, 5 mL of each supernatant was transferred into glass tubes that contained 5 mL of CaCl₂ solution (5 g L⁻¹) as a flocculent. Supernatant was then filtered through a 0.45 µm polytetrafluoroethylene (PTFE) syringe cartridge, and 1 mL of each filtered solution was transferred into an HPLC vial. Soil extracts were analyzed and quantified by HPLC.

2.6 Adapted Toxicity Characteristic Leaching Procedure (ATCLP) Extraction of TNT from Soil

In addition to extraction with ACN, TNT was also extracted from soil using an ATCLP (Haley et al., 1993) at the beginning of each definitive test. The ATCLP is a modification of the toxicity characteristic leaching procedure (TCLP) (40 CFR Part 268.41, Hazardous Waste Management, Method 1311). The procedure was modified by substituting CO₂-saturated water for acetic acid to acidify the water and thereby simulate the soil–water conditions that exist as a result of respiration by soil biota. To retain the effects of the natural buffering capacity of the soil, the CO₂-saturated water was not recharged once it had been added to the soil (unlike the acetic acid in the TCLP). All extractions were performed in triplicate. For each treatment concentration, 4 g of soil were transferred into 20 mL vials, and 16 mL of CO₂-saturated water (pH 3.8–4.0) was added. The vials were immediately sealed. Soil samples were vortexed for 45 s then mixed in darkness for 18 h on a rotary end-over-end mixer (30 rpm) at room temperature (40 CFR Part 268.41). The mixture was allowed to settle for at least 2 h before supernatants were filtered through 0.45 µm PTFE syringe cartridges. An equivalent volume of ACN was added to each filtered soil extract before HPLC analysis was performed.

ATCLP-based extractions were not conducted in studies with RDX because a portion of the range of RDX concentrations selected for toxicity tests with *F. candida* exceeded the aqueous solubility of RDX (42 mg L⁻¹ at 20 °C; Monteil-Rivera et al., 2004).

2.7 Analytical Determinations of TNT and RDX

Soil extracts were analyzed and EM concentrations were quantified using reversed-phase HPLC with a modified U.S. EPA Method 8330A. The method was modified by adjusting the flow rate of the 50:50 methanol–water mobile phase to 1.0 mL min⁻¹ rather than 1.5 mL min⁻¹. A 25 cm × 4.6 mm × 5 µm particle size C-18 column was used for all determinations. For HPLC, Beckman System Gold analytical instrumentation (Beckman Coulter; Brea, CA) was used, which consists of a model 126 programmable solvent module, a model 168 diode array detector, and a model 507 automatic sampler. Calibration curves were generated before each HPLC run by dissolving certified standards (AccuStandard) of each EM in a 50:50 water–ACN solution in a range of concentrations appropriate for each run. The method detection limits were 0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil. In addition, solution reagent blanks and standards were placed intermittently between samples. All chemical concentrations in soil were expressed on a dry-mass basis. Nominal and analytically determined concentrations used in the definitive tests are shown in Tables 2 through 21.

2.8 Toxicity Assessments

Several soil invertebrate toxicity tests, for which standardized protocols have been developed by the International Organization for Standardization (ISO, 1998a, 1998b), can effectively be used to assess the toxicity and derive protective benchmark values for energetic materials (Stephenson et al., 2002; Løkke and Van Gestel, 1998). In the present studies, we used the *Folsomia* Reproduction Test, which is an adaptation of the ISO 11267 bioassay, *Soil Quality—Inhibition of Reproduction of Collembola* (*Folsomia candida*) by Soil Pollutants (ISO, 1998b). This bioassay was selected on the basis of its ability to measure chemical toxicity to ecologically relevant test species during chronic assays and its inclusion of at least one reproduction component among the measurement endpoints. The ISO guideline for this assay was originally developed for use with Organisation for Economic Co-operation and Development (OECD, 1984) artificial soil (similar soil formulation was later adapted for U.S. EPA standard artificial soil [SAS]; U.S. EPA, 1996; and for ASTM artificial soil; ASTM E1676-04, 2004b). Research from our laboratory has shown that this test can also be conducted using natural soils (Phillips et al., 2002; Kuperman et al., 2006d, 2006f), and this adaptation was used in these studies. The measurement endpoints for the test included juvenile production and survival of *Collembola* as adults.

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

Glass jars (42 mm i.d. × 45 mm height) were used as test containers for toxicity testing. Before use in testing, the jars were cleaned with acetone, rinsed successively with tap water and ASTM Type I water, and air-dried. On the first day of a test, a 100 g soil batch from each treatment level was hydrated with ASTM Type I water to 88% of its WHC, then one-fifth of each batch was weighed and transferred into a test container. Baker's yeast (0.05 g) was added to the soil surface in each container. Ten 10–12 day old juveniles were placed in each test container and were lightly misted with ASTM Type I water. Transparent plastic wrap was stretched over the top of each container and secured with a rubber band. Three pinholes were made in the plastic wrap to facilitate air exchange. All test containers were placed in an environment-controlled incubator under a 16 h light–8 h dark photoperiod cycle with a mean photosynthetically active radiation light intensity of $12.8 \pm 0.7 \mu\text{M m}^{-2} \text{s}^{-1}$ (985 ± 52 lux) and a mean temperature of 21.6 ± 0.1 °C for the duration of the 28 day test. The containers were weighed once each week, and any mass loss was replenished with an appropriate amount of ASTM Type I water.

On day 28 of exposure, the test was terminated by adding approximately 15 mL of tap water to each test chamber. The containers were gently mixed with a spatula and allowed to sit for several minutes to fully hydrate the soil. An additional 10 mL of water was added to each container, and the contents were again mixed with a spatula. The contents of each test container were then examined under a dissecting microscope (at 15× magnification) for the presence of adults and juveniles. The total numbers of adults and juveniles that floated to the surface were counted and recorded.

Treatment concentrations for each definitive test with TNT or RDX were selected on the basis of the range-finding test results to bracket the 20 and 50% inhibition levels for juvenile production for comparison with juvenile production in the carrier controls for each soil. Definitive tests included negative controls (no chemicals added), carrier controls (acetone), positive controls, and SSL soil control (SSL hydrated with ASTM Type I water to 88% of the WHC). The positive control was prepared as a solution of beryllium sulfate in ASTM Type I water added to SSL to obtain a nominal beryllium concentration of 50 mg kg⁻¹. Performance of a *F. candida* culture was deemed acceptable if juvenile production in the positive control remained within the range of 30 to 50% of juvenile production in the SSL controls. Five replicates of each of the EM treatments and controls were used in the definitive tests. Validity criteria for the negative controls included the following performance parameters (ISO, 1998b):

- The adult mortality does not exceed 30% at the end of the 28 day test;
- The average number of juveniles per test container at the end of the test is greater than 80 springtails (*F. candida*); and
- The coefficient of variation for the mean number of juveniles is ≤30%.

2.9 Data Analyses

Adult survival and juvenile production data were analyzed independently using regression models selected from those described in the 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 the regression lines generated by the models were closest to the data points, the regression coefficients for point estimates were the greatest, the residuals were homoscedastic (i.e., had the most random scattering), and the means, standard errors (SEs), and variances of the residuals were the smallest. The models selected for data analyses in these studies included logistic (Gompertz; eq 1), exponential (eq 2), and logistic hormetic (3), which has an additional parameter to accommodate hormesis (an effect whereby low concentrations of the test chemical stimulate the performance of the test organisms compared to the control organisms):

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

$$Y = a \times e^{\{[\log(1 - p)] \div EC_p\} \times C} + b \quad (2)$$

$$Y = \{a \times [1 + (h \times C)]\} \div (1 + \{[p + (h \times EC_p)] \div (1 - p)\} \times (C \div EC_p)^b) \quad (3)$$

where

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

The EC_p parameters used in these studies included the TNT or RDX concentrations that produced a 20% (EC_{20}) or 50% (EC_{50}) decrease in the measurement endpoint compared with the carrier control. The EC_{20} parameter based on reproduction endpoint is the preferred parameter for establishing Eco-SSL benchmark data for deriving Eco-SSL values. The EC_{50} parameter, a commonly reported value, was included to enable comparisons of the results produced in these studies with results reported by other researchers. The 95% confidence intervals (CIs) associated with the point estimates were determined.

Analysis of variance was used to determine the bounded (when possible) no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC) values for adult survival and juvenile production data, respectively. When no-observed-adverse-effect concentration (NOAEC) or lowest-observed-adverse-effect concentration (LOAEC) values were determined, the same statistical methods were used. Mean separations were performed using Fisher’s least-significant difference (FLSD) pairwise-comparison tests. The relationships among the selected soil parameters and toxicity data were determined using Pearson’s correlation analysis. All analyses were performed using untransformed data and analytically determined TNT or RDX concentrations. A significance level of $p \leq 0.05$ (95% confidence level) was accepted for all statistical tests. Statistical analyses were performed using SYSTAT 11.0 (Systat Software; Chicago, IL).

3. RESULTS

3.1 Analytical Determinations of TNT in Soil

Concentrations of TNT within amended soil were determined at the beginning of each definitive toxicity test using both ACN- and ATCLP-based (water) extractions. Samples prepared for weathering-and-aging of TNT in test soils were analyzed to determine the initial TNT concentrations. These concentrations were then contrasted with TNT concentrations determined at the end of the weathering-and-aging procedure to assess the net effect of weathering-and-aging of TNT in soil on the exposure conditions for *F. candida* during respective toxicity tests, and to compare TNT FA versus W-A in soil. Analytically determined initial concentrations were also used for monitoring ACN-extractable TNT concentrations during the weathering-and-aging process to determine the time when TNT concentrations were effectively stabilized and/or had declined to $\leq 5\%$ of the initial concentration.

Performance of the weathering-and-aging procedures in the present studies revealed differential rates of decreases in extractable soil TNT concentrations over time according to soil type. TNT concentrations decreased more rapidly over time in the three clay loam soils (RCL, KCL, and WCL) than in the two sandy loam soils (TSL and SSL). Decreases in the analytically determined TNT concentrations in the nominal 100 mg kg^{-1} treatment level in the five soils are shown as a function of time in Figure 1. These changes in soil TNT concentrations were typical of other nominal TNT treatments measured periodically over the 3 month weathering-and-aging procedures, and are shown in Figure 1 as examples because in the present studies, the nominal 100 mg kg^{-1} was the only treatment level that was selected for use in all five soils tested. Based on the results for each TNT–soil pairing, day 82, from the initial hydration of each test soil to 60% of the WHC, was designated for termination of the weathering-and-aging procedures and for commencement of definitive toxicity testing with *F. candida* in each of the five soils.

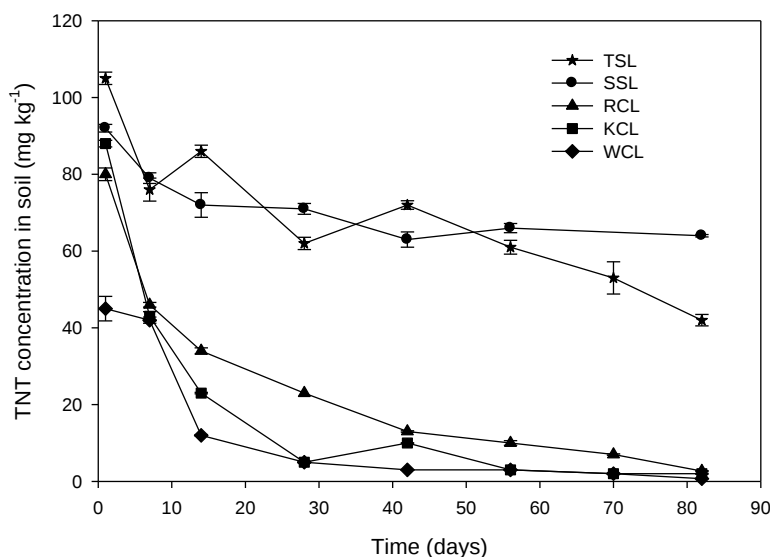


Figure 1. Concentrations of TNT in soil analytically determined during the 3 month weathering-and-aging process. Concentration values are means and SEs ($n = 3$). Natural soils TSL, SSL, KCL, RCL, and WCL were initially amended with 100 mg kg^{-1} nominal TNT concentration. Initial concentration was determined after a 24 h moisture equilibration of FA soils hydrated to 60% of the WHC of each soil.

3.1.1 TNT Concentrations in TSL Soil

Mean values for ACN-extractable TNT within FA TSL soil, expressed as percentage of amendment, ranged from 30% at nominal 1 mg kg^{-1} concentration to 88% at nominal 100 mg kg^{-1} concentration (Table 2). This indicated that a portion of TNT was rapidly transformed or degraded, strongly sorbed onto soil, or a combination of these processes during the initial 24 h period after soil hydration. Mean concentrations of ATCLP-extractable TNT within FA TSL soil ranged from 10 to 57% of the mean concentrations of ACN-extractable TNT (Table 2).

Table 2. Concentrations of TNT FA into TSL Soil Used
in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/ Nominal (%)	ATCLP Extraction (mg kg ⁻¹)	SE	ATCLP/ACN (%)
0	BDL	BDL	NA	BDL	BDL	NA
0.5	BDL	BDL	BDL	BDL	BDL	BDL
1	0.3	0.2	30	0.03	0.01	10
5	2	0.2	40	0.4	0.1	20
10	4	0.1	40	1	0.1	25
20	9	0.1	45	2.8	0.2	22
40	29	0.5	72	11	0.1	38
60	49	1.0	81	24	0.1	49
80	69	0.7	86	37	0.1	54
100	88	1.3	88	50	0.5	57

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) or water extraction (ATCLP) of TNT from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

As indicated in Table 3, mean values for TNT, W-A in TSL soil and remaining ACN-extractable, calculated as percentages of corresponding initial concentrations of ACN-extractable TNT in FA soils, ranged from 15% (at nominal 20 mg kg⁻¹) to 103% (at nominal 75 mg kg⁻¹) of initial values. The greatest percentage decrease (85%) occurred in the lowest nominal TNT treatment, nominal 20 mg kg⁻¹. The percentage decreases in ACN-extractable TNT during the weathering-and-aging procedure were lower and more uniform at greater nominal concentrations of 100 and 120 mg kg⁻¹. Mean concentrations of ATCLP-extractable TNT W-A in TSL soil ranged from 22 to 57% of the corresponding ACN-extractable TNT concentrations.

Table 3. Concentrations of TNT W-A in TSL Soil Used
in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/ Initial ACN (%)	W-A ATCLP (mg kg ⁻¹)	W-A ATCLP/ W-A ACN (%)
0	BDL	BDL	NA	BDL	NA
20	17 (4)	2.5 (0.06)	15	0.56 (0.01)	22
50	52 (5)	27 (0.7)	52	11 (0.03)	40
75	57 (5)	59 (0.5)	103	34 (0.3)	57
100	54 (4)	42 (1.5)	78	21 (0.4)	50
120	138 (4)	101 (2.4)	73	56 (0.3)	55

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A, ACN) or water extraction (ATCLP) of TNT from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

3.1.2 TNT Concentrations in SSL Soil

As shown in Table 4, mean values for ACN-extractable TNT within FA SSL soil averaged 80% (ranging from 60 to 89%) of nominal concentrations. Mean values for ATCLP-extractable TNT within FA SSL soil treatments, expressed as percentages of the concentrations of ACN-extractable TNT within corresponding treatments, averaged 59% (ranging from 40 to 73%) of ACN-extractable TNT.

Table 4. Concentrations of TNT FA into SSL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/Nominal (%)	ATCLP Extraction (mg kg ⁻¹)	SE	ATCLP/ACN (%)
0	BDL	BDL	NA	BDL	BDL	NA
5	3	0.1	60	1.2	0.08	40
10	7	0.1	70	3	0.1	43
25	21	1	84	11	0.03	52
50	40	0.4	80	25	0.2	63
60	51	1	85	35	0.8	69
75	62	2	83	40	0.5	65
100	85	0.2	85	59	1	69
150	134	6	89	98	0.6	73

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) or water extraction (ATCLP) of TNT from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

As shown in Table 5, mean values for TNT, W-A in SSL soil and remaining ACN-extractable, calculated as percentages of corresponding initial concentrations of ACN-extractable TNT in FA soils, ranged from 17% (at nominal 20 mg kg⁻¹ concentration) to 87% (at nominal 50 mg kg⁻¹ concentration) of initial values. Percentage decreases in ACN-extractable TNT within SSL during weathering-and-aging procedures were lower and more uniform at the greater nominal concentrations, 50 to 160 mg kg⁻¹.

Mean values for ATCLP-extractable TNT W-A in SSL soil, expressed as percentages of the concentrations of ACN-extractable TNT within corresponding treatments, ranged from 13% (at nominal 20 mg kg⁻¹ concentration) to 33% (at nominal 150 mg kg⁻¹ concentration) of the ACN-extractable TNT values. Mean ratios of ATCLP-extractable TNT versus ACN-extractable TNT decreased from an average of 59% within FA SSL soil to an average of 28% when TNT was W-A in SSL soil treatments.

Table 5. Concentrations of TNT W-A in SSL Soil Used
in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/ Initial ACN (%)	W-A ATCLP (mg kg ⁻¹)	W-A ATCLP/ W-A ACN (%)
0	BDL	BDL	NA	BDL	NA
20	18 (0.2)	3 (0.5)	17	0.4 (0.01)	13
50	46 (1)	40 (1)	87	12 (0.2)	30
75	73 (1)	46 (1)	63	14 (0.1)	30
100	92 (1)	66 (2)	72	20 (0.3)	30
110	108 (1)	68 (3)	63	22 (0.3)	32
120	101 (1)	53 (0.2)	52	13 (0.5)	25
140	129 (3)	82 (3)	64	25 (0.1)	30
150	139 (5)	94 (0)	68	31 (0.4)	33
160	150 (1)	105 (3)	70	31 (1.4)	30

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A, ACN) or water extraction (ATCLP) of TNT from soil. NA, not applicable.
BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

3.1.3 TNT Concentrations in KCL Soil

As indicated in Table 6, mean values for ACN-extractable TNT within FA KCL soil, expressed as percentage of amendment, averaged 82% (ranging from 70 to 88%) of nominal concentrations. Mean values for ATCLP-extractable TNT within FA KCL soil treatments, expressed as percentages of the concentrations of ACN-extractable TNT within corresponding treatments, averaged 43% (ranging from 28 to 57%) of ACN-extractable concentrations.

Table 6. Concentrations of TNT FA into KCL Soil Used
in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/ Nominal (%)	ATCLP Extraction (mg kg ⁻¹)	SE	ATCLP/ ACN (%)
0	BDL	BDL	NA	BDL	BDL	NA
10	7	0.1	70	2	0.1	28
20	15	0.2	75	5	0.06	33
40	34	0.4	85	13	0.2	38
50	41	0.3	82	18	0.2	44
60	50	1	83	22	0.5	44
80	65	2	81	34	0.7	52
100	88	1	88	45	1.9	51
150	132	4	88	75	1.9	57

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) or water extraction (ATCLP) of TNT from soil.
NA, not applicable.
BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

As shown in Table 7, mean values for TNT, W-A in KCL soil and remaining ACN-extractable, calculated as percentages of corresponding initial concentrations of ACN-extractable TNT in FA soils, ranged from 2% (at nominal 50 and 100 mg kg⁻¹ concentrations) to 9% (at nominal 60 and 150 mg kg⁻¹ concentrations) of initial values. Mean values for ATCLP-extractable TNT W-A in KCL soil, expressed as percentages of the concentrations of ACN-extractable TNT within corresponding treatments, ranged from 5% (at nominal 50 mg kg⁻¹ concentration) to 36% (at nominal 80 and 150 mg kg⁻¹ concentration) of ACN-extractable TNT.

Mean ratios of ATCLP-extractable TNT versus ACN-extractable TNT decreased from an average of 43% within FA KCL soil to an average of 27% when TNT was W-A in KCL soil treatments.

Table 7. Concentrations of TNT W-A in KCL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/Initial ACN (%)	W-A ATCLP (mg kg ⁻¹)	W-A ATCLP/W-A ACN (%)
0	BDL	BDL	NA	BDL	NA
10	7 (0.1)	0.3 (0.03)	4	BDL	BDL
20	15 (0.2)	0.5 (0.03)	3	BDL	BDL
40	34 (0.4)	1.6 (0.14)	5	0.44 (0.08)	28
50	41 (0.3)	0.8 (0.03)	2	0.04 (0.04)	5
60	50 (1)	4.3 (0.15)	9	1.1 (0.01)	25
80	65 (2)	5.3 (0.08)	8	1.9 (0.36)	36
100	88 (1)	2.1 (0.10)	2	0.5 (0.02)	24
150	132 (4)	11.5 (0.38)	9	4.2 (0.06)	36
200	179 (7)	5.5 (0.16)	3	1.8 (0.09)	33

Note: Analytically determined concentrations (means and SEs, *n* = 3) were based on ACN extraction (U.S. EPA Method 8330A, ACN) or water extraction (ATCLP) of TNT from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

3.1.4 TNT Concentrations in RCL Soil

As indicated in Table 8, mean percentages of ACN-extractable TNT within FA RCL soil averaged 63% (ranging from 40 to 91%) of nominal concentrations. Mean values for ATCLP-extractable TNT within FA RCL soil treatments, expressed as percentages of the ACN-extractable TNT concentrations within corresponding treatments, averaged 27% (ranging from 10 to 46%) of ACN-extractable concentrations.

Table 8. Concentrations of TNT FA into RCL Soil Used
in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/ Nominal (%)	ATCLP Extraction (mg kg ⁻¹)	SE	ATCLP/ ACN (%)
0	BDL	BDL	NA	BDL	BDL	NA
2.5	1	0.1	40	BDL	BDL	NA
5	2	0.1	40	0.2	0.03	10
7.5	5	0.4	67	0.8	0.07	16
10	4	0.1	40	0.3	0.04	10
25	15	0.2	60	3.1	0.11	21
50	35	0.2	70	11	0.03	33
75	68	0.3	91	28	0.2	41
100	80	1.6	81	34	0.6	42
125	103	1	82	47	1.1	46

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A, ACN) or water extraction (ATCLP) of TNT from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

As indicated in Table 9, mean values for TNT, W-A in RCL soil yet remaining ACN-extractable, calculated as percentages of corresponding initial concentrations of ACN-extractable TNT in FA soils, ranged from 16 to 25% of initial values. No ATCLP-extractable TNT was detected at or below the 25 mg kg⁻¹ nominal concentration. Mean values for detectable ATCLP-extractable TNT W-A in RCL soil, expressed as percentages of the ACN-extractable TNT concentrations within corresponding treatments, ranged from 26 to 41% of the ACN-extractable concentrations.

Overall, mean ratios expressed as percentages of ATCLP-extractable TNT versus ACN-extractable TNT were similar when the TNT was FA and W-A in RCL soil treatments (27 and 33%, respectively).

Table 9. Concentrations of TNT W-A in RCL Soil Used
in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/ Initial ACN (%)	W-A ATCLP (mg kg ⁻¹)	W-A ATCLP/ W-A ACN (%)
0	BDL	BDL	NA	BDL	NA
2.5	1 (0.1)	BDL	NA	BDL	NA
5	2 (0.1)	BDL	NA	BDL	NA
10	4 (0.1)	0.2 (0.04)	25	BDL	NA
25	15 (0.2)	0.12 (0.01)	20	BDL	NA
50	35 (0.2)	3.7 (0.03)	20	1.1 (0.21)	30
75	68 (0.3)	14 (0.14)	16	4.5 (0.07)	32
100	80 (1.6)	2.7 (0.08)	20	0.7 (0.00)	26
125	103 (1)	17 (0.5)	16	5.4 (0.09)	32
150	126 (1)	19 (0.2)	25	7.0 (0.03)	37
200	168 (2)	22 (0.3)	25	9.1 (0.07)	41

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A, ACN) or water extraction (ATCLP) of TNT from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

3.1.5 TNT Concentrations in WCL Soil

As shown in Table 10, mean values for ACN-extractable TNT within FA WCL soil, expressed as percentage of amendment, averaged 73% (ranging from 36 to 98%) of nominal concentrations. Mean values for detectable ATCLP-extractable TNT within FA WCL soil, expressed as percentages of ACN-extractable TNT concentrations within corresponding treatments, ranged from 0.4 to 41% of ACN-extractable concentrations.

Table 10. Concentrations of TNT FA into WCL Soil Used
in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/ Nominal (%)	ATCLP Extraction (mg kg ⁻¹)	SE	ATCLP/ ACN (%)
0	BDL	BDL	NA	BDL	BDL	NA
40	15	1	38	BDL	BDL	NA
60	23	1	38	0.1	0.03	0.4
80	29	1	36	0.3	0.07	1
100	45	3	45	1.2	0.12	3
150	86	9	57	11	1	13
200	155	7	78	41	1	26
225	198	10	88	55	2	28
250	245	11	98	83	1	34
275	235	9	85	72	5	31
300	284	17	95	100	5	35
325	302	9	93	118	1	39
350	334	6	95	126	4	38
400	387	6	97	159	1	41

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A, ACN) or water extraction (ATCLP) of TNT from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

As indicated in Table 11, mean values for TNT, W-A in WCL soil yet remaining ACN-extractable, calculated as percentages of corresponding initial concentrations of ACN-extractable TNT in FA soils, ranged from 1% (at concentration 40 mg kg⁻¹) to 17% (at nominal 60 and 350 mg kg⁻¹ concentrations) of initial values. Mean values for detectable ATCLP-extractable TNT W-A in WCL soil, expressed as percentages of the ACN-extractable TNT concentrations within corresponding treatments, ranged from 1 to 21% of ACN-extractable concentrations. No ATCLP-extractable TNT was detected at or below the 150 mg kg⁻¹ nominal concentrations.

Overall, mean ratios expressed as percentages of ATCLP-extractable TNT versus ACN-extractable TNT decreased from an average of 26% within FA WCL soil to an average of 13% when TNT was W-A in WCL soil treatments.

Table 11. Concentrations of TNT W-A in WCL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/Initial ACN (%)	W-A ATCLP (mg kg ⁻¹)	W-A ATCLP/W-A ACN (%)
0	BDL	BDL	NA	BDL	NA
40	15 (1)	0.2 (0.01)	1	BDL	NA
60	23 (1)	4 (0.5)	17	BDL	NA
80	29 (1)	3 (0.2)	10	BDL	NA
100	45 (3)	0.7 (0.0)	2	BDL	NA
150	86 (9)	7 (0.3)	8	BDL	NA
200	155 (7)	1.4 (0.1)	1	0.02 (0.02)	1
225	198 (10)	16 (1.4)	8	1.8 (0.1)	11
250	245 (11)	23 (1.3)	9	3 (0.03)	13
300	284 (17)	30 (0.3)	11	5 (0.6)	17
325	302 (9)	49 (11)	16	8 (0.2)	16
350	334 (6)	57 (0.1)	17	12 (0.1)	21
400	387 (6)	11 (0.3)	3	1.7 (0.1)	9

Note: Analytically determined concentrations (means and SEs, $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A, ACN) or water extraction (ATCLP) of TNT from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

3.2 Analytical Determinations of RDX in Soil

Definitive toxicity tests with multiple RDX concentrations were conducted separately with TSL, SSL, KCL, RCL, and WCL soils. RDX concentrations in amended soils were determined after the 24 h moisture equilibration at the beginning of each definitive toxicity test using ACN extractions and U.S. EPA Method 8330A (U.S. EPA, 2007). ATCLP-based extractions were excluded from studies with RDX because several concentrations selected for toxicity tests with *F. candida* greatly exceeded the aqueous solubility of RDX (42 mg L⁻¹ at 20 °C; Monteil-Rivera et al., 2004). Nominal and ACN-extractable RDX concentrations in all soils are shown in Tables 12–21. Mean values for all treatments and soils for ACN-extractable RDX within FA soils, expressed as percentages of amendment within corresponding treatments, averaged 116% (ranging from 32% at nominal 5 mg kg⁻¹ concentration in TSL to 245% at nominal 20 mg kg⁻¹ concentration in KCL) of nominal RDX concentrations. Mean values for RDX, W-A in soil and remaining ACN-extractable, calculated as percentages of corresponding initial concentrations of ACN-extractable RDX in respective FA soils, ranged across all soils from 42% (at nominal 144 mg kg⁻¹ concentration in SSL) to 131% (at nominal 1 mg kg⁻¹ concentration in TSL) of initial values. The greatest percentage decrease (22%) from initial concentrations of ACN-extractable RDX within FA soil occurred in SSL (Table 15). Mean values for RDX, W-A in soil and remaining ACN-extractable, remained relatively stable in KCL (111% of initial value; Table 17) and WCL (105% of initial value; Table 21).

Table 12. Concentrations of RDX FA into TSL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/Nominal (%)
0	BDL	BDL	NA
5	2	1.6	32
10	14	7	140
20	11	3	53
40	67	31	168
60	75	38	125
80	137	5	171
100	113	25	113
150	144	52	96
200	233	54	117

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 13. Concentrations of RDX W-A in TSL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/Initial ACN (%)
0	BDL	BDL	NA
1	1.6 (0.1)	2.1 (0.02)	131
5	7 (0.2)	6 (0.05)	86
10	10 (0.3)	12 (1.2)	120
20	31 (0.1)	27 (0.3)	87
40	36 (0.2)	34 (0.5)	94
60	66 (1)	65 (0.2)	98
80	85 (4)	83 (0.4)	98
100	108 (4)	108 (2)	100
200	218 (23)	216 (6)	99

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 14. Concentrations of RDX FA into SSL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/Nominal (%)
0	BDL	BDL	NA
1.5	2	0.06	133
3	3	0.2	100
9	10	0.2	111
18	20	1	111
36	44	3	122
120	139	3	116
360	356	6	99
720	745	3	103
2000	2121	32	106

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 15. Concentrations of RDX W-A in SSL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/Initial ACN (%)
0	BDL	BDL	NA
6	8 (0.1)	6 (1.5)	75
9	9 (0.5)	8 (1)	89
18	18 (1)	16 (0.2)	89
36	33 (2)	30 (1)	91
72	74 (8)	57 (3)	77
144	148 (5)	62 (2)	42
300	304 (19)	254 (9)	81
600	656 (19)	527 (4)	80

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 16. Concentrations of RDX FA into KCL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/Nominal (%)
0	BDL	BDL	NA
5	9	3	180
10	12	6	120
20	49	22	245
40	74	21	185
60	84	19	140
80	144	39	180
100	191	66	191
150	187	20	125
200	186	32	93

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 17. Concentrations of RDX W-A in KCL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/Initial ACN (%)
0	BDL	BDL	NA
10	13 (0.6)	16 (1)	123
15	19 (0.8)	21 (0.6)	110
20	19 (0.2)	20 (0.3)	105
30	29 (0.5)	28 (1)	97
40	41 (2)	43 (2)	105
60	61 (1)	65 (0.1)	107
80	78 (2)	85 (1)	109
100	84 (3)	111 (0.4)	132
200	194 (4)	209 (1)	108

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 18. Concentrations of RDX FA into RCL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/Nominal (%)
0	BDL	BDL	NA
5	6	0.03	120
10	9	0.2	90
15	14	0.4	93
20	17	0.5	85
25	21	0.3	84
30	27	1	90
35	33	1	94
40	34	1	85
60	56	1	93
80	65	1	81
100	94	2	94
250	234	4	94
500	440	9	88
750	632	75	84
1000	973	10	97

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 19. Concentrations of RDX W-A in RCL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/Initial ACN (%)
0	BDL	BDL	NA
5	6 (0.03)	5 (0.3)	83
10	9 (0.2)	8 (0.1)	89
15	14 (0.4)	12 (0.4)	86
20	17 (0.5)	17 (0.3)	100
25	21 (0.3)	20 (0.5)	95
30	27 (1)	24 (1)	89
35	33 (1)	33 (0.1)	100
40	34 (1)	32 (1)	94
60	56 (1)	56 (1)	100
80	65 (1)	66 (1)	102
100	94 (2)	82 (2)	87
250	234 (4)	238 (2)	102
500	440 (9)	462 (3)	105
750	632 (75)	698 (14)	110
1000	973 (10)	1010 (17)	104

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.
 NA, not applicable.
 BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 20. Concentrations of RDX FA into WCL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	ACN Extraction (mg kg ⁻¹)	SE	ACN/Nominal (%)
0	BDL	BDL	NA
5	11	3	220
10	10	7	100
20	14	8	70
40	21	2	53
60	35	15	58
80	55	7	69
100	203	88	203
150	236	6	157
200	326	77	163

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.
 NA, not applicable.
 BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 21. Concentrations of RDX W-A in WCL Soil Used in Definitive Toxicity Tests with *F. candida*

Nominal Concentration (mg kg ⁻¹)	Initial ACN (mg kg ⁻¹)	W-A ACN (mg kg ⁻¹)	W-A/Initial ACN (%)
0	BDL	BDL	NA
25	21 (2)	22 (1)	105
30	27 (2)	24 (1)	89
40	31 (5)	33 (2)	106
50	38 (5)	41 (0.2)	108
60	54 (2)	49 (2)	91
80	64 (3)	71 (0.5)	111
100	72 (12)	88 (3)	122
250	218 (4)	211 (2)	97
500	438 (17)	458 (6)	105
750	561 (29)	671 (17)	120
1000	914 (13)	885 (7)	97

Note: Analytically determined concentrations (means and SEs; $n = 3$) were based on ACN extraction (U.S. EPA Method 8330A) of RDX from soil.

NA, not applicable.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

3.3 Effects of TNT on the Collembolan *F. candida*

Definitive studies using the *Folsomia* reproduction test (ISO, 1998b) were conducted to assess the acute (adult mortality) and chronic (juvenile production) effects of TNT on the Collembolan *F. candida* in TSL, SSL, KCL, RCL, and WCL soils. Within each soil, *F. candida* juveniles were exposed to a range of TNT concentrations in independent investigations. Measurement endpoints were assessed using treatment concentrations determined from the range-finding studies and included the numbers of surviving adults and juveniles produced during the 28 day test. Exposure concentrations for definitive tests of each soil were selected from range-finding results to achieve concentration bracketing of significant effects on reproduction endpoints (i.e., production of juveniles). Reproduction endpoints are preferred for establishing benchmarks for the development of Eco-SSL values for soil invertebrates (U.S. EPA, 2005) and were therefore the main focus of these studies. The ranges of exposure concentrations were expanded to identify the concentrations that caused lethal effects to adults. All ecotoxicological parameters were estimated using these respective measurement endpoint values and TNT concentrations in soil, which were analytically determined using U.S. EPA Method 8330A (U.S. EPA, 2007).

As shown in Tables 22–26, test results complied with the validity criteria adapted from the ISO test guideline and those stipulated in Section 2.8 of this report. Mean adult survival in the negative controls ranged from 86 to 96% in tests with TNT either FA or W-A in soil. The mean numbers of juveniles produced by *F. candida* in negative controls ranged from 143 to 295. All coefficients of variation (CVs) for the numbers of juveniles produced by *F. candida* in

negative controls were <30%, as required by the ISO 11267 test guideline (ISO, 1998b), and ranged from 7 to 17%. The mean numbers of juveniles in positive controls of studies ranged from 71 to 213, corresponding to 56 and 28% decreases from the respective negative controls. Overall, the positive-control data were consistent with the baseline established for the laboratory culture of *F. candida*. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the TNT treatments.

Table 22. Adult Survival and Juvenile Production of *F. candida*
Exposed to TNT FA or W-A in TSL Soil

TNT FA Treatment (mg kg ⁻¹)	Number ^a		TNT W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	9.6 (0.2)	280 (22)	Negative control	8.6 (0.2)	150 (11)
Acetone control	9.6 (0.2)	265 (20)	Acetone control	9.0 (0.3)	165 (8)
Positive control	6.8 (0.4)	213 (13)	Positive control	7.8 (0.4)	136 (16)
BDL	9.8 (0.2)	277 (20)	3	8.6 (0.2)	160 (5)
0.3	9.2 (0.4)	250 (14)	27	6.6 (0.9)	82 (16)
2	9.2 (0.4)	249 (17)	59	0.8 (0.8)	3.0 (3)
4	8.2 (0.6)	221 (22)	42	0	0.6 (0.6)
9	7.8 (0.4)	200 (7)	101	0	0
29	4.4 (0.2)	74 (16)	NT	NT	NT
49	0.4 (0.2)	6 (2.7)	NT	NT	NT
69	0	0	NT	NT	NT
88	0	0	NT	NT	NT

Note: TNT concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs ($n = 5$).

NT, not tested in this study.

BDL, below detection limit (0.05 mg L⁻¹ in solution and 0.5 mg kg⁻¹ in soil).

Table 23. Adult Survival and Juvenile Production of *F. candida*
Exposed to TNT FA or W-A in SSL Soil

TNT FA Treatment (mg kg ⁻¹)	Number ^a		TNT W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	9.4 (0.2)	295 (12)	Negative control	9.2 (0.4)	219 (11)
Acetone control	9.6 (0.2)	295 (15)	Acetone control	9.2 (0.4)	235 (11)
Positive control	6.8 (0.4)	213(13)	Positive control	7.8 (0.4)	136 (16)
3	9.8 (0.2)	327 (20)	3	9.4 (0.2)	220 (11)
7	9.4 (0.2)	312 (11)	2	9.4 (0.2)	213 (16)
21	7.0 (0.6)	167 (41)	46	7.2 (1.8)	170 (44)
40	2.6 (0.7)	27 (14)	66	3.0 (1.7)	27 (14)
51	0.2 (0.2)	12 (12)	68	2.6 (1.6)	31 (19)
62	2.0 (1.3)	14 (10)	53	0.2 (0.2)	0
85	0	0	82	0.2 (0.2)	0
134	0	0	94	0	0

Note: TNT concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs (*n* = 5).

Table 24. Adult Survival and Juvenile Production of *F. candida*
Exposed to TNT FA or W-A in KCL Soil

TNT FA Treatment (mg kg ⁻¹)	Number ^a		TNT W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	9.2 (0.2)	155 (6)	Negative control	9.2 (0.4)	162 (9)
Acetone control	8.8 (0.4)	140 (8)	Acetone control	9.2 (0.4)	167 (8)
Positive control	7.8 (0.4)	136 (16)	Positive control	5.4 (0.5)	71 (6)
7	9.2 (0.4)	144 (9)	0.3	9.2 (0.4)	153 (8)
15	8.4 (0.2)	140 (9)	0.5	7 (0.4)	130 (11)
34	7 (0.5)	72 (20)	1.6	5.8 (0.6)	107 (22)
41	7 (0.9)	69 (14)	0.8	5.4 (0.4) ^b	82 (12) ^b
50	4.8 (0.4)	45 (8)	4.3	5.4 (0.6)	70 (8)
65	3.8 (1.1)	15 (7)	5.3	5.2 (0.4)	69 (8)
88	0.2 (0.2)	5 (5)	2.1	3.4 (0.5) ^b	51 (7) ^b
132	0	0	11.5	3.2 (0.4)	32 (8)
NT	NT	NT	5.5	3.0 (0.5) ^b	27 (6) ^b

Note: TNT concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs (*n* = 5).

^bToxicity data from treatments used for monitoring TNT concentration during W-A process; these data were excluded from regression analyses.

NT, not tested in this study.

Table 25. Adult Survival and Juvenile Production of *F. candida*
Exposed to TNT FA or W-A in RCL Soil

TNT FA Treatment (mg kg ⁻¹)	Number ^a		TNT W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	8.6 (0.4)	190 (13)	Negative control	9.0 (0.3)	143 (4)
Acetone control	8.8 (0.4)	256 (12)	Acetone control	9.2 (0.4)	144 (8)
Positive control	6.2 (0.4)	153 (6)	Positive control	6.2 (0.4)	93 (5)
1	9.0 (0.3)	250 (14)	BDL	8.8 (0.2)	134 (5)
2	9.0 (0.3)	214 (7)	BDL	9.0 (0.4) ^b	131 (7) ^b
5	8.4 (0.2)	188 (5)	0.2	8.8 (0.4)	124 (7)
4	8.2 (0.4)	179 (9)	0.12	8.0 (0.3) ^b	111 (8) ^b
15	7.8 (0.4)	152 (6)	3.7	7.4 (0.7)	89 (15)
35	7.8 (0.4)	123 (9)	14	6.6 (0.8)	76 (14)
68	7.0 (0.4)	93 (7)	2.7	4.8 (0.7) ^b	62 (14) ^b
80	1.8 (1.2)	16 (12)	19	2.0 (0.4)	0.6 (0.4)
103	0.2 (0.2)	0	22	1.0 (0.3)	3 (2.3)

Note: TNT concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs ($n = 5$).

^bToxicity data from treatments used for monitoring TNT concentration during W-A process; these data were excluded from regression analyses.

Table 26. Adult Survival and Juvenile Production of *F. candida*
Exposed to TNT FA or W-A in WCL Soil

TNT FA Treatment (mg kg ⁻¹)	Number ^a		TNT W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	9.6 (0.2)	263 (20)	Negative control	9.2 (0.2)	175 (7)
Acetone control	9.4 (0.2)	307 (11)	Acetone control	9.4 (0.2)	186 (7)
Positive control	6 (0.4)	152 (13)	Positive control	6.0 (0.3)	89 (11)
15	9.4 (0.2)	328 (16)	0.2	9.2 (0.2) ^b	182 (11) ^b
23	9.4 (0.2)	281 (6)	4	9.4 (0.2)	166 (8)
29	9.2 (0.4)	287 (13)	3	9.0 (0.3)	155 (7)
45	8.4 (0.2)	228 (22)	0.7	9.2 (0.4) ^b	168 (7) ^b
86	7.9 (0.2)	226 (9)	7	8.6 (0.2)	166 (8)
155	7.6 (0.2)	243 (18)	1.4	8.6 (0.5) ^b	166 (7) ^b
198	5.5 (0.8)	209 (11)	16	7.8 (0.4)	163 (8)
245	3.6 (0.8)	162 (23)	23	7.2 (0.4)	150 (8)
235	1.6 (0.6)	26 (15)	30	5.8 (0.9)	84 (23)
284	1.8 (0.6)	50 (27)	49	6.0 (0.3)	106 (11)
302	1.3 (0.5)	39 (24)	57	5.2 (0.6)	84 (14)
334	2.4 (0.7)	26 (16)	11	4.2 (0.4) ^b	46 (7) ^b
387	3.4 (0.9)	10 (5)	NT	NT	NT

Note: TNT concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs ($n = 5$).

^bToxicity data from treatments used for monitoring TNT concentration during W-A process; these data were excluded from regression analyses.

NT, not tested in this study.

3.3.1 Effects of TNT in TSL Soil

Ecotoxicological responses of *F. candida* to TNT FA and W-A in TSL soil are shown in Table 22. Both adult survival and juvenile production were affected in TNT-amended TSL soil within the concentration ranges selected for definitive tests. Ecotoxicological benchmarks for analytically determined TNT concentrations are summarized in Table 27. For adult survival, the bounded NOEC and LOEC values, based on ACN-extractable concentrations, were 2 and 4 mg kg⁻¹, respectively, for TNT FA in TSL, and 3 and 27 mg kg⁻¹, respectively, for TNT W-A in TSL soil. The bounded NOEC and LOEC values, based on ATCLP-extractable (aqueous) concentrations of TNT W-A in TSL soil, were 0.6 and 11 mg kg⁻¹, respectively (Table 28).

Table 27. Toxicological Benchmarks for TNT FA or W-A in TSL, SSL, KCL, RCL, and WCL Soils
Determined Using ACN Extraction in Definitive Tests with *F. candida*

Ecotoxicological Parameter	TNT (mg kg ⁻¹)									
	TSL		SSL		KCL		RCL		WCL	
	FA	W-A	FA	W-A	FA	W-A	FA	W-A	FA	W-A
<i>Adult Survival</i>										
NOEC	2	3	7	46	15	0.3	35	0.2	45	7
<i>p</i>	0.219	0.583	0.680	0.830	0.381	1.000	0.165	0.743	0.068	0.206
LOEC	4	27	21	66	34	0.5	68	3.7	86	16
<i>p</i>	0.001	0.004	<0.0001	0.001	0.005	0.002	0.015	0.013	0.012	0.014
EC ₂₀	13	26	17	58	37	0.7	69	14	216	16
95% CI	9–17	0–937	9–24	46–70	30–43	0–1	65–73	12–15	195–238	6–26
EC ₅₀	25	30	37	71	56	6	76	17	298	66
95% CI	21–28	0–3631	29–45	63–78	51–61	3–8	73–78	16–18	283–313	47–85
Model used	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz
<i>R</i> ²	0.988	0.979	0.980	0.979	0.972	0.974	0.979	0.973	0.988	0.985
<i>Juvenile Production</i>										
NOEC	2	2	7	46	15	0.3	1	0.2	29	16
<i>p</i>	0.220	0.699	0.445	0.240	0.610	0.429	0.660	0.176	0.347	0.163
LOEC	4	27	21	66	34	0.5	2	3.7	45	23
<i>p</i>	0.006	0.001	<0.0001	<0.0001	<0.0001	0.034	0.002	<0.0001	<0.0001	0.036
EC ₂₀	7	21	17	53	21	0.6	4	3	174	17
95% CI	3–10	7–35	14–21	44–63	14–29	0–1.2	1–7	0.04–5.7	142–205	3–31
EC ₅₀	17	27	25	61	37	3	23	8	259	54
95% CI	13–21	25–29	22–28	57–66	32–43	2–5	15–31	4–12	237–281	37–71
Model used	Gompertz	Gompertz	Hormetic	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz
<i>R</i> ²	0.978	0.977	0.983	0.981	0.955	0.958	0.975	0.932	0.972	0.963

Note: Concentrations were based on ACN extraction using U.S. EPA Method 8330A.

*R*², coefficient of determination.

Table 28. Toxicological Benchmarks for TNT W-A in TSL, SSL, KCL, RCL, and WCL Soils Determined Using Water Extraction in Definitive Tests with *F. candida*

Ecotoxicological Parameter	TNT (mg kg ⁻¹)				
	TSL	SSL	KCL	RCL	WCL
<i>Adult Survival</i>					
NOEC	0.6	12	<0.44	<1.1	<1.8
<i>p</i>	0.629	0.902	ND	ND	ND
LOEC	11	13	0.44	1.1	1.8
<i>p</i>	0.008	<0.0001	<0.0001	0.010	0.011
EC ₂₀	11	9	0.2	4	2
95% CI	0–165	2–15	0–0.5	3.2–5	0.8–3.9
EC ₅₀ ^a	13	15	2.6	5.8	16
95% CI	0–666	11–20	1–4	5.2–6.4	7–24
<i>R</i> ²	0.964	0.786	0.970	0.973	0.985
<i>Juvenile Production</i>					
NOEC	0.6	12	<0.44	<1.1	3
<i>p</i>	0.669	0.443	ND	ND	0.170
LOEC	11	13	0.44	1.1	4.7
<i>p</i>	<0.0001	<0.0001	0.005	<0.0001	<0.0001
EC ₂₀	8	9	0.2	1.2	3
95% CI	1–14	6–13	0–0.5	0.2–2.2	1–5
EC ₅₀ ^a	11	14	1.2	3.1	10
95% CI	10–12	11–17	0.6–1.9	1.8–4.4	6–14
<i>R</i> ²	0.976	0.820	0.951	0.939	0.963

Note: Concentrations were based on water extraction using ATCLP and U.S. EPA Method 8330A.

^aGompertz model was used for EC₅₀ determinations.

ND, not determined.

*R*², coefficient of determination.

The logistic Gompertz model provided the best fit for the adult survival data (Figure 2). The EC₂₀ and EC₅₀ values for adult survival based on ACN-extractable concentrations were 13 and 25 mg kg⁻¹, respectively, for TNT FA in TSL, and 26 and 30 mg kg⁻¹, respectively, for TNT W-A in TSL soil (Table 27). The EC₂₀ and EC₅₀ values for adult survival based on ATCLP-extractable concentrations were 11 and 13 mg kg⁻¹, respectively, for TNT W-A in TSL soil (Table 28). On the basis of both the EC₂₀ and EC₅₀ values and the respective 95% CIs (Table 27), weathering-and-aging of TNT in TSL soil did not significantly affect the acute toxicity to *F. candida* adults.

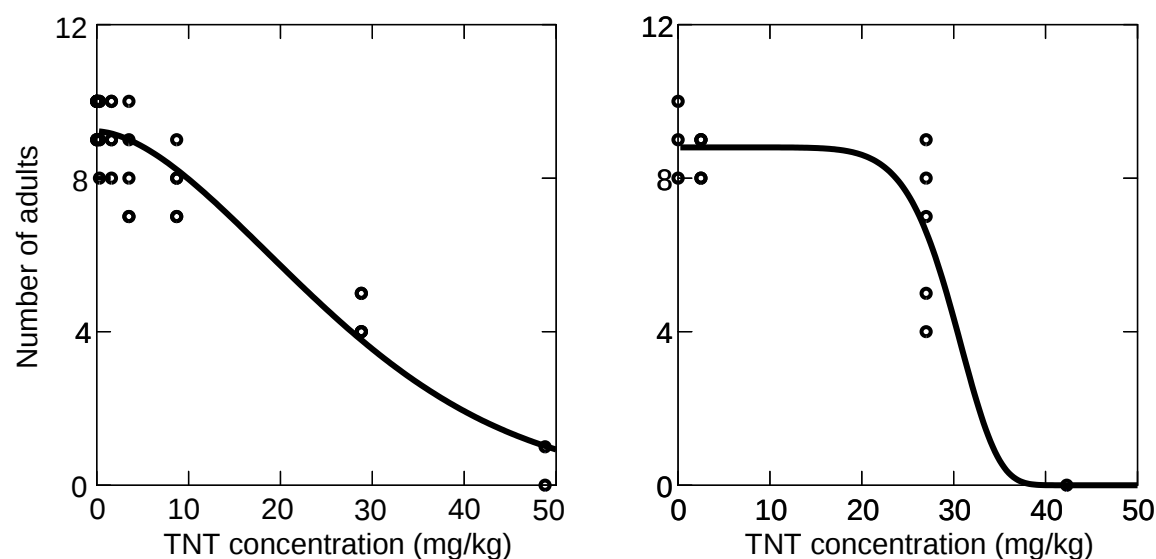


Figure 2. Effects of TNT FA (left) and W-A (right) in TSL soil on survival of adult *F. candida*.

The juvenile production-based bounded NOEC and LOEC values for TNT FA in TSL soil were 2 and 4 mg kg⁻¹, respectively, based on ACN-extractable TNT (Table 27). The logistic Gompertz model provided the best fit for the juvenile production data from toxicity tests with TNT FA in TSL soil (Figure 3). The EC₂₀ and EC₅₀ values for production of juveniles were 7 and 17 mg kg⁻¹, respectively.

The juvenile production-based bounded NOEC and LOEC values for TNT W-A in TSL soil were 2 and 27 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and 0.6 and 11 mg kg⁻¹, respectively (Table 28), based on ATCLP-extractable TNT. The logistic Gompertz model yielded the best fit for the juvenile production data from toxicity tests with TNT W-A in TSL soil (Figure 3). The EC₂₀ and EC₅₀ values for production of juveniles were 21 and 27 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and 8 and 11 mg kg⁻¹, respectively (Table 28), based on ATCLP-extractable TNT.

Coefficients of determination (R^2 values) from nonlinear regression analyses of the reproduction toxicity data for TNT W-A in TSL soil, based on ACN- or ATCLP-extractable TNT concentrations, were compared to determine which chemical measure of exposure better correlated with toxicity. These comparisons showed that coefficients were similar for both extraction types (Tables 27 and 28), which indicated that data from both extraction methods had excellent correlation with the toxicity data for juvenile production, and neither method had an advantage in characterizing TNT bioavailability to *F. candida* in TSL soil.

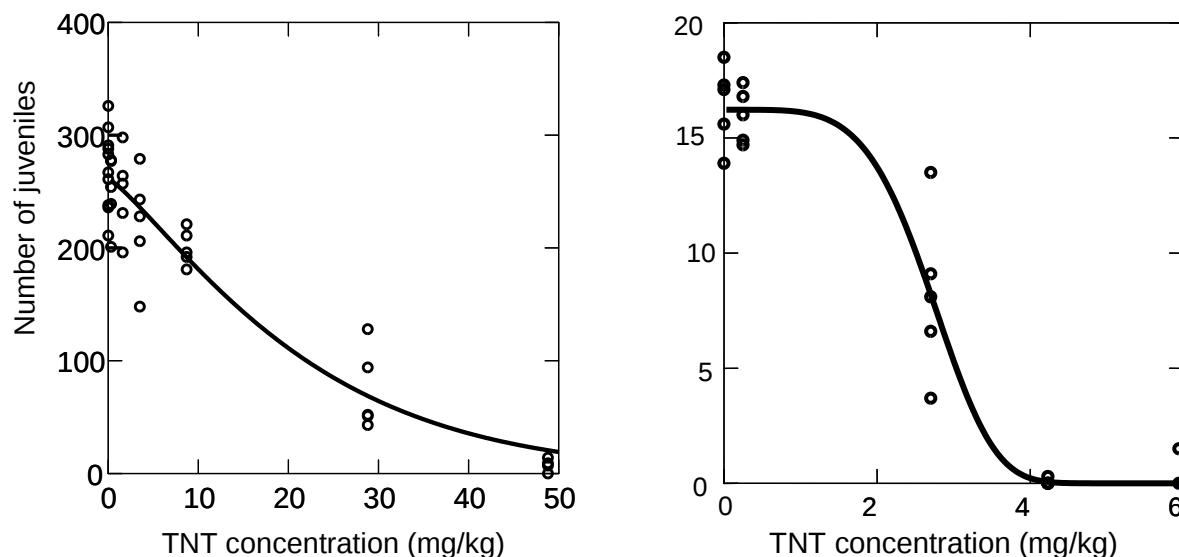


Figure 3. Effects of TNT FA (left) and W-A (right) in TSL soil on production of juveniles by *F. candida*.

3.3.2 Effects of TNT in SSL Soil

Ecotoxicological responses of *F. candida* to TNT FA and W-A in SSL soil are shown in Table 23. Both adult survival and juvenile production were affected in TNT-amended SSL soil within the concentration ranges selected for definitive tests. Ecotoxicological benchmarks for analytically determined TNT concentrations are summarized in Table 27. For adult survival, the bounded NOEC and LOEC values based on ACN-extractable concentrations were 7 and 21 mg kg⁻¹, respectively, for TNT FA in SSL, and 46 and 66 mg kg⁻¹, respectively, for TNT W-A in SSL soil. The bounded NOEC and LOEC values based on ATCLP-extractable concentrations of TNT W-A in SSL soil were 12 and 13 mg kg⁻¹, respectively (Table 28).

The logistic Gompertz model provided the best fit for the adult survival data (Figure 4). The EC₂₀ and EC₅₀ values for adult survival based on ACN-extractable concentrations were 17 and 37 mg kg⁻¹, respectively, for TNT FA in SSL, and 58 and 71 mg kg⁻¹, respectively, for TNT W-A in SSL soil (Table 27). The EC₂₀ and EC₅₀ values for adult survival based on ATCLP-extractable concentrations were 9 and 15 mg kg⁻¹, respectively, for TNT W-A in SSL soil (Table 28). On the basis of the EC₂₀ or EC₅₀ values and the respective 95% CIs, weathering-and-aging of TNT in SSL soil significantly decreased the acute toxicity to *F. candida* adults (Table 27).

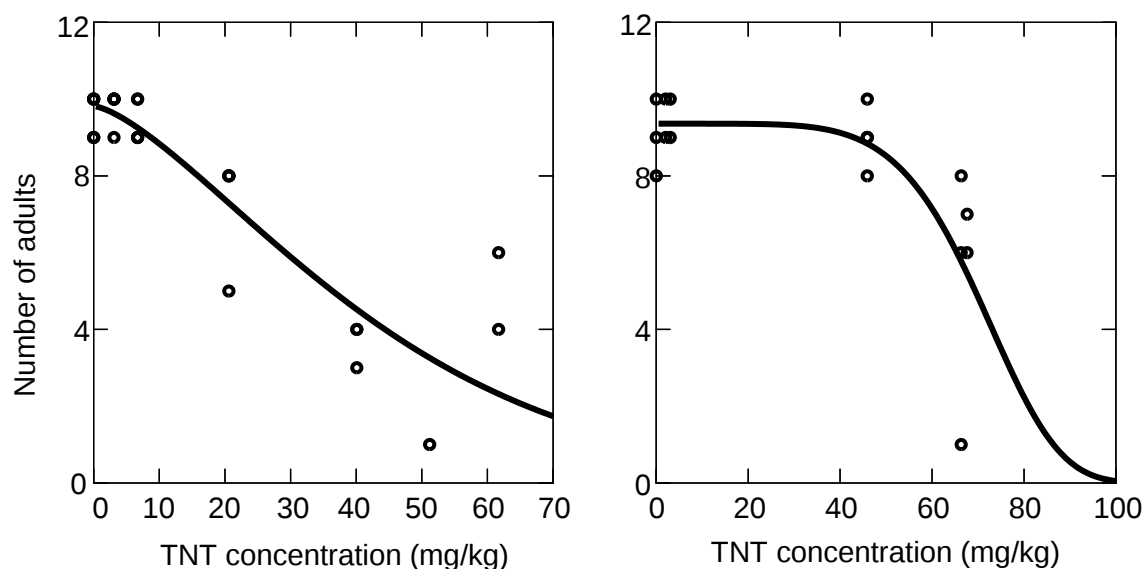


Figure 4. Effects of TNT FA (left) and W-A (right) in SSL soil on survival of adult *F. candida*.

As shown in Figure 5, juvenile production was stimulated in the lowest two TNT concentrations (3 and 7 mg kg⁻¹) in FA SSL, although the increases in juvenile numbers were not statistically significant ($p \geq 0.145$) when compared with the carrier control. Consequently, the bounded NOEC and LOEC values for TNT FA in SSL soil were 7 and 21 mg kg⁻¹, respectively, based on ACN-extractable TNT (Table 27). The logistic model with a hormetic parameter (the hormetic model) provided the best fit for the data from toxicity tests with TNT FA in SSL soil, due to stimulation of juvenile production at the lower treatment concentrations (Figure 5). The EC₂₀ and EC₅₀ values for juvenile production were 17 and 25 mg kg⁻¹, respectively.

The juvenile production-based bounded NOEC and LOEC values for TNT W-A in SSL soil were 46 and 66 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and 12 and 13 mg kg⁻¹, respectively (Table 28), based on ATCLP-extractable TNT. The logistic Gompertz model yielded the best fit for the juvenile production data from toxicity tests with TNT W-A in SSL soil (Figure 5). The EC₂₀ and EC₅₀ values for juvenile production were 53 and 61 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and 9 and 14 mg kg⁻¹, respectively (Table 28), based on ATCLP-extractable TNT.

The R^2 values from nonlinear regression analyses of the reproduction toxicity data for TNT W-A in SSL soil, based on ACN- or ATCLP-extractable TNT concentrations, were compared to determine which chemical measure of exposure better correlated with toxicity. These comparisons showed that the coefficient was greater for ACN-extractable TNT concentrations (Tables 27 and 28), although data from both extraction methods had excellent correlation with the toxicity data for juvenile production by *F. candida* in SSL soil.

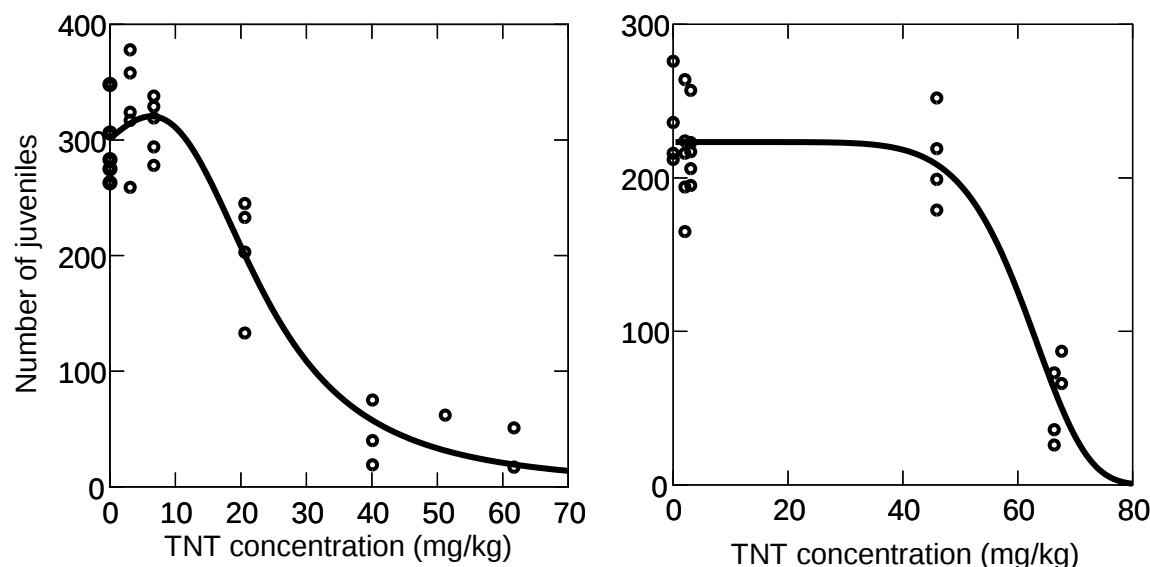


Figure 5. Effects of TNT FA (left) and W-A (right) in SSL soil on production of juveniles by *F. candida*.

3.3.3 Effects of TNT in KCL Soil

Ecotoxicological responses of *F. candida* to TNT FA and W-A in KCL soil are shown in Table 24. Both adult survival and juvenile production were affected in TNT-amended KCL soil within the concentration ranges selected for definitive tests (Table 24). Ecotoxicological benchmarks for analytically determined TNT concentrations are summarized in Table 27. For adult survival, the bounded NOEC and LOEC values based on ACN-extractable concentrations were 15 and 34 mg kg⁻¹, respectively, for TNT FA in KCL soil, and 0.3 and 0.5 mg kg⁻¹, respectively, for TNT W-A in KCL soil. The unbounded LOEC value based on ATCLP-extractable concentrations of TNT W-A in KCL soil was 0.44 mg kg⁻¹ (Table 28).

The logistic Gompertz model provided the best fit for the adult survival data (Figure 6). The EC₂₀ and EC₅₀ values for adult survival based on ACN-extractable concentrations were 37 and 56 mg kg⁻¹, respectively, for TNT FA in KCL soil, and 0.7 and 6 mg kg⁻¹, respectively, for TNT W-A in KCL soil (Table 27). The EC₂₀ and EC₅₀ values for adult survival based on ATCLP-extractable concentrations were 0.2 and 2.6 mg kg⁻¹, respectively, for TNT W-A in KCL soil (Table 28). On the basis of the EC₂₀ and EC₅₀ values and the respective 95% CIs, weathering-and-aging of TNT in KCL soil significantly increased the acute toxicity to adult *F. candida* (Table 27).

The juvenile production-based bounded NOEC and LOEC values for TNT FA in KCL soil were 15 and 34 mg kg⁻¹, respectively, based on ACN-extractable TNT (Table 27). The logistic Gompertz model yielded the best fit for the juvenile production data from toxicity tests with TNT FA in KCL soil (Figure 7). The EC₂₀ and EC₅₀ values for juvenile production were 21 and 37 mg kg⁻¹, respectively.

The juvenile production-based bounded NOEC and LOEC values for TNT W-A in KCL soil were 0.3 and 0.5 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and <0.44 and 0.44 mg kg⁻¹ (unbounded LOEC), respectively (Table 28), based on ATCLP-extractable TNT. The logistic Gompertz model provided the best fit for the juvenile production data from toxicity tests with TNT W-A in KCL soil (Figure 7). The EC₂₀ and EC₅₀ values for juvenile production were 0.6 and 3 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and 0.2 and 1.2 mg kg⁻¹, respectively (Table 28), based on ATCLP-extractable TNT.

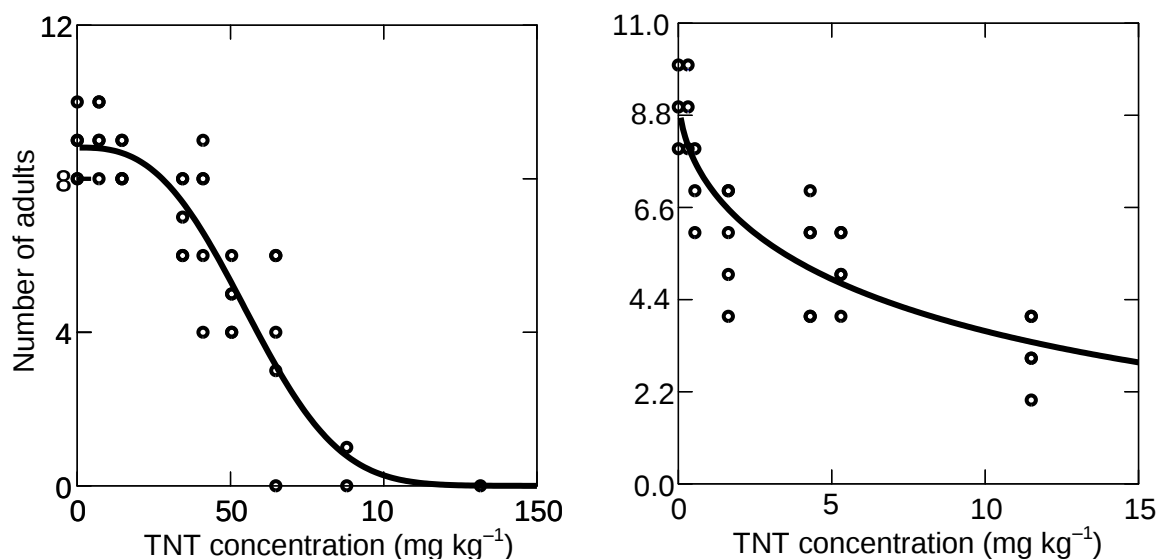


Figure 6. Effects of TNT FA (left) and W-A (right) in KCL soil on survival of adult *F. candida*.

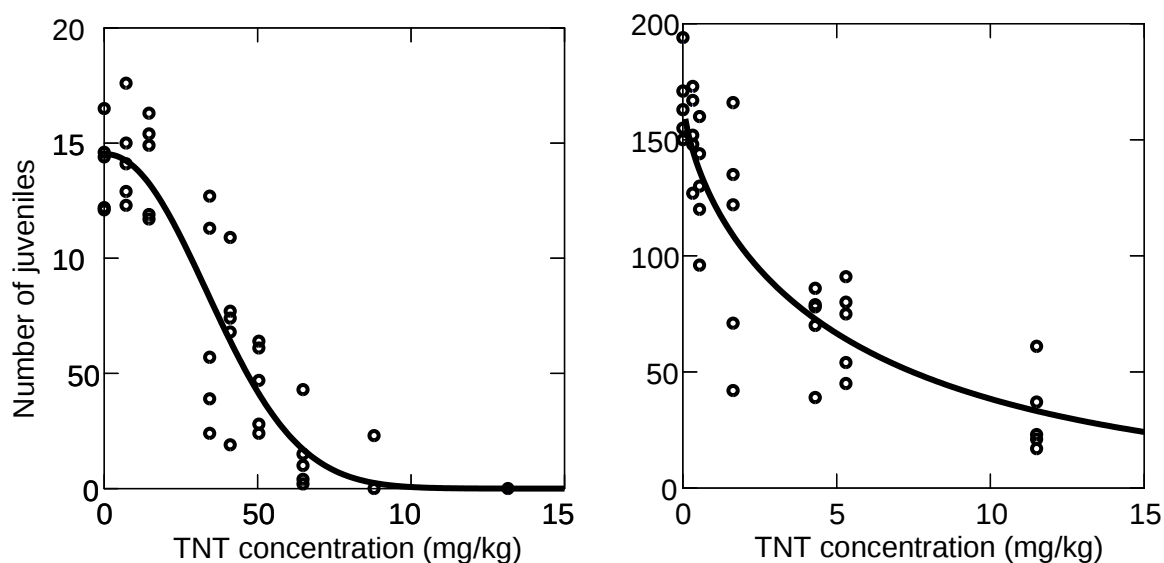


Figure 7. Effects of TNT FA (left) and W-A (right) in KCL soil on production of juveniles by *F. candida*.

The R^2 values from nonlinear regression analyses of the reproduction toxicity data for TNT W-A in KCL soil, based on ACN- or ATCLP-extractable TNT concentrations, were compared to determine which chemical measure of exposure better correlated with toxicity. These comparisons showed that the coefficients were similar for both extraction types (Tables 27 and 28), which indicated that data from both extraction methods had excellent correlation with the toxicity data for juvenile production, and that neither method had a distinct advantage in characterizing TNT bioavailability to *F. candida* in KCL soil.

3.3.4 Effects of TNT in RCL Soil

Ecotoxicological responses of *F. candida* to TNT FA and W-A in RCL soil are shown in Table 25. Both adult survival and juvenile production were affected in TNT-amended RCL soil within the concentration ranges selected for definitive tests (Table 25). Ecotoxicological benchmarks for analytically determined TNT concentrations are summarized in Table 27. For adult survival, the bounded NOEC and LOEC values based on ACN-extractable concentrations were 35 and 68 mg kg⁻¹, respectively, for TNT FA in RCL soil, and 0.2 and 3.7 mg kg⁻¹, respectively, for TNT W-A in RCL soil. The unbounded LOEC value based on ATCLP-extractable concentrations of TNT W-A in RCL soil was 1.1 mg kg⁻¹ (Table 28).

The logistic Gompertz model yielded the best fit for the adult survival data (Figure 8). The EC₂₀ and EC₅₀ values for adult survival based on ACN-extractable concentrations were 69 and 76 mg kg⁻¹, respectively, for TNT FA in RCL soil, and 14 and 17 mg kg⁻¹, respectively, for TNT W-A in RCL soil (Table 27). The EC₂₀ and EC₅₀ values for adult survival based on ATCLP-extractable concentrations were 4 and 5.8 mg kg⁻¹, respectively, for TNT W-A in RCL soil (Table 28). On the basis of the EC₂₀ and EC₅₀ values and the respective 95% CIs, weathering-and-aging of TNT in RCL soil significantly increased the acute toxicity to adult *F. candida* (Table 27).

The juvenile production-based bounded NOEC and LOEC values for TNT FA in RCL soil were 1 and 2 mg kg⁻¹, respectively, based on ACN-extractable TNT (Table 27). The logistic Gompertz model provided the best fit for the juvenile production data from toxicity tests with TNT FA in RCL soil (Figure 9). The EC₂₀ and EC₅₀ values for juvenile production were 4 and 23 mg kg⁻¹, respectively.

The juvenile production-based bounded NOEC and LOEC values for TNT W-A in RCL soil were 0.2 and 3.7 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and <1.1 and 1.1 mg kg⁻¹ (unbounded LOEC), respectively (Table 28), based on ATCLP-extractable TNT. The logistic Gompertz model yielded the best fit for the juvenile production data from toxicity tests with TNT W-A in RCL soil (Figure 9). The EC₂₀ and EC₅₀ values for juvenile production were 3 and 8 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and 1.2 and 3.1 mg kg⁻¹, respectively (Table 28), based on ATCLP-extractable TNT.

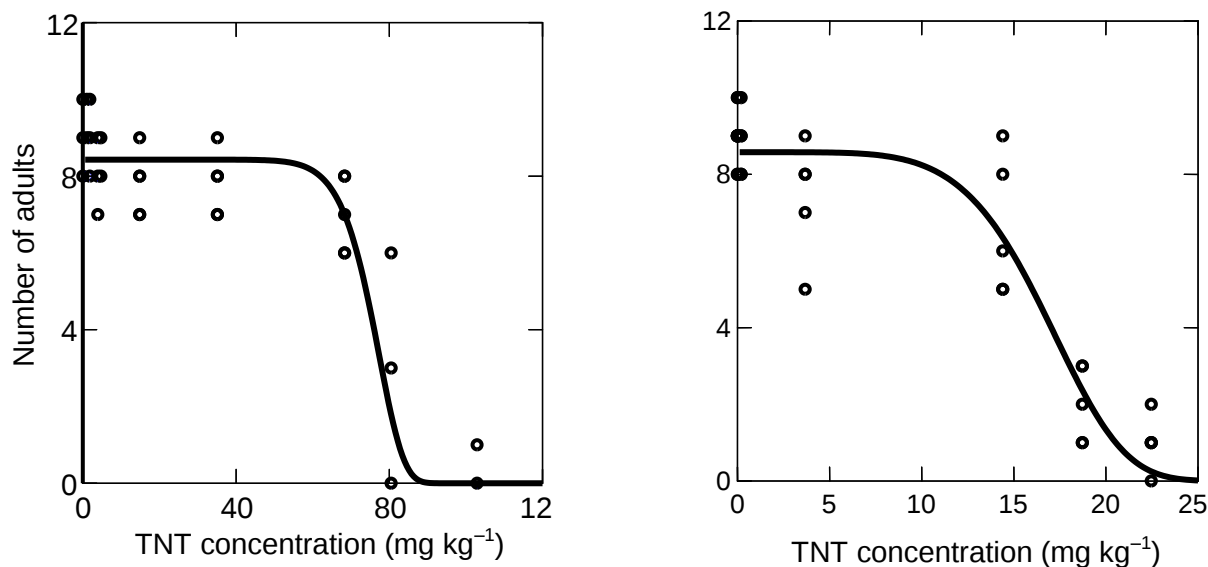


Figure 8. Effects of TNT FA (left) and W-A (right) in RCL soil on survival of adult *F. candida*.

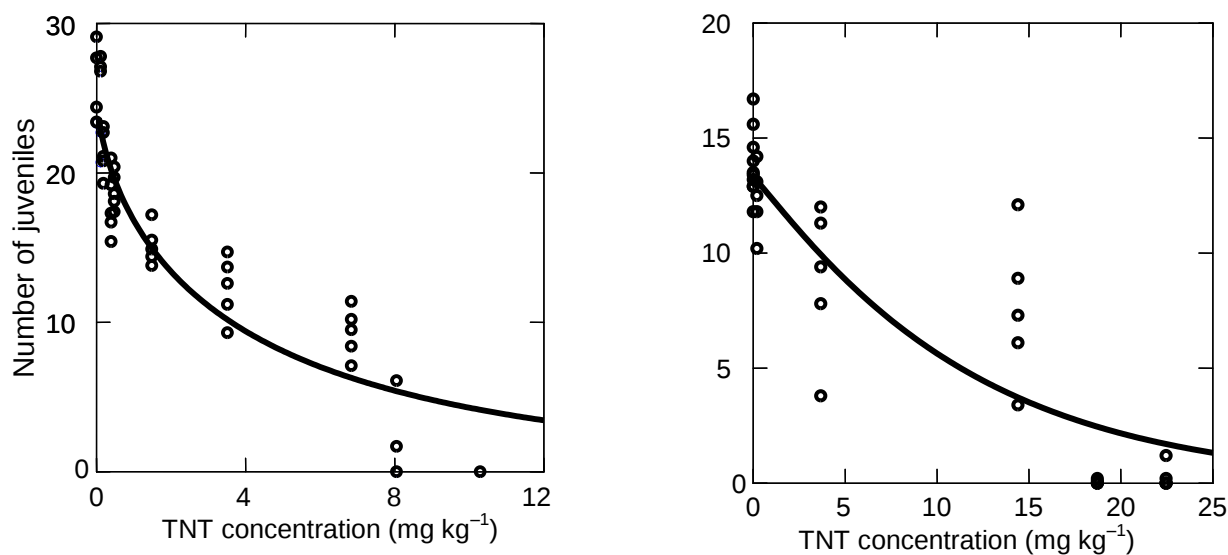


Figure 9. Effects of TNT FA (left) and W-A (right) in RCL soil on production of juveniles by *F. candida*.

The R^2 values from nonlinear regression analyses of the reproduction toxicity data for TNT W-A in RCL soil, based on ACN- or ATCLP-extractable TNT concentrations, were compared to determine which chemical measure of exposure better correlated with toxicity. These comparisons showed that the coefficients were similar for both extraction types (Tables 27 and 28), which indicated that data from both extraction methods had excellent correlation with the toxicity data for juvenile production, and neither method had a distinct advantage in characterizing TNT bioavailability to *F. candida* in RCL soil.

3.3.5 Effects of TNT in WCL Soil

Ecotoxicological responses of *F. candida* to TNT FA and W-A in WCL soil are shown in Table 26. Adult survival and juvenile production were affected in TNT-amended WCL soil within the concentration ranges selected for definitive tests (Table 26). Ecotoxicological benchmarks for analytically determined TNT concentrations are summarized in Table 27. For adult survival, the bounded NOEC and LOEC values based on ACN-extractable concentrations were 45 and 86 mg kg⁻¹, respectively, for TNT FA in WCL, and 7 and 16 mg kg⁻¹, respectively, for TNT W-A in WCL soil. The unbounded LOEC value based on ATCLP-extractable concentrations of TNT W-A in WCL soil was 1.8 mg kg⁻¹ (Table 28).

The logistic Gompertz model provided the best fit for the adult survival data (Figure 10). The EC₂₀ and EC₅₀ values for adult survival based on ACN-extractable concentrations were 216 and 298 mg kg⁻¹, respectively, for TNT FA in WCL, and 16 and 66 mg kg⁻¹, respectively, for TNT W-A in WCL soil (Table 27). The EC₂₀ and EC₅₀ values for adult survival based on ATCLP-extractable concentrations were 2 and 16 mg kg⁻¹, respectively, for TNT W-A in WCL soil (Table 28). On the basis of the EC₂₀ and EC₅₀ values and the respective 95% CIs, weathering-and-aging of TNT in WCL soil significantly increased the acute toxicity for *F. candida* (Table 27).

The juvenile production-based bounded NOEC and LOEC values for TNT FA in WCL soil were 29 and 45 mg kg⁻¹, respectively, based on ACN-extractable TNT (Table 27). The logistic Gompertz model yielded the best fit for the juvenile production data from toxicity tests with TNT FA in WCL soil (Figure 11). The EC₂₀ and EC₅₀ values for juvenile production were 174 and 259 mg kg⁻¹, respectively.

The juvenile production-based bounded NOEC and LOEC values for TNT W-A in WCL soil were 16 and 23 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and 3 and 4.7 mg kg⁻¹, respectively (Table 28), based on ATCLP-extractable TNT. The logistic Gompertz model yielded the best fit for the juvenile production data from toxicity tests with TNT W-A in WCL soil (Figure 11). The EC₂₀ and EC₅₀ values for production of juveniles were 17 and 54 mg kg⁻¹, respectively (Table 27), based on ACN-extractable TNT, and 3 and 10 mg kg⁻¹, respectively (Table 28), based on ATCLP-extractable TNT.

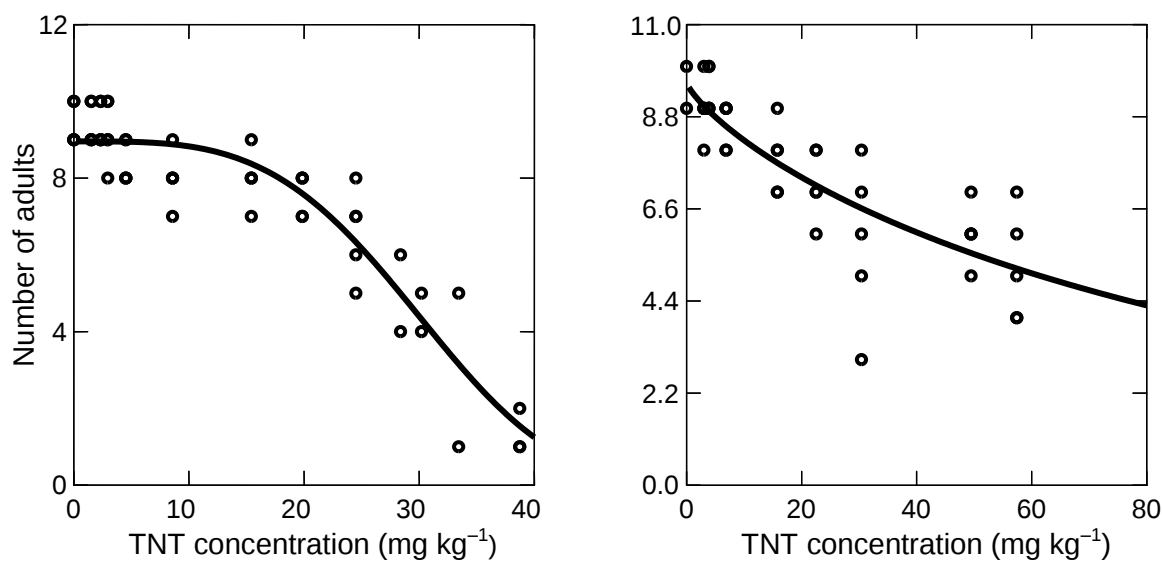


Figure 10. Effects of TNT FA (left) and W-A (right) in WCL soil on survival of adult *F. candida*.

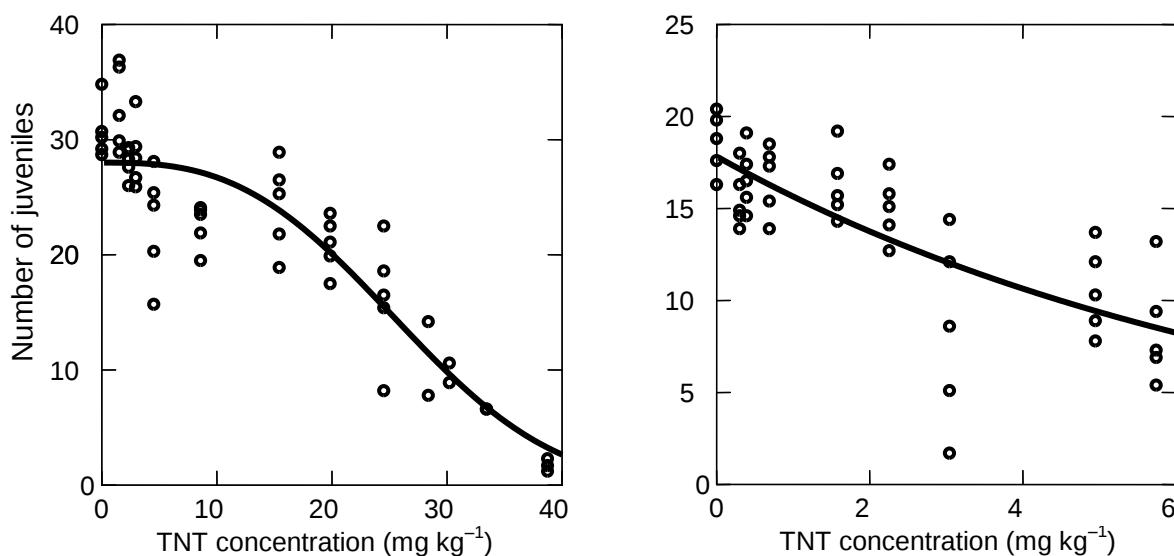


Figure 11. Effects of TNT FA (left) or W-A (right) in WCL soil on production of juveniles by *F. candida*.

The R^2 values from nonlinear regression analyses of the reproduction toxicity data for TNT W-A in WCL soil, based on ACN- or ATCLP-extractable TNT concentrations, were compared to determine which chemical measure of exposure better correlated with toxicity. These comparisons showed that the coefficients were similar for both extraction types (Tables 27 and 28), which indicated that data from both extraction methods had excellent correlation with the toxicity data for juvenile production, and neither method had a distinct advantage in characterizing TNT bioavailability to *F. candida* in WCL soil.

TNT toxicity varied across the selected soils. Soil-related differences were evident in acute (adult survival) and chronic (juvenile production) toxicity benchmarks for TNT FA or W-A in each of the five natural soils tested in these studies. On the basis of EC₅₀ values for TNT FA in soil, chronic toxicity to *F. candida* was in the order TSL > RCL > SSL > KCL > WCL; and for TNT W-A in soil, the order was KCL > RCL > TSL > WCL > SSL. The effect of soil on TNT toxicity was investigated by determining quantitative relationships between the concentration-response-based toxicity benchmark estimates (EC₂₀ and EC₅₀ values) for acute or chronic endpoints and the soil property measurements shown in Table 1. All linear correlations were performed on original (untransformed) data. Pearson's linear correlation coefficients (*r*) and their respective probability values are summarized in Table 29. There was no statistically significant collinearity (*r* = 0.777; *p* = 0.122) between soil organic matter (OM) and clay measurements, which are key soil constituents that could affect bioavailability of TNT. Multicollinearity among the soil sand, silt, and clay contents (*r* ≥ −0.950; *p* ≤ 0.013) was present (data are not shown) as expected, due to the method of determination of these constituents; however, it was deemed inconsequential for the purposes of these studies. There was significant correlation between clay content and soil pH (*r* = 0.878; *p* = 0.050; Table 29). However, none of the toxicity benchmark estimates correlated significantly with soil pH (Table 29).

Table 29. Pearson Correlation Coefficients for Key Soil Properties and TNT Toxicity Benchmarks for Acute (Adult Survival) and Chronic (Juvenile Production) Endpoints Determined in Definitive Tests with *F. candida*

Parameter	Clay		Soil OM Content		pH	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Clay	1.000	0.000				
OM	0.777	0.122	1.000	0.000		
pH	0.878	0.050	0.503	0.388	1.000	0.000
EC ₂₀ FA _{acute}	0.591	0.294	0.957	0.010	0.271	0.659
EC ₅₀ FA _{acute}	0.540	0.347	0.929	0.022	0.190	0.760
EC ₂₀ W-A _{acute}	−0.689	0.198	−0.537	0.350	−0.544	0.343
EC ₅₀ W-A _{acute}	−0.287	0.640	0.133	0.831	−0.439	0.459
EC ₂₀ FA _{chronic}	0.411	0.492	0.843	0.073	0.009	0.988
EC ₅₀ FA _{chronic}	0.443	0.455	0.870	0.055	0.051	0.935
EC ₂₀ W-A _{chronic}	−0.313	0.608	−0.316	0.604	0.032	0.959
EC ₅₀ W-A _{chronic}	0.162	0.794	0.237	0.701	0.430	0.470

Notes: Pearson correlation coefficients and corresponding probability values were determined using data from definitive toxicity tests with SSL, TSL, KCL, RCL, and WCL soils. Estimates of EC₂₀ and EC₅₀ values compared with acetone control were determined for TNT FA and W-A in soil.

OM content of the soil was strongly ($r \geq 0.929$) and significantly ($p \leq 0.022$) correlated with acute toxicity benchmarks for TNT FA in soil. OM content of the soil was also well correlated with chronic toxicity benchmarks for TNT FA in soil (for EC_{20} , $r = 0.843$ with $p = 0.073$; for EC_{50} , $r = 0.870$ with $p = 0.055$). However, these correlations were not sustained after weathering-and-aging of TNT in soil.

3.5 Effects of RDX on the Collembolan *F. candida*

Definitive studies using the Folsomia reproduction test (ISO, 1998b) were conducted to assess the acute (adult mortality) and chronic (juvenile production) effects of RDX on the Collembolan *F. candida* in TSL, SSL, KCL, RCL, and WCL soils. Within each soil, *F. candida* juveniles were exposed to a range of RDX concentrations in independent investigations. Measurement endpoints were assessed using treatment concentrations determined from the range-finding studies and included the number of surviving adults and the number of juveniles produced during the 28 day test. Exposure concentrations for definitive tests for each soil were selected from range-finding results to achieve concentration bracketing of significant effects on reproduction endpoints (i.e., production of juveniles). Reproduction endpoints are preferred for establishing benchmarks for the development of Eco-SSL values for soil invertebrates (U.S. EPA, 2005), and therefore were the main focus of these studies. The ranges of exposure concentrations were expanded to include determination of the concentrations that caused lethal effects to adults. All ecotoxicological parameters were estimated using these respective measurement endpoint values and concentrations of RDX in soil that were analytically determined utilizing U.S. EPA Method 8330A (U.S. EPA, 2007).

Test results complied with the validity criteria adapted from the ISO test guideline and those stipulated in Section 2.8 of this report. Mean adult survival in negative controls ranged from 84 to 98% in tests with RDX either FA or W-A in soil (Tables 30–34). The mean numbers of juveniles produced by *F. candida* in negative controls ranged from 136 to 473 (Tables 30–34). All CVs for the numbers of juveniles produced by *F. candida* in negative controls were <30%, as required by the ISO 11267 test guideline, and ranged from 8 to 22%. The mean numbers of juveniles in positive controls of studies ranged from 25 to 304, corresponding to 85 and 36% decreases from the respective negative controls. Overall, the positive-control data were consistent with the baseline established for the laboratory culture of *F. candida*. Compliance with the test validity criteria confirmed that the toxicological effects determined in the definitive tests were attributable to the RDX treatments.

Table 30. Adult Survival and Juvenile Production of *F. candida*
Exposed to RDX FA or W-A in TSL Soil

RDX FA Treatment (mg kg ⁻¹)	Number ^a		TNT W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	8.8 (0.4)	136 (13)	Negative control	9.2 (0.4)	253 (11)
Acetone control	9.2 (0.4)	140 (12)	Acetone control	9.6 (0.2)	296 (12)
Positive control	4.2 (0.4)	41 (9)	Positive control	5.0 (0.7)	137 (15)
2	9.0 (0.3)	152 (17)	2	9.6 (0.2)	296 (18)
14	7.6 (0.5)	154 (14)	6	9.6 (0.2)	294 (14)
11	8.6 (0.2)	122 (10)	11	9.4 (0.2)	287 (9)
67	4.4 (0.4)	45 (12)	27	9.2 (0.4)	228 (13)
75	3.2 (0.9)	27 (10)	34	8.4 (0.2)	181 (19)
136	4.0 (0.3)	38 (5)	65	7.8 (0.4)	187 (6)
113	3.2 (0.4)	17 (6)	83	5.2 (0.6)	147 (23)
144	2.8 (0.4)	21 (2)	108	6.2 (0.7)	138 (14)
233	2.6 (0.4)	16 (3)	216	4.8 (0.5)	106 (15)

Note: RDX concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs ($n = 5$).

Table 31. Adult Survival and Juvenile Production of *F. candida*
Exposed to RDX FA or W-A in SSL Soil

RDX FA Treatment (mg kg ⁻¹)	Number ^a		RDX W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	9.6 (0.2)	295 (16)	Negative control	9.2 (0.2)	473 (23)
Acetone control	9.4 (0.2)	285 (7)	Acetone control	9.8 (0.2)	500 (12)
Positive control	5.8 (0.2)	119 (10)	Positive control	9.8 (0.2)	304 (27)
2	9.6 (0.2)	299 (17)	6	9.8 (0.2)	474 (14)
3	9.2 (0.4)	273 (17)	8	10 (0)	480 (15)
10	9.2 (0.4)	289 (14)	16	9.6 (0.2)	482 (19)
20	9.0 (0.3)	273 (19)	30	10 (0)	463 (22)
44	8.0 (0.3)	226 (12)	57	9.2 (0.4)	451 (33)
139	7.6 (0.2)	171 (10)	62	9.6 (0.4)	428 (13)
356	6.8 (0.4)	165 (16)	254	9.4 (0.2)	319 (15)
745	6.2 (0.5)	115 (10)	527	9.4 (0.2)	308 (23)
2121	4.8 (0.4)	116 (13)	NT	NT	NT

Note: RDX concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs ($n = 5$).

NT, not tested in this study.

Table 32. Adult Survival and Juvenile Production of *F. candida*
Exposed to RDX FA or W-A in KCL Soil

RDX FA Treatment (mg kg ⁻¹)	Number ^a		RDX W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	8.8 (0.4)	170 (14)	Negative control	8.8 (0.4)	192 (10)
Acetone control	9.2 (0.4)	190 (15)	Acetone control	9.4 (0.2)	197 (10)
Positive control	5.4 (0.7)	25 (6)	Positive control	5.8 (0.4)	95 (9)
9	8.6 (0.2)	173 (9)	16	9.2 (0.4)	188 (8)
12	8.4 (0.2)	158 (14)	21	8.6 (0.2)	184 (7)
49	8.2 (0.2)	126 (18)	20	8.8 (0.2)	178 (6)
74	8.2 (0.2)	125 (7)	28	9.0 (0.3)	189 (7)
84	7.6 (0.2)	112 (15)	43	8.0 (0.3)	167 (5)
144	6.8 (0.7)	78 (8)	65	6.8 (0.4)	141 (4)
191	7.0 (0.4)	74 (9)	85	6.0 (0.3)	132 (5)
187	6.0 (0.3)	64 (8)	111	5.4 (0.5)	93 (18)
186	4.4 (0.2)	34 (8)	209	5.2 (0.6)	75 (9)

Note: RDX concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs ($n = 5$).

Table 33. Adult Survival and Juvenile Production of *F. candida*
Exposed to RDX FA or W-A in RCL Soil

RDX FA Treatment (mg kg ⁻¹)	Number ^a		RDX W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	9.0 (0.4)	207 (14)	Negative control	9.6 (0.2)	154 (6)
Acetone control	8.8 (0.4)	201 (22)	Acetone control	9.0 (0.3)	156 (10)
Positive control	4.0 (0.6)	53 (12)	Positive control	6.0 (0.3)	73 (7)
6	9.0 (0.4)	190 (26)	5	9.0 (0.3)	153 (6)
9	8.8 (0.4)	190 (29)	8	9.6 (0.2)	158 (6)
14	7.0 (0.7)	202 (23)	12	5.8 (0.4)	46 (7)
17	8.2 (0.4)	190 (10)	17	5.2 (0.4)	38 (4)
21	8.2 (1)	189 (13)	20	4.4 (0.5)	20 (2)
27	6.8 (0.4)	196 (10)	24	3.4 (0.2)	13 (2)
33	7.2 (0.4)	186 (13)	33	3.4 (0.7)	7.8 (2.6)
34	6.8 (0.4)	178 (18)	32	4.0 (0.3)	8.0 (1.1)
56	6.6 (0.5)	185 (14)	56	2.6 (0.9)	1.8 (1.1)
65	7.4 (0.4)	171 (24)	66	3.6 (0.5)	5.8 (2.6)
94	4.8 (0.6)	135 (11)	82	2.8 (0.6)	1.4 (0.6)
234	5.6 (0.5)	102 (22)	238	2.2 (0.8)	0.6 (0.4)
440	5.2 (0.4)	107 (21)	460	3.2 (0.7)	0.6 (0.6)
633	5.2 (0.7)	92 (15)	700	1.4 (0.7)	1.0 (0.6)
973	3.2 (0.6)	52 (10)	1000	2.0 (0.6)	1.8 (1)

Note: RDX concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs (*n* = 5).

Table 34. Adult Survival and Juvenile Production of *F. candida*
Exposed to RDX FA or W-A in WCL Soil

RDX FA Treatment (mg kg ⁻¹)	Number ^a		RDX W-A Treatment (mg kg ⁻¹)	Number ^a	
	Adults	Juveniles		Adults	Juveniles
Negative control	8.4 (0.2)	140 (11)	Negative control	9.8 (0.2)	232 (10)
Acetone control	9.2 (0.4)	140 (12)	Acetone control	9.6 (0.2)	242 (11)
Positive control	6.0 (0.4)	69 (18)	Positive control	5.8 (0.4)	90 (10)
11	8.6 (0.4)	138 (14)	22	9.4 (0.2)	239 (11)
10	8.8 (0.4)	143 (12)	24	9.0 (0.3)	219 (12)
11	8.2 (0.4)	132 (10)	33	9.6 (0.2)	221 (11)
21	7.2 (0.4)	112 (15)	41	7.8 (0.2)	139 (22)
35	7.4 (0.5)	106 (15)	49	7.6 (0.4)	185 (8)
55	6.4 (0.9)	102 (25)	71	2.2 (0.7)	77 (17)
203	5.6 (0.5)	70 (13)	88	2.4 (0.6)	61 (14)
236	3.8 (1.0)	49 (14)	211	2.2 (0.5)	35 (15)
326	3.6 (0.9)	18 (5)	458	3.0 (0.5)	46 (13)
NT	NT	NT	671	2.4 (0.2)	50 (9)
NT	NT	NT	885	2.6 (0.4)	41 (15)

Note: RDX concentrations were based on ACN extraction using U.S. EPA Method 8330A.

^a*F. candida* numbers are means and SEs ($n = 5$).

NT, not tested in this study.

3.5.1 Effects of RDX in TSL Soil

Ecotoxicological responses of *F. candida* to RDX FA and W-A in TSL soil are shown in Table 30. Adult survival and juvenile production were affected in RDX-amended TSL soil within the concentration ranges selected for definitive tests (Table 30). Ecotoxicological benchmarks for analytically determined RDX concentrations are summarized in Table 35. For adult survival, the bounded NOEC and LOEC values were 11 and 14 mg kg⁻¹, respectively, for RDX FA in TSL, and 27 and 34 mg kg⁻¹, respectively, for RDX W-A in TSL soil. The logistic Gompertz model provided the best fit for the adult survival data (Figure 12). The EC₂₀ and EC₅₀ values for adult survival were 11 and 65 mg kg⁻¹, respectively, for RDX FA in TSL, and 44 and 172 mg kg⁻¹, respectively for RDX W-A in TSL soil (Table 35). On the basis of the EC₅₀ values and the respective 95% CIs (Table 35), weathering-and-aging of RDX in TSL soil significantly decreased the acute toxicity for *F. candida*.

The juvenile production-based bounded NOEC and LOEC values were 14 and 67 mg kg⁻¹, respectively, for RDX FA in TSL soil, and 12 and 27 mg kg⁻¹, respectively, for RDX W-A in TSL soil (Table 35). The logistic Gompertz model yielded the best fit for the juvenile production data from toxicity tests with RDX either FA or W-A in TSL soil (Figure 13). The EC₂₀ and EC₅₀ values for juvenile production were 17 and 46 mg kg⁻¹, respectively for RDX FA in TSL soil, and 16 and 90 mg kg⁻¹, respectively, for RDX W-A in TSL soil.

Table 35. Toxicological Benchmarks for RDX FA or W-A in TSL, SSL, KCL, RCL, and WCL Soils
Determined Using ACN Extraction in Definitive Tests with *F. candida*

Ecotoxicological Parameter	RDX (mg kg ⁻¹)									
	TSL		SSL		KCL		RCL		WCL	
	FA	W-A	FA	W-A	FA	W-A	FA	W-A	FA	W-A
<i>Adult Survival</i>										
NOEC	11	27	20	527	12	28	21	8	35	33
<i>p</i>	0.348	0.484	0.415	0.264	0.110	0.444	0.417	0.304	0.265	1.000
LOEC	14	34	44	>527	49	43	27	12	55	41
<i>p</i>	0.015	0.040	0.006	ND	0.048	0.010	0.009	<0.001	0.029	0.002
EC ₂₀	11	44	134	>527	122	41	29	4	52	14
95% CI	2–21	21–67	18–250	ND	81–163	19–64	0–70	0.2–7	0–110	9–18
EC ₅₀	65	172	1991	>527	275	177	509	24	238	42
95% CI	43–86	130–214	1211–2771	ND	199–350	133–221	199–819	14–34	144–332	29–56
Model used	Gompertz	Gompertz	Exp	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Exp
<i>R</i> ²	0.968	0.985	0.992	0.997	0.985	0.987	0.968	0.925	0.965	0.938
<i>Juvenile Production</i>										
NOEC	14	12	20	57	12	28	65	8	55	33
<i>p</i>	0.330	0.681	0.535	0.079	0.057	0.523	0.270	0.767	0.067	0.259
LOEC	67	27	44	62	49	43	94	12	203	41
<i>p</i>	<0.001	0.003	0.005	0.012	<0.001	0.024	0.018	<0.001	0.001	<0.001
EC ₂₀	17	16	29	113	31	43	68	7	36	14
95% CI	4–29	4–28	0–66	29–197	8–54	25–62	0–147	6–9	0–79	10–18
EC ₅₀	46	90	489	770	110	130	379	12	135	44
95% CI	30–63	63–115	230–747	444–1097	79–141	105–153	194–564	10–13	62–208	31–57
Model used	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Gompertz	Exp
<i>R</i> ²	0.932	0.977	0.978	0.991	0.957	0.985	0.948	0.956	0.929	0.937

Note: Concentrations were based on ACN extraction using U.S. EPA Method 8330A.

Exp, exponential.

ND, not determined.

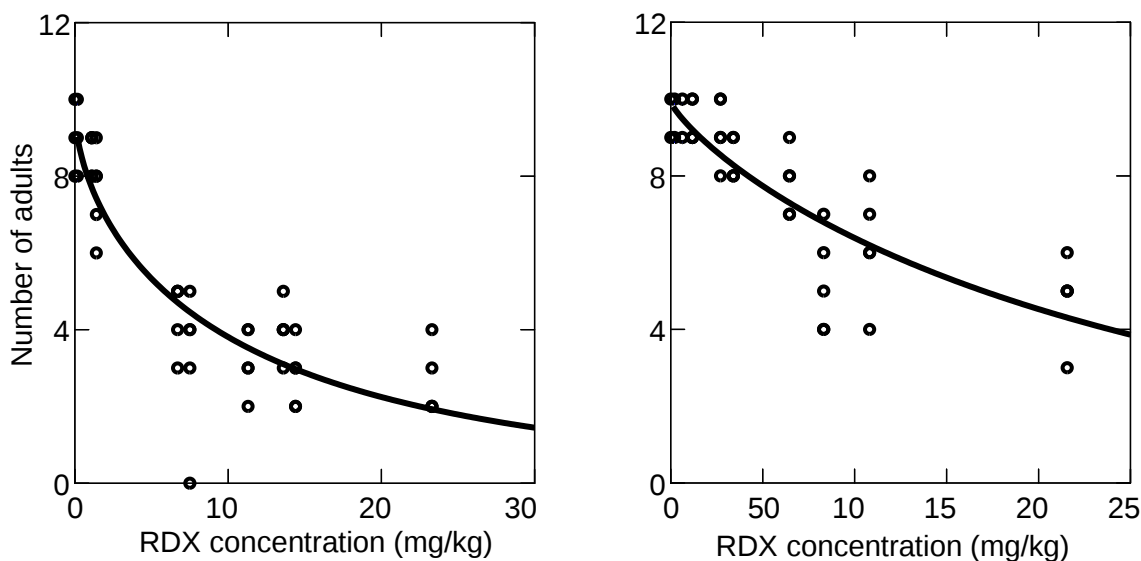


Figure 12. Effects of RDX FA (left) and W-A (right) in TSL soil on survival of adult *F. candida*.

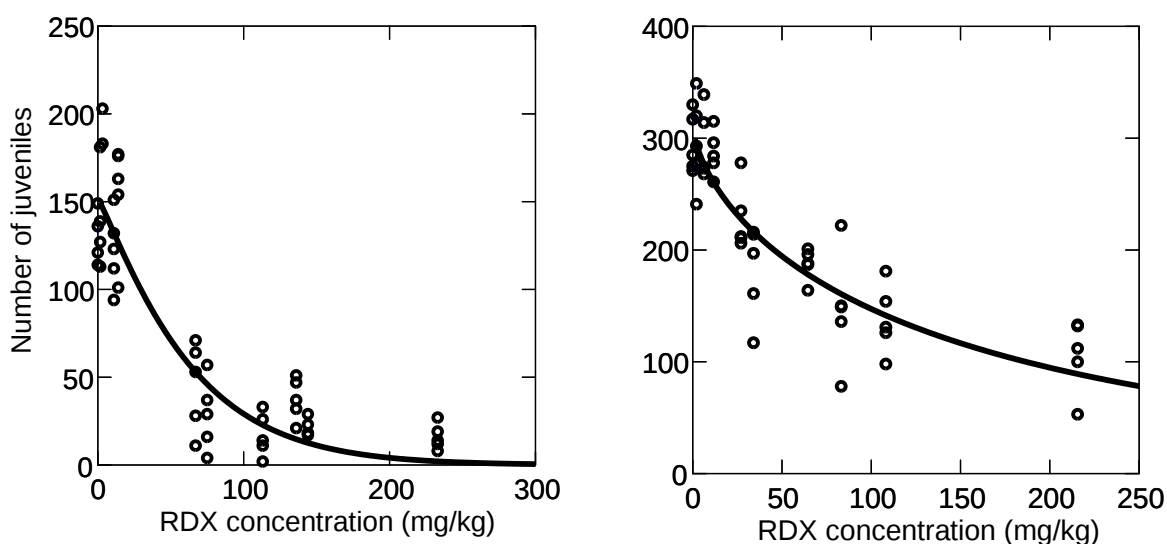


Figure 13. Effects of RDX FA (left) and W-A (right) in TSL soil on production of juveniles by *F. candida*.

3.5.2 Effects of RDX in SSL Soil

Ecotoxicological responses of *F. candida* to RDX FA and W-A in SSL soil are shown in Table 31. Both adult survival and juvenile production were affected in RDX-amended SSL soil within the concentration ranges selected for definitive tests (Table 31). Ecotoxicological benchmarks for analytically determined RDX concentrations are summarized in Table 35. For adult survival, the NOEC and LOEC values were 20 and 44 mg kg⁻¹, respectively, for RDX FA in SSL, and 527 (unbounded NOEC) and >527 mg kg⁻¹, respectively, for RDX W-A in SSL soil.

The exponential model provided the best fit for the adult survival data from study with FA SSL (Figure 14) and yielded the EC_{20} and EC_{50} values 134 and 1991 $mg\ kg^{-1}$ (Table 35). Weathering-and-aging of RDX in SSL soil significantly decreased the acute toxicity for *F. candida* (Table 35).

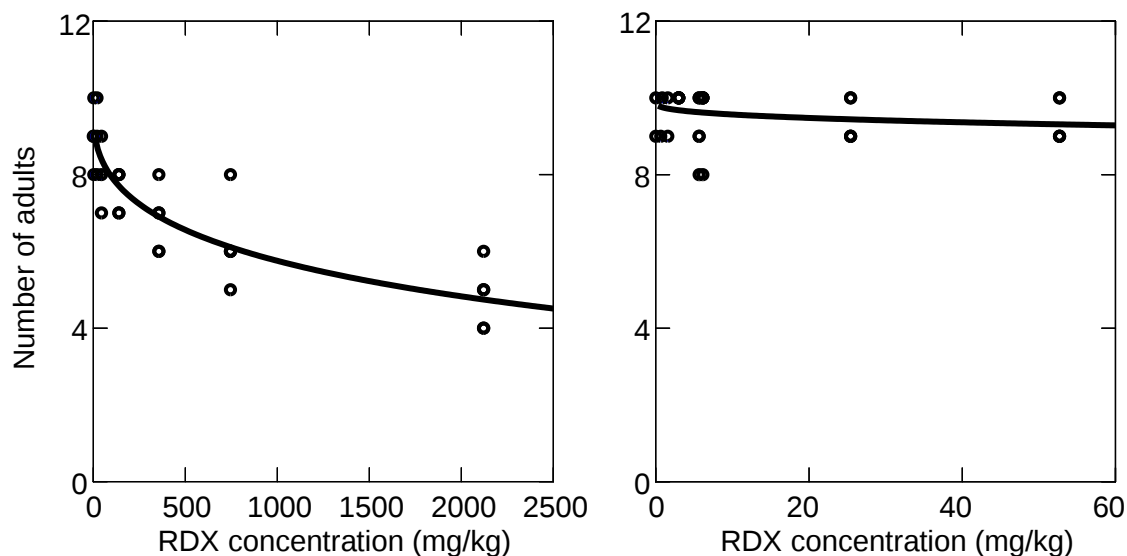


Figure 14. Effects of RDX FA (left) or W-A (right) in SSL soil on survival of adult *F. candida*.

The juvenile production-based bounded NOEC and LOEC values were 20 and 44 $mg\ kg^{-1}$, respectively, for RDX FA in SSL soil, and 57 and 62 $mg\ kg^{-1}$, respectively, for RDX W-A in SSL soil (Table 35). The logistic Gompertz model provided the best fit for the juvenile production data from toxicity tests with RDX either FA or W-A in SSL soil (Figure 15). The EC_{20} and EC_{50} values for juvenile production were 29 and 489 $mg\ kg^{-1}$, respectively for RDX FA in SSL soil, and 113 and 770 $mg\ kg^{-1}$, respectively, for RDX W-A in SSL soil. On the basis of the EC_{20} or EC_{50} values and the respective 95% CIs (Table 35), weathering-and-aging of RDX in SSL soil did not have a statistically significant effect on the toxicity for *F. candida* reproduction.

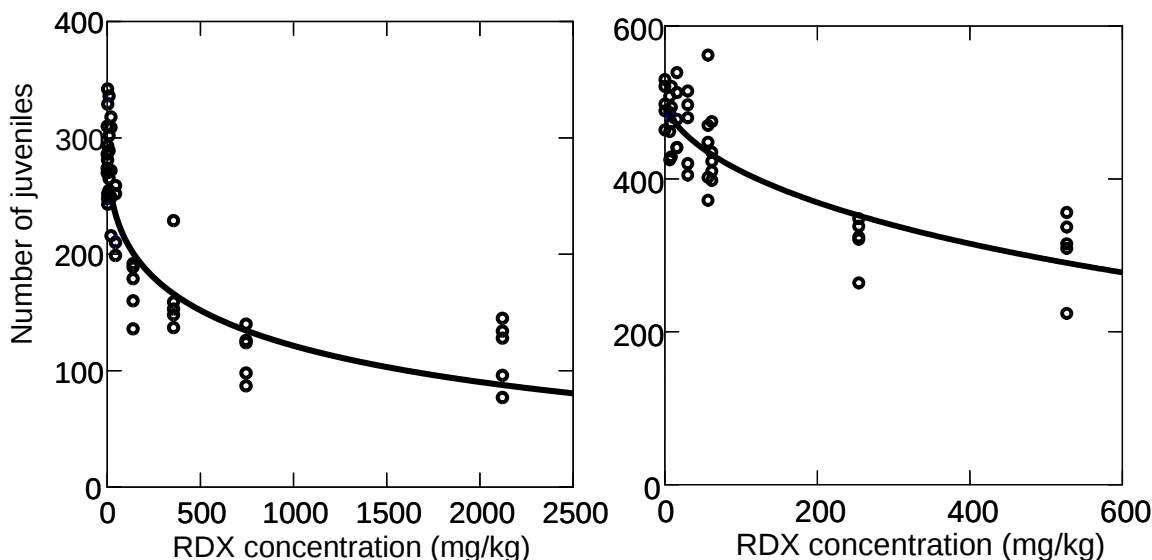


Figure 15. Effects of RDX FA (left) and W-A (right) in SSL soil on production of juveniles by *F. candida*.

3.5.3 Effects of RDX in KCL Soil

Ecotoxicological responses of *F. candida* to RDX FA and W-A in KCL soil are shown in Table 32. Both adult survival and juvenile production were affected in RDX-amended KCL soil within the concentration ranges selected for definitive tests (Table 32). Ecotoxicological benchmarks for analytically determined RDX concentrations are summarized in Table 35. For adult survival, the bounded NOEC and LOEC values were 12 and 49 mg kg⁻¹, respectively, for RDX FA in KCL, and 28 and 43 mg kg⁻¹, respectively, for RDX W-A in KCL soil. The logistic Gompertz model yielded the best fit for the adult survival data (Figure 16). The EC₂₀ and EC₅₀ values for adult survival were 122 and 275 mg kg⁻¹, respectively, for RDX FA in KCL, and 41 and 177 mg kg⁻¹, respectively, for RDX W-A in KCL soil (Table 35). On the basis of the EC₂₀ or EC₅₀ values and the respective 95% CIs (Table 35), weathering-and-aging of RDX in KCL soil significantly increased the acute toxicity for *F. candida*.

The juvenile production-based bounded NOEC and LOEC values were 12 and 49 mg kg⁻¹, respectively, for RDX FA in KCL soil, and 28 and 43 mg kg⁻¹, respectively, for RDX W-A in KCL soil (Table 35). The logistic Gompertz model provided the best fit for the juvenile production data from toxicity tests with RDX either FA or W-A in KCL soil (Figure 17). The EC₂₀ and EC₅₀ values for juvenile production were 31 and 110 mg kg⁻¹, respectively, for RDX FA in KCL soil, and 43 and 130 mg kg⁻¹, respectively, for RDX W-A in KCL soil. Weathering-and-aging of RDX in KCL soil did not significantly affect the reproduction toxicity for *F. candida* (Table 35).

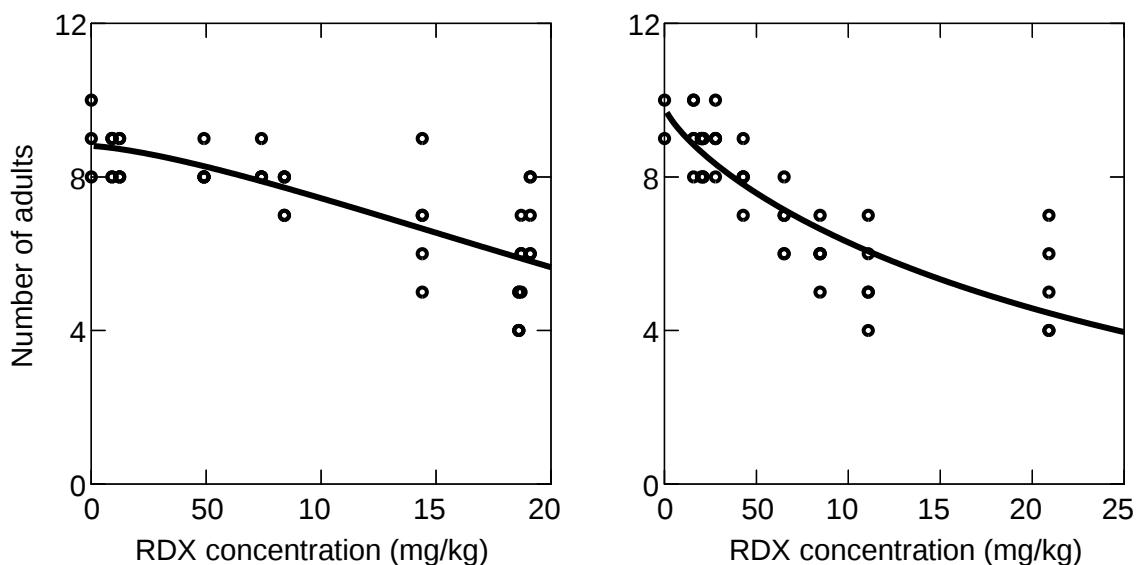


Figure 16. Effects of RDX FA (left) and W-A (right) in KCL soil on survival of adult *F. candida*.

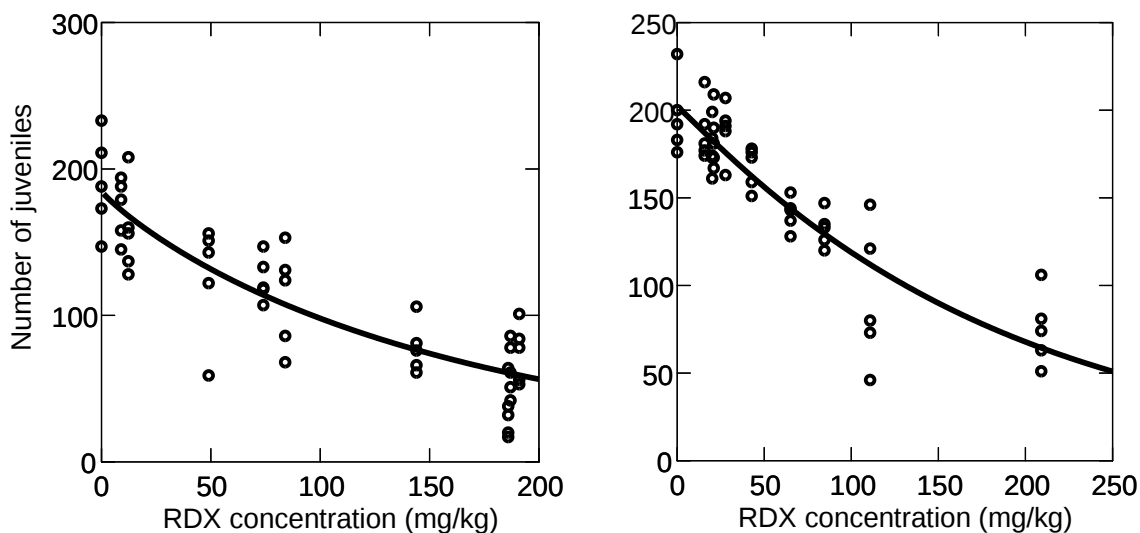


Figure 17. Effects of RDX FA (left) and W-A (right) in KCL soil on production of juveniles by *F. candida*.

3.5.4 Effects of RDX in RCL Soil

Ecotoxicological responses of *F. candida* to RDX FA and W-A in RCL soil are shown in Table 33. Adult survival and juvenile production were affected in RDX-amended RCL soil within the concentration ranges selected for definitive tests (Table 33). Ecotoxicological benchmarks for analytically determined RDX concentrations are summarized in Table 35. For adult survival, the bounded NOEC and LOEC values were 21 and 27 mg kg⁻¹, respectively, for RDX FA in RCL, and 8 and 12 mg kg⁻¹, respectively, for RDX W-A in RCL soil. The logistic

Gompertz model yielded the best fit for the adult survival data (Figure 18). The EC_{20} and EC_{50} values for adult survival were 29 and 509 $mg\ kg^{-1}$, respectively, for RDX FA in RCL, and 4 and 24 $mg\ kg^{-1}$, respectively, for RDX W-A in RCL soil (Table 35). On the basis of the EC_{50} values and the respective 95% CIs (Table 35), weathering-and-aging of RDX in RCL soil significantly increased the acute toxicity for *F. candida*.

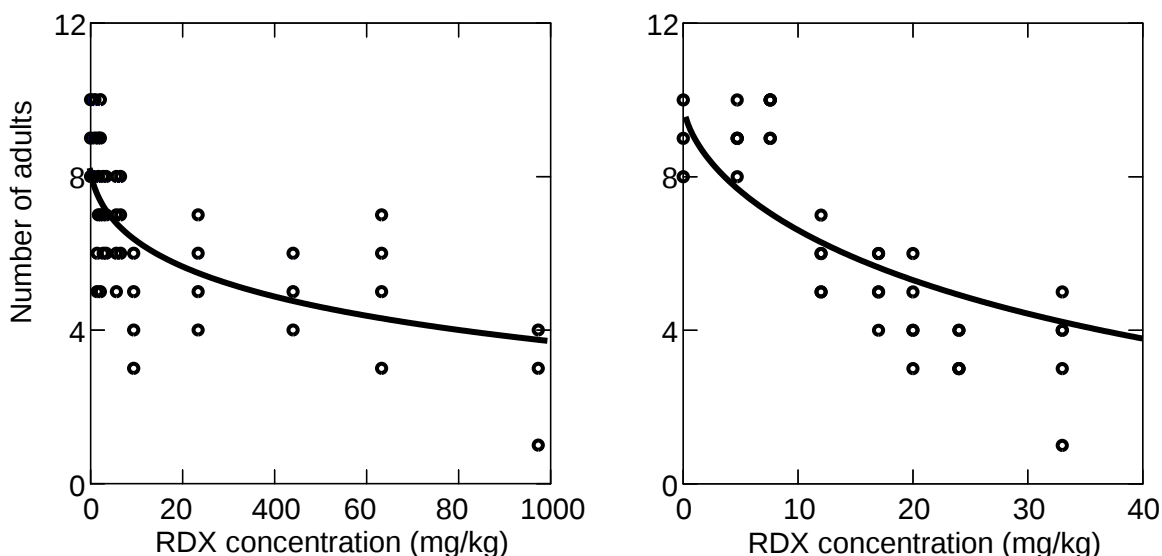


Figure 18. Effects of RDX FA (left) and W-A (right) in RCL soil on survival of adult *F. candida*.

The juvenile production-based bounded NOEC and LOEC values were 65 and 94 $mg\ kg^{-1}$, respectively, for RDX FA in RCL soil, and 8 and 12 $mg\ kg^{-1}$, respectively, for RDX W-A in RCL soil (Table 35). The logistic Gompertz model yielded the best fit for the juvenile production data from toxicity tests with RDX either FA or W-A in RCL soil (Figure 19). The EC_{20} and EC_{50} values for juvenile production were 68 and 379 $mg\ kg^{-1}$, respectively, for RDX FA in RCL soil, and 7 and 12 $mg\ kg^{-1}$, respectively, for RDX W-A in RCL soil. On the basis of the EC_{50} values and the respective 95% CIs for juvenile production (Table 35), weathering-and-aging of RDX in RCL soil significantly increased the reproduction toxicity for *F. candida*.

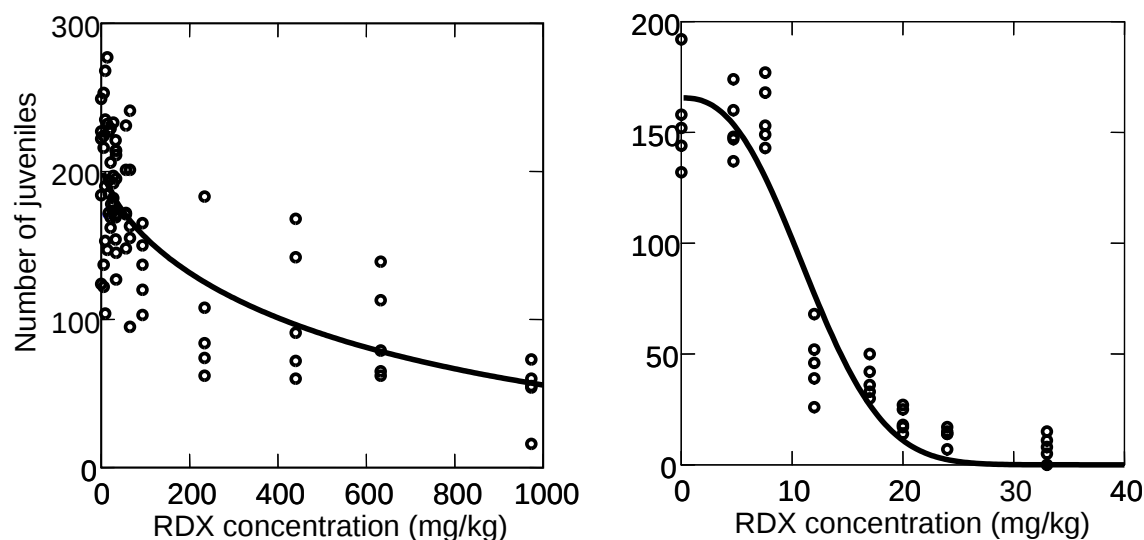


Figure 19. Effects of RDX FA (left) and W-A (right) in RCL soil on production of juveniles by *F. candida*.

3.5.5 Effects of RDX in WCL Soil

Ecotoxicological responses of *F. candida* to RDX FA and W-A in WCL soil are shown in Table 34. Adult survival and juvenile production were affected in RDX-amended WCL soil within the concentration ranges selected for definitive tests (Table 34). Ecotoxicological benchmarks for analytically determined RDX concentrations are summarized in Table 35. For adult survival, the bounded NOEC and LOEC values were 35 and 55 mg kg⁻¹, respectively, for RDX FA in WCL, and 33 and 41 mg kg⁻¹, respectively, for RDX W-A in WCL soil. The logistic Gompertz model yielded the best fit for the adult survival data (Figure 20). The EC₂₀ and EC₅₀ values for adult survival were 52 and 238 mg kg⁻¹, respectively, for RDX FA in WCL, and 14 and 42 mg kg⁻¹, respectively, for RDX W-A in WCL soil (Table 35). On the basis of the EC₅₀ values and the respective 95% CIs (Table 35), weathering-and-aging of RDX in WCL soil significantly increased the acute toxicity for *F. candida*.

The juvenile production-based bounded NOEC and LOEC values were 55 and 203 mg kg⁻¹, respectively, for RDX FA in WCL soil, and 33 and 41 mg kg⁻¹, respectively, for RDX W-A in WCL soil (Table 35). The logistic Gompertz model provided the best fit for the juvenile production data from toxicity tests with RDX in FA WCL soil and yielded EC₂₀ and EC₅₀ values of 36 and 135 mg kg⁻¹, respectively. The exponential model provided the best fit for the juvenile production data from toxicity tests with RDX W-A in WCL soil (Figure 21) and yielded EC₂₀ and EC₅₀ values of 14 and 44 mg kg⁻¹, respectively. On the basis of the EC₅₀ values and the respective 95% CIs for juvenile production (Table 35), weathering-and-aging of RDX in WCL soil significantly increased the reproduction toxicity for *F. candida*.

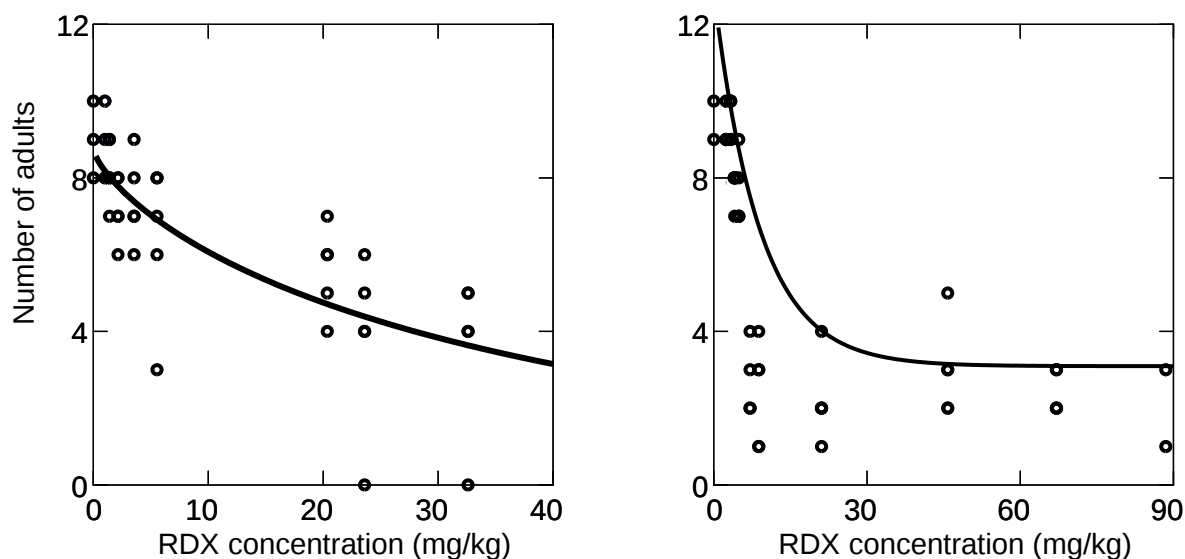


Figure 20. Effects of RDX FA (left) and W-A (right) in WCL soil on survival of adult *F. candida*.

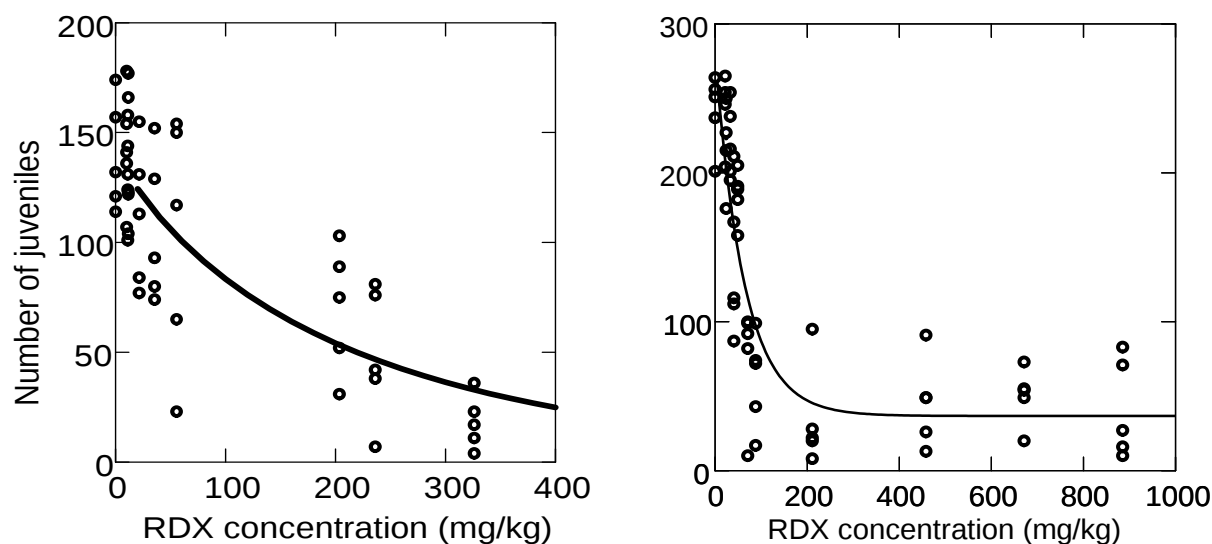


Figure 21. Effects of RDX FA (left) and W-A (right) in WCL soil on production of juveniles by *F. candida*.

3.6 Effects of Soil Properties on RDX Toxicity for *F. candida*

RDX toxicity varied across the selected soils (Table 35). On the basis of the EC_{50} values for RDX FA in soil, chronic toxicity to *F. candida* was in the order $TSL > KCL > WCL > RCL > SSL$; and for RDX W-A in soil, the order was $RCL > WCL > TSL > KCL > SSL$. The effect of soil on RDX toxicity was investigated by determining quantitative relationships between the concentration-response-based toxicity benchmark estimates (EC_{20} and EC_{50} values) for acute or chronic endpoints and the soil property measurements shown in Table 1. All linear

correlations were performed on original (untransformed) data. Pearson's linear correlation coefficients and their respective probability values are summarized in Table 36. Correlation analyses indicated strong statistically significant ($r = 0.904$; $p = 0.035$) relationships between soil pH and acute toxicity benchmarks (EC_{20} for adult survival) for RDX FA in soil. There were no similar significant ($p \leq 0.05$) correlations among any other toxicity benchmarks for RDX and soil properties (Table 36).

Table 36. Pearson Correlation Coefficients for Key Soil Properties and RDX Toxicity Benchmarks for Acute (Adult Survival) and Chronic (Juvenile Production) Endpoints Determined in Definitive Tests with *F. candida*

Parameter	Clay		Soil OM Content		pH	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
$EC_{20}FA_{acute}$	0.106	0.865	-0.251	0.684	0.904	0.035
$EC_{50}FA_{acute}$	-0.300	0.624	-0.445	0.453	0.295	0.630
$EC_{20}WA_{acute}$	-0.708	0.181	-0.793	0.110	-0.316	0.604
$EC_{50}WA_{acute}$	-0.396	0.510	-0.524	0.365	-0.379	0.529
$EC_{20}FA_{chronic}$	0.662	0.223	0.425	0.476	0.032	0.959
$EC_{50}FA_{chronic}$	-0.008	0.990	-0.231	0.708	-0.129	0.837
$EC_{20}WA_{chronic}$	-0.377	0.532	-0.561	0.326	-0.719	0.172
$EC_{50}WA_{chronic}$	-0.476	0.417	-0.575	0.311	-0.493	0.398

Notes: Pearson correlation coefficients and corresponding probability values were determined using data from definitive toxicity tests with SSL, TSL, KCL, RCL, and WCL soils. Estimates of EC_{20} and EC_{50} values compared with acetone control were determined for RDX FA and W-A in soil.

4. DISCUSSION

Development of ecotoxicological benchmarks for energetic soil contaminants has become a critical need in recent years (Kuperman et al., 2009b). These benchmarks are required for derivation of Eco-SSLs for use in ERAs of contaminated sites (U.S. EPA, 2005). Eco-SSLs represent concentrations of chemicals in soil that, when not exceeded, are theoretically protective of terrestrial ecosystems within specific soil boundary conditions from unacceptable harmful effects. These values can be used in a SLERA to identify those contaminants that are not of potential ecological concern in soils and therefore do not require further evaluation in a BERA. This can potentially result in cost savings during ecologically based site assessments and remedial investigations. Such screening values can also help site managers make operational decisions regarding long-term sustainability of testing and training ranges. An extensive literature review (Kuperman et al., 2009a) revealed that insufficient information exists about TNT or RDX to generate Eco-SSLs for soil invertebrates. The toxicity studies described herein were specifically designed to fill these knowledge gaps. Natural soils that meet the criteria for Eco-SSL development were used, primarily because these soils have characteristics supporting high relative bioavailability of TNT and RDX. The weathering-and-aging procedure applied to soils amended with ranges of TNT or RDX concentrations allowed us to determine the net ecotoxicological effects of complex fate processes in soil that affect bioavailability of TNT and RDX for the soil invertebrate *F. candida*, and to more realistically assess the toxicity under conditions closely resembling the potential exposure situations in the field.

The exposure concentrations of TNT and RDX in soil were analytically determined at the beginning of each definitive toxicity test using ACN extraction and U.S. EPA Method 8330A (U.S. EPA, 2007). This method quantifies the “total” extractable concentration of each explosive, which includes the nonaccessible (nondissolved crystalline plus adsorbed) and water-soluble fractions of TNT or RDX. Consequently, use of U.S. EPA Method 8330A includes the potential to overestimate the amount of explosive available to the exposed organism because the bioavailability of an organic compound having an octanol–water partition coefficient ($\log K_{ow}$) of <5 (1.6 for TNT and 0.90 for RDX; Monteil-Rivera et al., 2009) for uptake by a soil organism is primarily determined by the fraction dissolved in the soil interstitial water (Belfroid et al., 1994, 1996; Savard et al., 2010). Therefore, in addition to ACN extraction, the water-soluble fraction of TNT was extracted from soil using the ATCLP method (Haley et al., 1993). The TNT concentrations determined by this method better simulate field soil–water conditions that exist due to respiration by soil biota; also, this method is perceived to measure the intensity factor of the bioavailable fraction of chemicals in soil. The ATCLP-based extraction was not applied to RDX because all concentrations selected for toxicity tests with *F. candida* exceeded the aqueous solubility of RDX (42 mg L^{-1} at 20°C ; Monteil-Rivera et al., 2004), and RDX partitioning in the soil interstitial water is expected to control RDX uptake by soil invertebrates from soil only up to the limit of RDX saturation in the interstitial water (i.e., below 100 mg kg^{-1}). This was recently confirmed for earthworms by Savard et al. (2010).

The R^2 values for ACN- and ATCLP-based extractions determined in nonlinear regression analyses of the reproduction toxicity data from studies with TNT W-A in soils were compared to determine which chemical measure of exposure correlated better with TNT toxicity. These comparisons showed that both extraction methods had excellent correlation with the toxicity data for juvenile production (a reproduction endpoint), and that neither extraction method had an advantage for characterizing bioavailability of TNT to *F. candida*. This result supported a decision to develop a draft Eco-SSL for TNT for soil invertebrates on the basis of ACN extraction. The ACN extraction-based Eco-SSL values will be especially practical for ERAs at contaminated sites because TNT concentrations determined during site characterization are typically based on ACN extraction using U.S. EPA Method 8330A.

TNT recovery using ACN extraction was $71 \pm 3\%$ (mean $\pm$ SE; $n = 46$) of the nominal concentrations across all of the FA soils tested, which indicated good correlation between nominal and measured TNT concentrations determined in our studies after a 24 h moisture-equilibration period for soils hydrated to 60% of the WHC. TNT concentrations were below the detection limits (0.05 mg L^{-1} in solution and 0.5 mg kg^{-1} in soil) in the lowest nominal 0.5 mg kg^{-1} treatment in TSL. A 30% recovery of TNT was determined in the second-lowest nominal 1 mg kg^{-1} treatment in TSL. Results of present studies are consistent with findings by Rocheleau et al. (2006), who reported recoveries greater than 80% for nominal TNT treatments ranging from 20 to 500 mg kg^{-1} but lower recoveries for nominal TNT treatments of $\leq 10 \text{ mg kg}^{-1}$ in studies with FA SSL soil. Decreased TNT recoveries in low nominal treatments ($<20 \text{ mg kg}^{-1}$) suggest that a portion of TNT can be rapidly transformed, degraded, or sorbed to soil matrix during the initial 24 h period of soil hydration. These mechanisms are corroborated by the findings of Myers et al. (1998), who reported that sorption of TNT in soils with a wide

range of physical properties was rapid and occurred on a time scale of a few minutes. Dodard et al. (2003) reported an average of 99% recovery of TNT from OECD artificial soil amended with a comparable range of nominal TNT concentrations. Such high recovery of TNT can be attributed to both the properties of the components of this formulated soil and the insufficient or absence of microbial transformation of TNT in dry OECD artificial soil (treatments were extracted immediately after preparation) compared with hydrated natural soils. Major et al. (1992) suggested that the time-dependent disappearance of explosives may be due to covalent or other non-equilibrium bonding to natural soil components, and therefore, analytical results for soils that were amended with explosives, air-dried, then immediately extracted, primarily test the “potential” efficiency of the extraction process. Overall, our chemical analysis results confirmed that the soil amendment procedure used in toxicity tests was appropriate, and that U.S. EPA Method 8330A was efficient for quantifying the amount of TNT in soil.

The 3 month weathering-and-aging of TNT in soils decreased TNT concentrations in all soils tested. The residual concentrations were representative of TNT concentrations found in contaminated soils at some former ammunition plants (Simini et al., 1995) and military training ranges (Hewitt et al., 2007; Jenkins, 2007; Jenkins et al., 2006; Walsh et al., 2007). The overall TNT recovery was $48 \pm 5\%$ (mean $\pm$ SE; $n = 32$) of initial concentrations in hydrated FA soils. The percent recovery of TNT was on the order of (means $\pm$ SEs shown) KCL ($81 \pm 2\%$) > SSL ($79 \pm 3\%$) > WCL ($73 \pm 7\%$) > RCL ($63 \pm 7\%$) > TSL ($60 \pm 8\%$). The resulting TNT concentrations after weathering-and-aging in soils were generally related to soil properties defining the QRB scores for organic chemicals (U.S. EPA, 2005). The amount of TNT remaining in soil after the 3-month weathering-and-aging period was greater in sandy loam soils (TSL and SSL) with “very high” QRB scores compared with clay loam soils (KCL, RCL, and WCL) with “medium” or “low” QRB scores, according to the Eco-SSL criteria (Table 1; U.S. EPA, 2005).

In contrast with the fate of TNT in amended soils, RDX concentrations in the five soils tested in the present studies did not appreciably decrease during the 3 month weathering-and-aging process. These results are consistent with other studies in which the fates and ecotoxicological effects of RDX in soils were investigated under aerobic conditions. RDX recoveries averaged 93, 95, and 83% after similarly performed weathering-and-aging procedures using SSL soil (Kuperman et al., 2003; Rocheleau et al., 2005; and Simini et al., 2006). Sheremata et al. (2001) reported little RDX degradation in batch cultures in a natural soil under aerobic conditions. Extensive degradation occurred only under anaerobic conditions after several weeks; the RDX metabolites hexahydro-3,5-dinitro-1-nitroso-1,3,5-triazine; hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine; and hexahydro-1,3,5-trinitroso-1,3,5-triazine were not found until after extensive anaerobic degradation had occurred. The authors also measured relatively low sorption (K_d^s) values (0.83 L kg^{-1}), although the sorption that occurred was nearly irreversible (Sheremata et al., 2001; Checkai et al., 1993). Sorption of RDX to soils was low, as demonstrated by low K_d^s values, and RDX was therefore typically highly mobile in soils, governed by interactions with soil minerals rather than by association with soil OM (Monteil-Rivera et al., 2009). Consequently, RDX readily leaches through the vadose zone, which presents a high risk for groundwater contamination.

This project was undertaken to produce scientifically defensible toxicity benchmark data for the development of soil invertebrate-based Eco-SSL values for TNT and RDX, and to investigate and characterize predominant soil physicochemical parameters that can affect the bioavailability and resulting toxicity of TNT or RDX to soil invertebrates. To achieve the first objective, studies were designed to meet specific criteria (U.S. EPA, 2005). Eco-SSL test acceptance criteria were met or exceeded in these investigations by ensuring that:

- (1) Tests were conducted in natural soils having physicochemical characteristics that support high relative bioavailability of TNT or RDX;
- (2) Experimental designs for laboratory studies were documented and appropriate;
- (3) Both nominal and analytically determined concentrations of chemicals of interest were reported;
- (4) Tests included both negative and positive controls;
- (5) Chronic or life cycle tests were used;
- (6) Appropriate chemical dosing procedures were reported;
- (7) Concentration-response relationships were reported;
- (8) Statistical tests used to calculate the benchmark and level of significance were described; and
- (9) The origin of test species was specified and appropriate.

Results of definitive studies using the springtail *F. candida* exposures in TSL or SSL soils established new ecotoxicological benchmarks for TNT or RDX effects on soil invertebrates under conditions of very high relative bioavailability for organic chemicals in soil (as defined in U.S. EPA, 2005). Toxicological benchmarks for TNT established in the present studies with *F. candida* were generally consistent with data for soil invertebrates reported in a comprehensive review by Kuperman et al. (2009a). The chronic EC₅₀ values for TNT summarized by Kuperman et al. (2009a) ranged from 23 to 919 mg kg⁻¹ for different soil types, test species, and degree of weathering-and-aging of TNT in soil, and were derived using either nominal or analytically determined soil TNT concentrations. Dodard et al. (2003) determined that the EC₂₀ and EC₅₀ values for TNT were 59 and 111 mg kg⁻¹ for juvenile production by *Enchytraeus albidus* in OECD artificial soil. These values were similar to those determined in our study with FA TSL or SSL soil. In a study with multiple soil types, Schäfer (2002) found that *Enchytraeus crypticus* was less sensitive to TNT exposure than *F. candida* and also determined that the respective reproduction EC₅₀ values were 501 and 64 mg kg⁻¹ in Lufa 2.2 soil (2.2% organic carbon), and 277 and 23 mg kg⁻¹ in Lufa 2.3 soil (0.7% organic carbon). However, these values may not be representative because problems with the performance of the test organisms in the soils used were encountered in those studies, including failure to meet validity criteria in several control treatments, high data variability, and low reproduction rates of the species tested (Schäfer, 2002). Exposure to TNT in OECD artificial soil affected the earthworm *Eisenia andrei* with respect to adult survival (the 14 day LC₅₀ value was 365 mg kg⁻¹ [Robidoux et al., 1999]) and reproduction (the 56 day LOEC value was 111 mg kg⁻¹ [Robidoux et al., 2000]). For *E. andrei* exposed to a sandy forest soil (3.8% OM, 83% sand, and 8% clay; pH 7.6), greater TNT toxicity was determined as compared with values for OECD artificial soil; the EC₂₀ value for juvenile

production was 52 mg kg⁻¹ (Robidoux et al., 2002). These results were comparable to findings for *F. candida* in the present studies with FA TSL or SSL soils. Acute and subacute (weight change) effects of TNT on the earthworm *Eisenia fetida* have been reported by Phillips et al. (1993) for exposures in SAS and in a natural forest soil that contained 5.9% OM. In those studies, the LOEC values based on the earthworm weight loss and nominal TNT concentrations were 140 and 150 mg kg⁻¹ for SAS and forest soil, respectively. Phillips et al. (1993) also reported 100% mortality of *E. fetida* in SAS fortified with a mixture of EMs that included 30, 50, 62.5, and 20 mg kg⁻¹ of TNT, 1,3,5-trinitrobenzene (TNB), 2,4-dinitrotoluene (2,4-DNT), and 2,6-dinitrotoluene (2,6-DNT), respectively. Statistically significant ($p < 0.01$) subacute effects on earthworms (weight loss) were observed at concentrations of 6, 10, 12.5, and 4 mg kg⁻¹ of TNT, TNB, 2,4-DNT, and 2,6-DNT, respectively. These results showed a greater TNT toxicity compared with the present studies; however, direct comparisons of data from these studies are inappropriate because of differences in the experimental designs, and particularly because of the presence of EM contaminant mixtures in the studies by Phillips et al. (1993).

The juvenile production endpoint used in the present studies was generally a more sensitive measure of TNT and RDX toxicities to *F. candida* compared with the adult survival endpoint. This comports with results reported in the literature for potworms (Dodard et al., 2003; Schäfer, 2002; Schäfer and Achazi, 1999; Kuperman et al., 1999, 2003, 2005, 2004a, 2004b, 2006b, 2006c, 2006e), earthworms (Phillips et al., 1993; Robidoux et al., 2000, 2001, 2002; Simini et al., 2003, 2006), and Collembola (Schäfer, 2002; Schäfer and Achazi, 1999). This finding supported the Eco-SSL requirement of using reproduction endpoints for toxicity benchmark development (U.S. EPA, 2005).

Overall, the present definitive studies using *F. candida* exposures in TSL or SSL soils resulted in the development of TNT and RDX ecotoxicological benchmarks in compliance with Eco-SSL test acceptance criteria (U.S. EPA, 2005) and will contribute to the benchmarks dataset used to derive the soil invertebrate-based Eco-SSL values for TNT and RDX.

4.3 Effects of Soil Properties on TNT or RDX Toxicities

The important role of soil properties in affecting bioavailability and toxicities of energetic soil contaminants to soil invertebrates has been emphasized in several studies (Kuperman et al., 2003, 2004a, 2005, 2006b, 2006c, 2006e; Schäfer, 2002; Simini et al., 2003, 2006; Phillips et al., 1993; Robidoux et al., 2002). To achieve the second objective of the present studies, toxicity testing was conducted with additional natural soils, including KCL, RCL, and WCL, to extend the range of comparison for soil physicochemical characteristics that were hypothesized to affect the EM toxicity to soil invertebrates. The QRB scores for organic chemicals in natural soils were considered “very high” for TSL and SSL, “medium” for KCL and WCL, and “low” for RCL soil, according to the Eco-SSL criteria (U.S. EPA, 2005). Soil-related differences were evident in acute (adult survival) and chronic (juvenile production) toxicity benchmarks for TNT FA or W-A in each of the five natural soils tested in these studies. On the basis of EC₅₀ values for TNT FA in soil, chronic toxicity to *F. candida* was in the order TSL > RCL > SSL > KCL > WCL; and the order for TNT W-A in soil was KCL > RCL > TSL > WCL > SSL. In these results, no correspondence existed between the soil QRB scores and the order for TNT toxicity to *F. candida*.

The quantitative analyses of relationships among the acute or chronic toxicity benchmarks for TNT and soil property measurements revealed that the OM content of the soil affected TNT toxicity to *F. candida* in FA treatments. However, this correlation was not sustained after weathering-and-aging TNT in soil. Results of the present studies comport with findings of several published studies suggesting that the bioavailability of TNT and related nitroaromatic compounds (NACs) can be affected by the soil OM content (Anzhi et al., 1997; Eriksson and Skyllberg, 2001; Singh et al., 2010). Other studies implicated clay content (Emery et al., 2001; Haderlein et al., 1996; Singh et al., 2008) or a combination of the soil OM and clay contents (Jaenig, 2006).

TNT and its metabolites were shown to react and sorb to OM in the soil (Achtnich et al., 1999; Anzhi et al., 1997; Dawel et al., 1997; Drzyzga et al., 1998; Eriksson and Skyllberg, 2001; Esteve-Núñez et al., 2001; Simpson, 2006; Singh et al., 2010; Thorn and Kennedy, 2002; Thorn et al., 2002; Xing and Pignatello, 1997; Weiß et al., 2004). Sorption studies with low-polarity organic compounds, including nitroaromatic energetic materials, have shown that binding of these compounds to both soil OM (Xing and Pignatello, 1997) and silicate clays (Haderlein et al., 1996) is competitive, selective, nonlinear, and frequently reversible. Both specific (electrostatic interactions/covalent bond formation reactions between functional groups in the organic contaminant and OM) and nonspecific (hydrophobic partitioning reaction between nonpolar organic contaminants and nonpolar moieties of OM) adsorption mechanisms are possible for TNT and soil OM. In a study by Singh et al. (2010) the carbonyl carbon content of OM was responsible for 98% variation in TNT sorption, possibly because positively charged carbonyl carbon is electrostatically associated with the negatively charged nitro groups of TNT. The importance of negatively charged functional groups in TNT adsorption was confirmed by an increase in TNT sorption to humic acids with an increase in soil pH and, therefore, with dissociation of hydroxylic and carboxylic groups in OC (Li et al., 1997; Ainsworth et al., 1993; Eriksson and Skyllberg, 2001).

Correlation analyses showed a relationship between soil pH and acute toxicity benchmarks (EC₂₀ for adult survival) for RDX FA in soil. There were no significant correlations among any other toxicity benchmarks for RDX and soil properties. These results contrast with the findings of Savard et al. (2010), who reported that variations in RDX bioavailability to the earthworm *E. Andrei*, measured as the biota soil accumulation factor (BSAF) values, were soil-specific and decreased in the order of Defence Research and Development Canada (Canadian sandy soil; 1% clay, 1.2% OM) > TSL (similar to soil used in our studies) > KCL (19% clay, 1.5% OM) > WCL (similar to soil used in our studies) at RDX concentrations $\leq 100 \text{ mg kg}^{-1}$. The smallest BSAF was determined for WCL soil, which had the greatest OM content and the lowest RDX bioavailability among soils tested in those studies. However, at RDX concentrations in soil ranging from 100 to 10,000 mg kg^{-1} , which were more relevant to the results of our studies, the trend was less clear (Savard et al., 2010).

4.4 Effects of Weathering-and-Aging Explosives in Soil on Toxicity

In addition to contaminant loading, the attainment of sorption-desorption equilibria for explosives and other EMs and their transformation in contaminated soils are time-dependent processes that ultimately determine EM bioavailability to soil organisms. These processes can decrease the amount of chemical that is bioavailable compared to freshly

contaminated soils or increase the toxicity due to the presence of more toxic transformation products than parent compound freshly introduced into soil (Alexander, 2000; Hawari et al., 1998, 2000; Preuß and Rieger, 1995; Gorontzy, et al., 1994; Spain et al., 2000; Schäfer, 2002; Kaplan, 1992; Kuperman et al., 2005, 2006b, 2006e; Sunahara et al., 2001). Therefore, the present studies included weathering-and-aging of TNT or RDX in soil in the experimental designs to determine the net ecotoxicological effects of these complex processes and to more closely approximate the exposure effects in the field. These studies revealed alterations in toxicity for *F. candida* after weathering-and-aging of TNT or RDX in soil, and these alterations were soil- and endpoint-specific. On the basis of the EC₅₀ values for adult survival and the respective 95% CIs (Table 27), compared with respective toxicities in FA soils, weathering-and-aging of TNT in soil significantly decreased acute toxicity for *F. candida* in SSL but significantly increased acute toxicity in KCL, RCL, and WCL soils. On the basis of the EC₅₀ values for juvenile production and the respective 95% CIs (Table 27), compared with respective toxicities in FA soils, weathering-and-aging of TNT in soil significantly decreased chronic toxicity for *F. candida* in TSL and SSL soils but significantly increased chronic toxicity in KCL, RCL, and WCL soils. Weathering and aging of RDX in soil significantly (based on EC₅₀ values and respective 95% CIs) decreased both acute and chronic toxicities for *F. candida* in TSL, but significantly increased both acute and chronic toxicities for *F. candida* in RCL and WCL (Table 35).

As discussed previously, different products that are formed during biotic and abiotic transformation of TNT in soil under aerobic conditions can alter the exposure effects for *F. candida* compared with those effects that can be observed when the parent compound is freshly introduced into the soil. These transformation products can include 2-amino-4,6-dinitrotoluene (2-ADNT); 4-amino-2,6-dinitrotoluene (4-ADNT); 2,4-diaminotoluene (2,4-DANT); and 2,6-diaminotoluene (2,6-DANT) (Ainsworth et al., 1993; Dodard et al., 2004; Esteve-Núñez et al., 2001; Fernando et al., 1990; Hawari et al., 2000; McCormick et al., 1976; Monteil-Rivera et al., 2009). In addition, 2,4-DNT and 2,6-DNT are common byproducts found in munitions as impurities resulting from TNT manufacturing (Major et al., 2002). The reduction of amines goes through the formation of nitroso derivatives (ArNO; 2-NO-DNT and 4-NO-DNT), and hydroxylamine derivatives (ArNHOH; 2-HADNT and 4-HADNT), which can be further transformed to azoxy-TNT compounds (Monteil-Rivera et al., 2009 and references therein). Photolysis of TNT can lead to formation of additional NACs, including 3,5-dinitroaniline; 2,4,6-trinitrophenol; 2,4,6-trinitrobenzyl alcohol; 2,4,6-trinitrobenzoic acid; and TNB (Monteil-Rivera et al., 2009). Several of these products were identified in TNT-contaminated soil (Daun et al., 1998; Frische, 2002), and a few, including 2-ADNT, 4-ADNT, 2,4-DANT, and 2,6-DANT, have been detected in earthworms *E. andrei* and *Lumbricus terrestris* exposed to TNT-contaminated soils (Johnson et al., 2000; Renoux et al., 2000; Robidoux et al., 2000). Some of these NACs are potentially more bioavailable and toxic than their precursors (Rieger and Knackmuss, 1995). Kuperman et al. (2006b) reported greater toxicities of TNB, 2,6-DNT, and 2,4-DNT compared with TNT toxicity to *E. crypticus* in SSL soil. In a study investigating relative TNT toxicities and reduced TNT metabolites in a sandy loam forest soil (8% clay, 3.8% OM), Lachance et al. (2004) demonstrated that acute toxicity of 4-ADNT to the earthworm *E. andrei* was greater compared with the toxicity of the parent compound, whereas exposures to equimolar concentrations of 2-ADNT, 2,4-DANT, and 2,6-DANT were less toxic for *E. andrei* adults compared with TNT. In our previous, similarly

designed studies, 2-ADNT and 4-ADNT were detected at all concentrations of TNT W-A in SSL soil but were identified in greater amounts at TNT concentrations between 50 and 200 mg kg⁻¹ (Rocheleau et al., 2006). The amino-nitrotoluene intermediates can be formed by soil bacteria in either aerobic or anaerobic conditions (Hawari et al., 1998, 2000; Monteil-Rivera et al., 2009). They are the most commonly detected products of TNT transformation, and they can contribute to the alteration of toxicity to *F. candida* after weathering-and-aging of TNT in natural soils. Identification of TNT transformation products in soils subjected to a weathering-and-aging procedure was not included in the scope of the current investigation; studies reported herein focused primarily on the net toxic effects of *F. candida* exposure to TNT in aerobic upland soils, thus meeting primary study objectives. Our ongoing studies with amino-nitrotoluene intermediates of TNT transformation will provide additional information required to definitively resolve current uncertainties regarding relative toxicities of TNT and its transformation products, especially as they relate to chronic exposure effects for soil invertebrates.

The net effects of weathering-and-aging of contaminant EMs in soil on the resulting exposure effects for soil invertebrates were investigated in several studies (Kuperman et al., 2003, 2004a, 2005, 2006b, 2006c, 2006e; Schäfer, 2002; Simini et al., 2003, 2006). Kuperman et al. (2005, 2006b, 2006c) reported that weathering-and-aging in SSL soil significantly increased the toxicities of TNT; 2,6-DNT; and 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (also known as CL-20) to *E. crypticus*, whereas the toxicities of 2,4-DNT or TNB were unaffected. In contrast, decreased toxicity of TNT after aging in soil has been reported for *E. albidus* in OECD artificial soil (Dodard et al., 2003) and for *F. candida* in Lufa 2.2 soil (Schäfer, 2002) or SSL soil (Kuperman et al., 2006f). No effects of weathering-and-aging of RDX or octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in SSL soil on toxicity were reported for *E. crypticus* (Kuperman et al., 2003) and *E. fetida* (Simini et al., 2003). Several factors may contribute to the differential effects of TNT weathering-and-aging in soils on the toxicity to *F. candida* observed in the present studies, versus the toxicity to *E. crypticus* or *F. candida* as determined in studies by Dodard et al. (2003) and Schäfer (2002). These include differences in the properties of soils used in the studies, the weathering-and-aging procedures employed (including soil aerobicity during processing and testing), and the resulting effects on the bioavailability of TNT and its transformation products for the organisms tested. Additional studies would be required to resolve the current uncertainties in our understanding of the mechanisms contributing to the increased or decreased toxicity of EMs after their weathering-and-aging in soil. These studies should be conducted using different soil types with relatively large ranges of properties that affect the fate and bioavailability of EMs. This would provide a better understanding of the complex interactions among physical, chemical, and biological components that jointly contribute to the outcome of ecotoxicity testing.

5. CONCLUSIONS

This project was undertaken to produce scientifically defensible toxicity benchmark data for the development of soil invertebrate-based Eco-SSL values for TNT and RDX and to investigate and characterize predominant soil physicochemical parameters that can affect the bioavailability and resulting toxicities of TNT or RDX to soil invertebrates. The present studies produced ecotoxicological benchmarks for TNT and RDX using the ecologically relevant soil invertebrate species *F. candida*. Reproduction was a more sensitive endpoint than adult survival for evaluation of exposure effects; therefore, reproduction endpoint-based toxicity benchmarks should be used to set soil invertebrate screening criteria for TNT and RDX. This finding also supports the Eco-SSL requirement of using reproduction endpoints for toxicity benchmark development (U.S. EPA, 2005).

The natural soils TSL and SSL were used in toxicity tests reported herein to develop ecotoxicological benchmark data for use in derivation of soil invertebrate Eco-SSLs. These soils had low OM and clay contents, which fulfilled the U.S. EPA requirement of using soil with characteristics that support high relative bioavailability of organic contaminants for developing realistic yet conservative Eco-SSL values (U.S. EPA, 2005). The exposure concentrations of TNT or RDX in soil were analytically determined at the beginning of each definitive toxicity test; consequently, the ecotoxicological benchmarks were determined using measured TNT or RDX concentrations. This complied with the U.S. EPA preference for establishing benchmarks for derivation of Eco-SSL values on the basis of measured soil concentration (rather than nominal concentration) of a chemical (U.S. EPA, 2005). Chemical analyses of FA soils using U.S. EPA Method 8330A showed generally good correlation between nominal and measured ACN-extracted concentrations, which confirmed that the soil amendment procedure used in the definitive toxicity tests was appropriate and that this method was efficient for quantifying amounts of TNT or RDX in soil. Overall, the definitive studies using *F. candida* exposures in TSL or SSL soils developed ecotoxicological benchmarks for TNT and RDX in compliance with Eco-SSL test acceptance criteria (U.S. EPA, 2005); thus the first objective of this investigation was achieved. All ecotoxicological benchmarks determined in these studies will be provided to the Eco-SSL Work Group for quality-control review before inclusion in the Eco-SSL database for later use in the development of individual soil invertebrate-based Eco-SSL values for TNT and RDX.

In addition to ACN extraction, the water-soluble fraction of TNT was extracted from soil using the ATCLP method, which was perceived to measure the intensity factor, which is the immediately bioavailable fraction of chemical in soil pore water. The present studies showed that both extraction methods had excellent correlation with the toxicity data for juvenile production and that neither extraction method had a distinct advantage for characterizing bioavailability of TNT to *F. candida*. These results support a decision to develop a draft Eco-SSL for TNT for soil invertebrates on the basis of ACN extraction. The ACN extraction-based Eco-SSL values will be especially practical for ERA at contaminated sites because TNT concentrations determined during site characterization are typically based on ACN extraction and the U.S. EPA Method 8330A.

Toxicity testing was conducted using natural soils with a range of physicochemical characteristics that were hypothesized to affect the EM toxicity to soil invertebrates. Soil-related differences were evident in acute and chronic toxicity benchmarks for TNT. On the basis of the EC₅₀ values for TNT FA in soil, chronic toxicity to *F. candida* (the primary focus of these studies) was in the order TSL > RCL > SSL > KCL > WCL; and for TNT W-A in soil, the order was KCL > RCL > TSL > WCL > SSL. On the basis of the EC₅₀ values for RDX FA in soil, chronic toxicity to *F. candida* was in the order TSL > KCL > WCL > RCL > SSL; and for RDX W-A in soil, the order was RCL > WCL > TSL > KCL > SSL. Analyses of quantitative relationships between the toxicity benchmarks for TNT and the soil property measurements indicated that OM was the dominant property mitigating TNT toxicity for adult survival in FA soil. Correlation analyses also showed a relationship between soil pH and acute toxicity benchmarks (EC₂₀ for adult survival) for RDX FA in soil. However, these correlations were not sustained after weathering-and-aging of TNT or RDX in soil. These analyses confirmed the importance of including weathering-and-aging of EMs in soil into the experimental designs of toxicity tests that aim to assess the effects of soil properties on bioavailability and toxicity of EMs in diverse field soils at historically contaminated sites.

The present studies included weathering-and-aging of TNT or RDX in soil in the experimental designs to produce a soil microenvironment more similar to field conditions and thereby more closely approximate the exposure effects at contaminated sites. Results of chemical analyses showed that exposure conditions of *F. candida* to EMs W-A in soils differed from those of FA soils. Toxicity alterations after the weathering-and-aging process were soil- and endpoint-specific. On the basis of the EC₅₀ values for adult survival and the respective 95% CIs, weathering-and-aging of TNT in soil significantly decreased acute toxicity for *F. candida* in SSL but significantly increased acute toxicity in KCL, RCL, and WCL soils, compared with the respective toxicities in FA soils. On the basis of the EC₅₀ values for juvenile production and the respective 95% CIs, weathering-and-aging of TNT in soil significantly decreased chronic toxicity for *F. candida* in TSL and SSL soils but significantly increased chronic toxicity in KCL, RCL, and WCL soils, compared with respective toxicities in FA soils. Weathering-and-aging of RDX in soil significantly (based on the EC₅₀ values and the respective 95% CIs) decreased both acute and chronic toxicities for *F. candida* in TSL but significantly increased both acute and chronic toxicities for *F. candida* in RCL and WCL. Overall results of the present studies showed that special consideration given to the effects of weathering-and-aging of EMs in soil for assessing toxicity was well justified. Toxicity benchmarks generated in the present studies will contribute to development of Eco-SSL values that better represent the exposure conditions of soil invertebrates at contaminated sites. Our findings of altered reproduction toxicity for *F. candida* with TNT W-A in soil and the findings reported in the literature clearly show that additional studies are required to more completely investigate and resolve the toxicity of TNT transformation and degradation products. Analogously, further investigation of the more toxic transformation compounds that arise within soils amended with TNT should also have a weathering-and-aging component, so that the level of persistence and long-term impact of the ecotoxicity of these toxic transformation products may also be assessed. These studies should also be designed to generate benchmark data for transformation products, so that research results may be used to derive draft Eco-SSL values for these chemicals and provide more complete information on the ecotoxicological effects of energetic contaminants in soil for risk assessors and site managers.

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ACRONYMS AND ABBREVIATIONS

ACN	acetonitrile
2-ADNT	2-amino-4,6-dinitrotoluene
4-ADNT	4-amino-2,6-dinitrotoluene
ATCLP	adapted toxicity characteristic leaching procedure
BDL	below detection limit
BERA	baseline ecological risk assessment
BSAF	biota-soil-accumulation factor
CAS	Chemical Abstracts Service
CI	confidence interval
CV	coefficient of variation
2,4-DANT	2,4-diaminotoluene
2,6-DANT	2,6-diaminotoluene
2,4-DNT	2,4-dinitrotoluene
2,6-DNT	2,6-dinitrotoluene
EC	effective concentration
EC ₂₀	concentration that produces 20% decrease in measurement endpoint
EC ₅₀	concentration that produces 50% decrease in measurement endpoint
ECBC	U.S. Army Edgewood Chemical Biological Center
Eco-SSL	ecological soil screening level
ECp	estimate of effect concentration for a specified percent effect
EM	energetic material
ERA	ecological risk assessment
FA	freshly amended
FLSD	Fisher's least-significant difference
HPLC	high-performance liquid chromatography
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
ISO	International Organization for Standardization
KCL	Kirkland clay loam
K _{ow}	octanol–water partition coefficient
K _d ^s	soil adsorption coefficient
LOAEC	lowest-observed-adverse-effect concentration
LOEC	lowest observed-effect concentration
NA	not applicable
NAC	nitroaromatic compound
ND	not determined
NOAEC	no-observed-adverse-effect concentration
NOEC	no-observed-effect concentration
OECD	Organisation for Economic Co-operation and Development
OM	organic matter
PTFE	polytetrafluoroethylene
QRB	qualitative relative bioavailability
<i>r</i>	Pearson's linear correlation coefficient
<i>R</i> ²	coefficient of determination
RCL	Richfield clay loam

RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
SAS	standard artificial soil
SE	standard error
SLERA	screening level ecological risk assessment
SSL	Sassafras sandy loam
TCLP	toxicity characteristic leaching procedure
TNB	1,3,5-trinitrobenzene
TNT	2,4,6-trinitrotoluene
TSL	Teller sandy loam
U.S. EPA	U.S. Environmental Protection Agency
W-A	weathered-and-aged
WCL	Webster clay loam
WHC	water-holding capacity

