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TOXICITY OF SELENIUM, WEATHERED AND AGED IN SOIL, TO THE COLLEMBOLAN *FOLSOMIA CANDIDA*

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14. ABSTRACT

We investigated the toxicity of selenium (Se) to the soil invertebrate *Folsomia candida* using the Folsomia Reproduction Test (ISO 11267:1999). Studies were designed to generate ecotoxicological benchmarks for developing the ecological soil screening levels (Eco-SSLs) for risk assessments of contaminated soils. For this study, we selected aerobic upland soil, Sassafras sandy loam (SSL), with soil characteristics (low levels of clay and organic matter, soil pH adjusted high) that support high relative bioavailability of the anionic Se species that is typically found in aerobic soil. Se was amended into soil as sodium selenate, subjected to weathering-and-aging using 21 days of alternating cycles of air-drying/rehydration to 60–75% of the water-holding capacity of the SSL soil, under ambient greenhouse conditions. Effective concentration at 20 and 50% (EC_{20} and EC_{50}) levels were 4.7 and 10.9 mg of Se/kg of soil, respectively, as determined on the basis of production of juveniles (reproduction) by *F. candida* exposed to Se weathered-and-aged in SSL soil. Toxicity benchmarks established in this study were submitted to the U.S. Environmental Protection Agency Eco-SSL Workgroup, and the EC_{20} value was used in developing soil invertebrate-based Eco-SSL for Se.

15. SUBJECT TERMS

Ecological soil screening level (Eco-SSL)	Selenium	Selenite	Natural soil
Folsomia candida	Selenate	Sandy loam	Toxicity assessment

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PREFACE

The work described in this report was authorized under U.S. Environmental Protection Agency (USEPA), Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA), USEPA Interagency Agreement (IAG) identification number DW-21-93926501-0. The work was started in July 2002 and completed in May 2006.

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TOXICITY OF SELENIUM, WEATHERED AND AGED IN SOIL, TO THE COLLEMBOLAN *FOLSOMIA CANDIDA*

1. INTRODUCTION

Selenium (Se) occurs naturally in the environment due to the weathering of rocks and volcanic activity; consequently, Se is released into the soil, water, and air. Se is also produced anthropogenically from burning coal or oil. Se found in air is commonly attached to fly ash and suspended particles. Airborne selenium particles can settle on soil or water surfaces. Disposal of Se in commercial products and waste can also contribute to Se contaminant levels in soil. Se was found in at least 508 of the 1623 current or former hazardous waste sites on the National Priorities List (Agency for Toxic Substances and Disease Registry [ATSDR], 2003). An average Se concentration in the earth's crust was estimated at 0.05–0.09 mg/kg with greater concentrations up to and including 120 mg of Se/kg of soil found in volcanic rocks in the western United States (ATSDR, 2003) and in the vicinity of sandstone-type uranium deposits at concentrations as high as 1000 mg of Se/kg of soil (Shamberger, 1981). Most seleniferous soils contained <2 mg of Se/kg of soil, with a maximum Se concentration of <100 mg/kg reported by Rosenfeld and Beath (1964). Overexposure to Se can have a detrimental effect on human and animal health (ATSDR, 2003).

Se can occur in the soil environment in the Se^{6+} (selenate), Se^{4+} (selenite), Se^{0} (elemental), and Se^{2-} (selenide) oxidation states depending upon the redox (reduction–oxidation) values (Eh) and pH values (Brown et al., 1999). Se is relatively insoluble and immobile in reduced forms. However, in oxidized forms, particularly Se^{6}, Se is mobile in aqueous solutions and poses a significant risk to organisms. Naturally occurring forms of Se include selenates [i.e., compounds containing $(Se^{6+}O_4^{8-})^{2-}$], selenites [containing $(Se^{4+}O_3^{8-})^{2-}$], elemental selenium (Se^{0}), various metal selenides [e.g., berzelianite (Cu_2Se) or umangite (Cu_3Se_2)], and organic selenides.

Notwithstanding the evidence for Se persistence in soil, data in the published literature were insufficient to establish the screening-level concentrations for the assessment of ecological risks at Se-contaminated sites. Much of the previous research published on the ecotoxicology of Se focused primarily on aquatic or wetland systems (Maier and Knight, 1994; USEPA, 1998; Sappington, 2002; Zawislanski and McGrath, 1998) or microbial transformations of Se (Losi and Frankenberger, 1998). The minimal information that is available on the effects of Se on terrestrial invertebrates was established with insect-feeding studies (Hladun et al., 2012; Jensen and Trumble, 2003; Vickerman et al., 2004; Vickerman and Trumble, 1999). Even less information existed on the ecotoxicological effects of Se on soil invertebrates. Fischer and Koszorus (1992) investigated the sublethal effect of Se on the earthworm *Eisenia fetida*, but the exposures were conducted in a mixture of peaty marshland soil and horse manure (1:1 m/m), which is not representative of Se bioavailability conditions in upland aerobic soils. To fill the existing data gaps, we conducted a definitive study, designed specifically to meet the U.S. Environmental Protection Agency (USEPA) criteria for derivation of toxicity benchmarks acceptable for inclusion in the development of ecological soil screening levels (Eco-SSLs; USEPA, 2005) for Se released into upland aerobic soil environments. The Eco-SSLs are concentrations of contaminants in soil that are protective of the 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 screening-level ecological risk assessments (SLERAs) 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). Eco-SSLs are consistent national screening values that can be applied during ecologically based site assessments and remedial investigations, which can result in potential cost savings. Use of Eco-SSL values can help site managers to distinguish those sites that do not pose significant environmental risks from those that do, prioritize contaminated sites by the level of risk posed, quantify the relative risks at each site, and decide whether further investigation in a BERA is merited to determine appropriate remedial actions.

2. MATERIALS AND METHODS

2.1 Soil Collection and Characterization

We used natural soil, Sassafras sandy loam (SSL: fine–loamy, siliceous, semiactive, mesic Typic Hapludult; U.S. Department of Agriculture, 1975), collected from an open grassland field in the coastal plain on the property of the U.S. Army Aberdeen Proving Ground, Harford County, MD. This soil was selected for developing the ecotoxicological values that are protective of soil organisms because (1) it was previously used to establish ecotoxicological benchmarks for organic and inorganic chemicals in standardized soil invertebrate toxicity tests (Kuperman et al., 2003, 2004a, 2004b, 2005, 2006a, 2006b, 2006c, 2006d, 2012, 2013a, 2013b; Simini et al., 2003); and (2) it has physical and chemical characteristics that support qualitatively "high" relative bioavailability for metals in natural soils (USEPA, 2005). The pH of this slightly acidic soil was adjusted to represent conditions of an alkaline aerobic upland soil to promote the formation of water-soluble forms of Se (Lemly, 1997), thus increasing the relative bioavailability of Se (USEPA, 2005) in toxicity tests.

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 screen, air-dried for at least 72 h, mixed periodically to ensure uniform drying, passed through a 2 mm sieve, and then stored at room temperature before use in testing. Soil was then analyzed for physico-chemical characteristics (Table 1). The water-holding capacity (WHC) of SSL soil was determined to be 18% of the soil dry weight.

Table 1. Physical and Chemical Characteristics of SSL Soil, before and after Adding CaO

Soil Parameter	SSL	SSL (w/ 0.1% CaO)
Sand (%)	70	70
Silt (%)	13	13
Clay (%)	17	17
Texture	sandy loam	sandy loam
CEC cmol/kg	5.49	9.8
Organic matter (%)	1.3	1.0
pH	5.2	7.1

CEC: cation exchange capacity.

2.2 Chemicals and Reagents

Calcium oxide (CaO; Chemical Abstracts Service [CAS] no. 1305-78-8; lot no. 4521MZ; purity 98%; Aldrich Chemical Company; Milwaukee, WI) was used to raise the soil pH level to ≥ 7. Sodium selenate (anhydrous $H_2O_4Se \cdot 2Na$; CAS no. 13410-01-0; lot no. G14105; purity 99.8%; Alfa Aesar; Ward Hill, MA) was used to prepare soil treatment concentrations. Beryllium sulfate ($BeSO_4 \cdot 4H_2O$; CAS no. 7787; purity 99.99%) was used as the positive control in definitive testing. ASTM Type I water (18 MΩ cm at 25 °C; ASTM International, 2004a) was used throughout the toxicity studies. It was obtained using a Milli-RO 10 Plus followed by a Milli-Q PF Plus system (Millipore Corporation; 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 MΩ cm at 25 °C), analytical reagent-grade nitric acid 1% (v/v), and ASTM Type I water.

2.3 Soil Preparation

To enhance Se bioavailability for this study, the SSL soil was amended with CaO to raise the pH level to ≥7. Aliquots of SSL soil were amended with different levels of CaO to determine the concentration needed to raise pH from 5.3 to ≥7. Soil was moistened to initiate CaO reaction with soil. Soil pH was monitored daily during the first week and then weekly thereafter. Results showed that 0.1% CaO was needed to raise the pH of the SSL soil to ≥7. Sufficient soil for both range-finding and definitive studies was amended with 0.1% CaO. Soil pH stabilized at the mean value of 7.1. This prepared soil was then used in toxicity tests to establish ecotoxicological benchmarks for the effects of Se on the test soil invertebrate species. Physico-chemical properties of SSL soil were again characterized following the addition of 0.1% CaO (Table 1).

Portions of air-dried SSL soil at pH 7.1 were prepared in mass quantities for each Se treatment level and then amended with sodium selenate to establish target treatment concentrations. Aqueous (ASTM Type I water) stock solutions of 100 and 1000 mg/L sodium selenate were prepared. Appropriate aliquots of these stock solutions were diluted with ASTM Type I water and then added to the soil to achieve the target treatment concentrations of Se. The SSL soil was hydrated to 17.1% dry weight (equivalent to 95% of the WHC of SSL soil), mixed thoroughly, and allowed to equilibrate for 24 h. Portions of these amended SSL soils were used in a range-finding test. The remainder of the amended soils was used in the weathering-and-aging process described in Section 2.4.

2.4 Weathering-and-Aging Se in Soil

Se treatments in SSL soil, used in the definitive toxicity testing, were subjected to a weathering-and-aging process before commencing tests to provide the appropriate benchmark data for Eco-SSL development. Standardized methods for weathering-and-aging of chemicals in soil were not available. We developed procedures that simulated, at least partially, the natural 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, as compared with tests conducted using freshly amended chemicals or with tests conducted following a short equilibration period (e.g., 24 h) (Kuperman et al., 2004a, 2005, 2006b, 2006c, 2006d;

Simini et al., 2003). Before definitive toxicity testing, samples of soil, which had been freshly amended with each Se treatment level, were first rehydrated with ASTM Type I water to 60% of the WHC to initiate the weathering-and-aging of Se in soil in open glass containers. The soil was then subjected to alternating hydrating and air-drying cycles at ambient temperatures in a greenhouse for a period of 21 days. All soil treatments were reweighed and readjusted to equal their initial mass by adding ASTM Type I water. Hydration frequency varied from one to two times each week, depending on the rate of soil drying. After completion of the Se weathering-and-aging procedures, all soil treatments were brought to 88% of the WHC of SSL soil 24 h before starting the definitive toxicity testing.

2.5 Analytical Determinations of Se in Soil

Soil from each Se treatment level was collected, lyophilized, and stored at –40°C, at the beginning and end of the 28 day test period. Three replicate soil samples (17.5 g each) were collected randomly from the homogenized soil in each soil-treatment level, including controls that did not receive Se. These soil samples were placed in 50 mL polypropylene centrifuge tubes with screw caps and frozen in a liquid nitrogen bath. After the bubbling of the liquid nitrogen was reduced to a low simmer (after approximately 15 min), the tubes were removed from the bath, and the screw caps were replaced by caps with vent holes. Soil was lyophilized for 24 to 48 h using a LABCONCO 6 L benchtop system (Labconco Corporation; Kansas City, MO). Vented caps were replaced by solid caps after lyophilization was complete. Soils were stored at –20 °C. Frozen samples were packed in dry ice in an insulated cooler and shipped overnight to Dr. Richard Higashi (who was at University of California–Davis [Davis, CA] at that time) for nitric–perchloric digestion and analytical determinations of Se and its speciation using liquid chromatography and inductively-coupled argon plasma mass spectrometry. These determinations included analyses for total extractable Se (range-finding and definitive tests) or inorganic Se speciation (range-finding tests only).

2.6 Toxicity Assessments

Several soil invertebrate toxicity tests, for which standardized protocols have been developed (ASTM, 2004b; International Organization for Standardization [ISO] 1998, 2004), can be used to effectively assess toxicity and derive protective benchmark values (Stephenson et al., 2002; Løkke and Van Gestel, 1998). We used the Folsomia Reproduction Test to assess the effects of Se on the survival and reproduction of the collembolan *Folsomia candida*. The Folsomia Reproduction Test is a chronic assay. The test we utilized is an adaptation of the ISO bioassay ISO/11267 (ISO, 1999). The ISO guideline for this assay was originally developed for use with artificial soil (USEPA standard artificial soil). Research in our laboratory has shown that this test can also be conducted using natural soils (Phillips et al., 2002, 2013; Kuperman et al., 2004b). The measurement endpoints for the test included adult survival and juvenile production. Adult *F. candida* were exposed to a range of Se concentrations in SSL soil. The total number of juveniles and the number of surviving adults were each counted after 28 days. Results for reproduction and for the survival of adults exposed to Se were compared to those of the control treatments to quantify ecotoxicological parameters.

The U.S. Army Edgewood Chemical Biological Center (ECBC) laboratory culture of *F. candida* was established in 2001 from a stock culture obtained from Dr. Paul Henning Krogh at

the Soil Fauna and Ecotoxicology Research Unit, Department of Terrestrial Ecology, National Environmental Research Institute (Vejlsøvej 25, DK-8600, Silkeborg, Denmark). The ECBC culture was maintained in culture jars on a mixture of charcoal and plaster of Paris in the dark at 20 °C. The *F. candida* were fed baker's yeast and kept moist by routine misting approximately twice per week with ASTM Type I water. 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, after which juveniles were transferred to new jars. These synchronized juveniles were then held for 10 days, thus providing the 10–12 day old juveniles that were used in these tests.

Glass jars (42 mm i.d. × 45 mm height) were used as test containers. Before the jars were used in the toxicity tests, they were cleaned with acetone, rinsed successively with tap water and ASTM Type I water and then air-dried. To prepare five replicates of each treatment and controls, 100 g of each air-dried soil treatment was hydrated to 88% of the SSL soil WHC. Then 20 g (dry mass basis) of each batch of hydrated treatment soil was transferred into a test container, and 0.05 g of baker's yeast was added to the surface of the soil. Ten 10–12 day old *F. candida* springtail juveniles were placed in each test container, and the soil with springtails was lightly misted with ASTM Type I water. A piece of transparent plastic wrap was placed over each container opening and held in place with a rubber band. The mass of each container was then recorded to monitor soil moisture loss during the test. The containers were misted with ASTM Type I water weekly to maintain soil moisture level.

The test containers were randomly placed in an incubator at 20 ± 0.5 °C with a relative humidity of 88 ± 5%. To terminate a test, approximately 15 mL of tap water was added to a test container and allowed to sit for several minutes to fully hydrate the soil. After gentle mixing with a spatula, an additional 10 mL of water was added. The contents of the test container were given a final mixing and examined under a dissecting microscope (15× magnification) for the presence of *F. candida* juveniles and adults. The juveniles and adults that floated to the surface were counted.

Se concentrations that were selected for definitive toxicity testing were chosen on the basis of the results of the range-finding test, which was performed to bracket the 20 and 50% inhibition in juvenile production, and compared with juvenile production in the negative-control soil. Definitive testing included the following replicated treatments: Se treatments (CaO-treated SSL soil with Se added), negative control (CaO-treated SSL soil with no Se added), pH control (SSL soil with no CaO or Se added), and positive control (toxicity tests with a reference toxicant in SSL soil). Positive-control soil was prepared using a solution of beryllium sulfate in ASTM Type I water to attain a 50 mg/kg Be nominal concentration in SSL soil (no CaO added). The effects of the reference toxicant were determined by comparing the results obtained in Be treatment of SSL soil with the results of the pH-control treatment of SSL soil. Validity criteria for the negative control included the following performance parameters (ISO, 1999):

- The adult mortality does not exceed 30% after 28 days;
- The average number of juvenile potworms per test container should reach 80 instars at the end of the 28 day test; and

- The coefficient of variation for the mean number of juveniles should be ≤30%.

2.7 Data Analyses

Data for adult *F. candida* survival and production of juveniles were analyzed using regression models selected from among those described in an Environment Canada (EC) guidance document (EC, 2005). During the statistical 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 to data 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 most-random scattering); and the means, standard errors, and variances of the residuals were the smallest. For analysis of adult *F. candida* survival and juvenile production data determined in the definitive test, we selected the logistic Gompertz model

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

where

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

The ECp parameters used in this study included the Se concentration producing a 20% (EC_{20}) or 50% (EC_{50}) decrease in the measurement endpoint as compared with the negative control. The EC_{20} parameter, based on a reproduction endpoint, is the preferred parameter for deriving Eco-SSL values. The EC_{50} parameter, a commonly reported value, was included to enable comparisons of the results produced in this study 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 *F. candida* survival and juvenile production data, respectively. Mean separations were determined using Fisher's least-significant difference pairwise comparison tests. When the no-observed-adverse-effect-concentration (NOAEC) or lowest-observed-adverse-effect-concentration (LOAEC) were determined, the same statistical analyses were applied. All analyses were performed using untransformed data and analytically determined total extractable Se 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, Inc.; San Jose, CA).

3. RESULTS

3.1 Range-Finding Test

A range-finding test was conducted to determine the range of Se concentrations in soil from a NOEC level to >50% reduction in production of juveniles by *F. candida*. Soil from the range-finding test was analyzed for total extractable Se, selenate (SeO_4), and selenite (SeO_3). Results of the chemical analyses are shown in Table 2. SeO_4 accounted for 25 to 86% of the extractable Se at the start of the experiment and for 7 to 65% of the extractable Se at the end of the experiment. The SeO_4 percentage increased with concentration (Table 2). Concentrations of SeO_3 were below levels of detection, both at the start and at the end of the experiment (Table 2). Therefore, SeO_4 was considered to be the principle available form of Se under these experimental conditions.

Table 2. Nominal and Measured Se Concentrations in Soil at the Start and End of the Range-Finding Test with *F. candida*

Nominal Se Concentration (mg/kg)	Start: Mean Se Concentration in Soil (mg/kg)			End: Mean Se Concentration in Soil (mg/kg)			Start: pH*
	Se Total	SeO_4	SeO_3	Se Total	SeO_4	SeO_3	
0	0.16	0.004	BDL	0.90	BDL	BDL	7.07
0.1	0.20	0.05	BDL	0.54	0.04	BDL	7.08
1	0.67	0.39	BDL	1.54	0.35	BDL	7.08
10	5.56	4.11	BDL	8.84	4.25	BDL	7.09
100	74.07	45.78	BDL	100.25	44.76	BDL	6.89
1000	740.75	635.90	BDL	876.79	568.91	BDL	6.70

Notes: Se concentrations included total extractable (Se total), SeO_4, and SeO_3 fractions in milligrams of Se per kilograms of soil.
BDL: below detection limit.
*Soil pH was measured at the initiation of the bioassay experiments, which followed Se additions as sodium selenate (Na_2SeO_4).

In this study, these results indicated that available Se was primarily in the form of SeO_4, as would be expected in aerobic soils sustaining high relative bioavailability of Se. Results of the range-finding test for the toxicity of Se in soil to *F. candida* are presented in Table 3. Adult *F. candida* survival and reproduction were not affected in soil concentrations up to and including 0.67 mg of Se/kg of soil, measured at the start of the test. At 5.56 mg of Se/kg of soil, survival of adult *F. candida* decreased by 62% as compared with negative-control treatment (Table 3). The production of juveniles by *F. candida* was decreased by 43.4% at 5.56 mg of Se/kg of soil.

Table 3. Effects of Se in Soil (Initially Added into Soil as SeO_4) on *F. candida* in Range-Finding Toxicity Testing

Measured Se Concentration (mg/kg)	Adults Mean (S.E.)	Juveniles Mean (S.E.)
0.16[†]	9.7 (0.3)	123.0 (7.6)
0.20	9.0 (0.6)	124.0 (24)
0.67	10.0 (0.0)	137.0 (5.5)
5.56	3.7 (1.5)	69.7 (31)
74.10	3.0 (0.6)	6.3 (9.5)
741.00	3.0 (0.6)	0

S.E.: standard error.
[†]Analytically determined background concentration of Se in SSL soil used in range-finding toxicity testing.

3.2 Definitive Test

Definitive testing using the adapted ISO 11267 (ISO 1999) Folsomia Reproduction Test was conducted to assess the effects of Se on acute (surviving adults) and chronic (number of juveniles produced) measurement endpoints for *F. candida* after 28 days in SSL soil. Treatment concentrations used in the definitive testing were based on the results of the range-finding studies. Exposure concentrations were selected for definitive testing to achieve bracketing of significant effects on the reproduction endpoint (i.e., production of *F. candida* juveniles). Reproduction endpoints are preferred for the development of Eco-SSL values for soil invertebrates (USEPA, 2005); therefore, these were the main focus of this study. All ecotoxicological parameters were estimated using these measurement endpoint values and analytically determined concentrations of Se in soil.

Test results complied with the validity criteria defined in the ISO 11267 test guideline, including those mentioned in Section 2.6 of this report. The validity criteria for test results from the negative-control treatment were as follows: mean adult survival 98%, mean number of juveniles produced 305, and a coefficient of variation (CV) of 12%. Juvenile *F. candida* production in the positive-control treatment was decreased by 46%, as compared with the pH control (SSL soil with no CaO added), and was within the baseline established for the ECBC laboratory culture of *F. candida*. Test compliance with the validity criteria confirmed that the toxicological effects determined in the definitive testing were attributable to the Se treatments.

The results of definitive testing are shown in Table 4, and the ecotoxicological responses of *F. candida* to Se are summarized in Table 5. Both adult *F. candida* survival and juvenile production were affected in Se-amended soils within the concentration ranges that were selected for definitive testing. Numbers of surviving adult *F. candida* were not significantly ($p = 0.724$) different between the negative control and 2.32 mg of Se/kg of soil (bounded NOAEC) treatments. Adult *F. candida* survival was significantly ($p = 0.039$) decreased at 4.03 mg of Se/kg of soil (bounded LOAEC) treatment as compared with the negative control.

Table 4. Effects of Se, Weathered-and-Aged in SSL Soil, on Toxicity to *F. candida* as Determined in Definitive Toxicity Testing

Nominal Se Concentration (mg/kg)	Measured Se‡ Concentration (mg/kg)	Adults Mean (S.E.)	Juveniles Mean (S.E.)
0 (pH control)§	NA	9.6 (0.24)	252 (12)
0 (negative control)	0.35	9.8 (0.20)	305 (16)
0.5	0.68	9.8 (0.20)	345 (22)
1	1.02	9.6 (0.24)	358 (20)
2	2.32	9.6 (0.24)	349 (24)
4	4.03	8.6 (0.51)	227 (16)
8	9.01	7.2 (0.37)	241 (21)
12	11.35	4.8 (0.58)	144 (21)
24	21.46	2.4 (0.51)	60 (10)
48	42.03	2.2 (0.49)	8.8 (4.1)
96	100.51	1.8 (0.37)	14 (7.1)
Positive control (Be 50 mg/kg)	NA	5.6 (0.51)	135 (11)

Notes: ‡Total extractable concentrations of Se, initially added as sodium selenate (Na_2SeO_4).
§SSL soil with no CaO or Se added.
NA: not applicable.

Table 5. Ecotoxicological Benchmarks for Adult Survival and Juvenile Production by *F. candida* Exposed to Se, Weathered-and-Aged in SSL Soil

Ecotoxicological Parameter	Adult Survival (mg/kg)	Juvenile Production (mg/kg)
NOAEC	2.32	2.32
p	0.724	0.079
LOAEC	4.03	4.03
p	0.039	0.003
EC_{20}	3.2	4.7
CI (95%)	1.05–5.44	2.49–6.89
EC_{50}	12.7	10.9
CI (95%)	8.9–16.4	8.7–13.2
R^2	0.976	0.965

R^2: coefficient of determination.

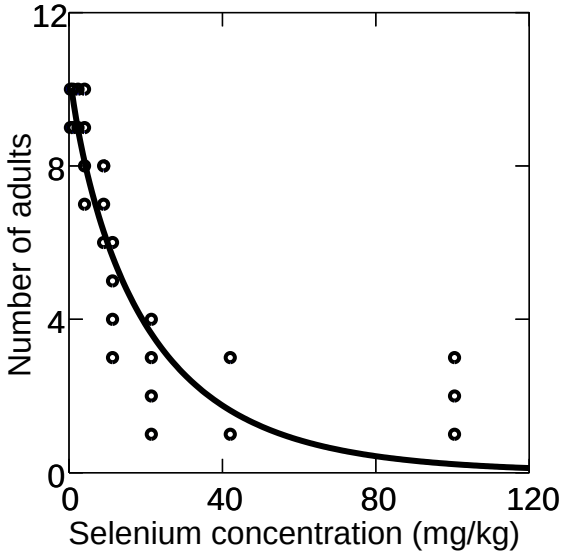

Figure 1. Effect of Se, weathered-and-aged in SSL soil,
on the survival of adult *F. candida*.

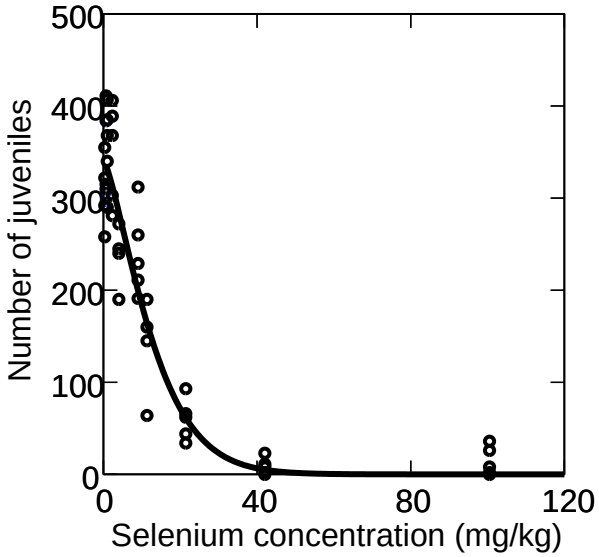

Figure 2. Effect of Se, weathered-and-aged in SSL soil,
on the production of juveniles by *F. candida*.

The range of Se concentrations selected for the test was sufficient to establish the concentration–response relationship based on survival of *F. candida* adults. The logistic Gompertz model had the best fit to the data (Figure 1). The R^2 value determined for the adult survival toxicity endpoint was 0.976, which indicated a very good fit of the model to toxicity

data. Nonlinear regression analysis of toxicity data yielded EC_{20} and EC_{50} values in milligrams of Se per kilogram of soil and corresponding 95% CIs (in parentheses) for adult *F. candida* survival of 3.2 (1.05–5.44) and 12.7 (8.9–16.4), respectively (Table 5).

Juvenile *F. candida* production was significantly ($p < 0.038$) increased in the 1.02 mg of Se/kg of soil concentration treatment as compared with the number of juveniles in the negative control, and significantly ($p < 0.006$) decreased in the 4.03 mg of Se/kg of soil treatment as compared with the negative control. This data resulted in bounded NOEC, LOEC, NOAEC, and LOAEC values of 0.68, 1.02, 2.32, and 4.03 mg of Se/kg of soil, respectively. The range of Se concentrations selected for the definitive test was sufficient to establish the concentration–response relationship based on juvenile production by *F. candida*. The effect of Se on juvenile production is shown in Figure 2. The ecotoxicological responses of *F. candida* to Se in SSL soil are summarized in Table 5. The logistic Gompertz model had the best fit to the data. The R^2 value determined for the juvenile production toxicity endpoint was 0.965, which indicated a very good fit of the model to toxicity data. Nonlinear regression analysis of toxicity data by the logistic Gompertz model yielded the EC_{20} and EC_{50} values (mg of Se/kg of soil) and corresponding 95% CI (in parentheses) for juvenile production of 4.7 (2.49–6.89) and 10.9 (8.7–13.2), respectively (Table 5).

4. DISCUSSION AND CONCLUSIONS

These studies were designed to develop scientifically defensible toxicity data that are required for the successful management of contaminated sites and for knowledge-based decision making. The main objective of this study was to generate toxicity data and establish benchmarks that are appropriate for use in deriving the soil invertebrate-based Eco-SSL values for Se. Ecotoxicological testing was specifically designed to successfully meet the criteria for Eco-SSL derivation outlined in the Eco-SSL Guideline (USEPA, 2005). Eco-SSL test acceptance criteria were met or exceeded in this investigation by ensuring that:

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

The preference for reproduction benchmarks and for a relatively low effect level (i.e., EC_{20}) ensured that Eco-SSL values would be protective of populations of the majority of

ecological receptors in soil and would provide confidence that Se concentrations that posed an unacceptable risk were not screened out early in the Ecological Risk Assessment (ERA) process during the SLERA. The exposure concentrations of Se in soil were analytically determined at the beginning of definitive toxicity testing; consequently, the ecotoxicological benchmarks were determined using measured Se concentrations. This complied with the USEPA preference for derivation of Eco-SSL values on the basis of measured concentrations of a chemical in soil, over those based on nominal concentrations (USEPA, 2005).

These studies included weathering-and-aging of Se in soil in the experimental design to produce a soil microenvironment that was similar to field conditions and would more closely approximate the exposure effects at contaminated sites. Therefore, toxicity benchmarks that were generated in these studies contributed to development of Eco-SSL value for Se that better represents the exposure conditions of soil invertebrates at contaminated sites.

Definitive testing in these studies established new ecotoxicological data for the effects of Se exposure under conditions of high relative bioavailability, as defined by the USEPA (2005), on soil invertebrate survival and reproduction (*F. candida*) in an upland aerobic sandy loam soil. The EC_{20} and EC_{50} values based on the toxicity of Se to reproduction of *F. candida* (Table 5) were remarkably similar to those values established for other soil invertebrates commonly used in standardized toxicity tests: earthworm *Eisenia andrei* (3.4 and 3.9 mg of Se/kg of soil, respectively) and the potworm *Enchytraeus crypticus* (4.4 and 6.2 mg of Se/kg of soil, respectively; Kuperman et al. 2016; USEPA, 2007). EC_{20} benchmarks, based on reproduction endpoints, are preferred for deriving Eco-SSLs for soil invertebrates (USEPA, 2005). The EC_{50} values for adult survival (acute toxicity endpoint) and for the production of juveniles (chronic toxicity endpoint) are provided for relative comparison to the EC_{50} values for other anionic contaminants because EC_{50} values are more commonly reported in the literature. The results of present research, along with the Se benchmarks developed for *E. andrei* and *E. crypticus* in our other separate studies, were submitted to the Eco-SSL National Task Group for quality-control review. Subsequently, these toxicity data were included in the Eco-SSL database, and the EC_{20} chronic toxicity endpoint data preferred by the USEPA were used in developing a Se Eco-SSL of 4.1 mg of Se/kg of soil for soil invertebrates (USEPA, 2007). This Eco-SSL value for Se is comparable with the Canadian Council of Ministers of the Environment (CCME) Se environmental soil quality guideline values of 1 mg of Se/kg of soil for agricultural and residential or parkland uses and 2.9 mg of Se/kg of soil for commercial and industrial land uses (CCME, 2009).

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ABBREVIATIONS AND ACRONYMS

ATSDR	Agency for Toxic Substances and Disease Registry
BDL	below detection limit
BERA	baseline ecological risk assessment
CAS	Chemical Abstracts Service
CCME	Canadian Council of Ministers of the Environment
CEC	cation exchange capacity
CI	confidence interval
CV	coefficient of variation
EC	Environment Canada
ECBC	U.S. Army Edgewood Chemical Biological Center
Eco-SSL	ecological soil-screening level
ECp	effective concentration of a specified percent
ERA	Ecological Risk Assessment
ISO	International Organization for Standardization
LOAEC	lowest-observed-adverse-effect-concentration
LOEC	lowest-observed-effect-concentration
NA	not applicable
NCEA	National Center for Environmental Assessment
NOAEC	no-observed-adverse-effect concentration
NOEC	no-observed-effect concentration
R^2	coefficient of determination
redox	reduction–oxidation
S.E.	standard error
SLERA	screening-level ecological risk assessment
SSL	Sassafras sandy loam
USEPA	U.S. Environmental Protection Agency
WHC	water-holding capacity

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