ECOTOXICITY EVALUATION IN SUPPORT OF THE DERIVATION OF TIER 2 VALUES FOR TEBUTHIURON

File No. 160960313

Prepared for:

EBA Engineering Consultants Limited
Riverbend Atrium One
115, 200 Rivercrest Drive Southeast
Calgary, AB T2C 2X5

Prepared by:

Stantec Consulting Ltd.
361 Southgate Drive
Guelph, ON N1G 3M5

July 2008

ECOTOXICITY EVALUATION IN SUPPORT OF TEBUTHIURON TIER 2 BENCHMARK DERIVATION

Table of Contents

List of Tables

ECOTOXICITY EVALUATION IN SUPPORT OF TEBUTHIURON TIER 2 BENCHMARK DERIVATION

Table of Contents

List of Appendices

1.0 Introduction

A request was made of Stantec Consulting Ltd. (Stantec) by EBA Engineering Consultants Limited (EBA) to conduct an ecotoxicity assessment of soil contaminated with a soil sterilant, tebuthiuron. Testing was conducted with four plant species, one earthworm species and one collembola species. The goal of the project was to generate scientifically-defensible toxicity data that could be used to derive Tier 2 site-specific soil quality criteria for tebuthiuron.

The ecotoxicity assessment test designs included field-collected soil contaminated historically with tebuthiuron, two field-collected reference control soils, and field-collected reference and contaminated soils amended with formulated tebuthiuron. One of the reference soils was a surface (top) soil (i.e., control topsoil or CTS) and the other reference soil was a subsurface soil (i.e., control subsurface soil or CSS). Both reference soils were free of contamination; however, only the subsurface reference soil had physical and chemical characteristics similar to those of the contaminated site soil (SS). In each test, reference and/or contaminated site soil was amended with formulated tebuthiuron in order to generate a tebuthiuron concentration gradient. Test organism performance in the tebuthiuron-amended or contaminated treatments was assessed relative to test organism performance in the uncontaminated control subsurface (CSS) treatment so that soil physico-chemical characteristics would not confound the toxicity test results. This experimental approach generated data that could be used to calculate point estimates (e.g., ICp, LCx, ECx) of tebuthiuron toxicity to plants and invertebrates in soils that are specific to the site.

The test species used in this ecotoxicity assessment included earthworm (*Eisenia andrei*), collembola (*Folsomia candida*) and plant (durum wheat (*Triticum durum*) and blue gramma grass (*Bouteloua gracilis*) species recommended in the Environment Canada test methods (EC, 2004, 2005a and 2007). In addition to these four species, two additional plant species were tested that are not included in the Environment Canada suite of species. These were western wheatgrass (*Pascopyrum smithii*) and silver sagebrush (*Artemisia cana* subsp. *cana*). Western wheatgrass and silver sagebrush were tested to provide additional toxicity data for site-specific species that are more ecologically relevant. Two other species were considered as possible candidate site-specific species, (i.e., Junegrass (*Koeleria macrantha*) and needle-and-thread (*Hesperostipa comata*)); however, practical constraints prohibited the use of these species in the ecotoxicity assessment.

In all tests conducted, an experimental negative control soil was also included in the test design; an artificial soil (AS) was formulated from constituents as recommended in the Environment Canada biological test methods (EC 2004, 2005a and 2007). The AS was included in the assessment as an internal QA/QC measure of test organism health and performance, technician proficiency, and experimental conditions.

Soil physico-chemical characteristics and tebuthiuron concentrations were measured by Access Laboratories (Calgary, AB) to characterize the test soils and to quantify the initial and final tebuthiuron exposure concentrations in invertebrate and plant tests. Pedological characterization of soil samples was done at the Soil and Nutrient Laboratory, University of Guelph (Guelph, ON).

1.1 TEST SOILS

1.1.1 Reference and Site Soils

The Stantec Consulting, Ltd. Soils Laboratory (361 Southgate Drive, Guelph, ON, N1G 3M5) received 13 buckets (20-L) containing either reference control soil (e.g., CTS and CSS) or contaminated soil (SS) collected from a site in Alberta in August 2007 (Table 1). Both the reference and contaminated soils were composites of soil samples collected from different locations within a site.

The soil samples, upon arrival at the Stantec laboratory, were assigned unique identification numbers and were entered into a logbook (Table 1). The soils did not require sieving. Soil from the three CTS subsamples were homogenized together and then stored in their original buckets until testing. The three CSS subsamples were also homogenized together and stored in their original buckets until testing. All seven SS subsamples were homogenized together and then stored in their original buckets until testing. The samples were stored at room temperature until tested (20.8 ± 1.5 °C).

The soil moisture content and water-holding capacity were determined for each soil prior to testing and the soils were prepared for testing in September 2007.

Table 1 Description of the reference and site soil samples received at the Stantec Soils Laboratory (Guelph) from EBA Engineering.

Soil Sample Descriptor	Date Received	Number of Samples	Unique Sample ID
Control Top Soil 1 of 3	2007-08-16	1	0757-1-CTS
Control Top Soil 2 of 3	2007-08-16	1	0757-2-CTS
Control Top Soil 3 of 3	2007-08-16	1	0757-3-CTS
Control Subsoil 1 of 3	2007-08-16	1	0756-1-CSS
Control Subsoil 2 of 3	2007-08-16	1	0756-2-CSS
Control Subsoil 3 of 3	2007-08-16	1	0756-3-CSS
Site Subsoil 1 of 6	2007-08-16	1	0755-1-SS
Site Subsoil 2 of 6	2007-08-16	1	0755-2-SS
Site Subsoil 3 of 6	2007-08-16	1	0755-3-SS
Site Subsoil 4 of 6	2007-08-16	1	0755-4-SS
Site Subsoil 5 of 6	2007-08-16	1	0755-5-SS
Site Subsoil 6 of 6	2007-08-16	1	0755-6-SS
Site Subsoil 7 Extra	2007-08-16	1	0755-7-SS

1.1.2 Physical and Chemical Characterization of Test Soils

Prior to shipping soil samples to Stantec, EBA collected and submitted subsamples for physical and chemical characterization of all test soils. Soil texture, fertility and carbon content were measured, along with salts, pH, conductivity and salinity. In addition, the soils were analyzed for concentrations of tebuthiuron, the BTEX compounds, petroleum hydrocarbons and a suite of metals (Tables 1 and 2; Appendix H, Table 2; Appendix I).

Subsamples of the artificial soil were submitted by Stantec for pedologic characterization to the University of Guelph Soil and Nutrient Laboratory (Guelph, ON) (Tables B.5, C.5, D.6, E.6, F.6 and G.6; Appendices B, C, D, E, F and G, respectively).

The pedologic characteristics of the artificial, reference and contaminated site soils were measured to satisfy the requirements of the Environment Canada test methods (EC 2004, 2005a and 2007). The Environment Canada test methods also require that pH, moisture content and water-holding capacity be measured for all test soils; these parameters were measured at the Stantec Soils Laboratory and are reported in Appendices B to G (Tables B.4, C.4, D.5, E.5, F.5 and G.5; Appendices B, C, D, E, F and G, respectively). Descriptions of the methods used to determine water-holding capacity, soil pH, moisture content and electrical conductivity are provided in Appendix A.

On Day 0 of the durum wheat, silver sagebrush and collembola tests, Stantec collected subsamples of soil for tebuthiuron analyses from the reference, contaminated soil, and tebuthiuron-amended treatments in order to quantify the initial tebuthiuron exposure concentrations in the soils and to determine the relationship between nominal and measured tebuthiuron concentrations (Tables 2 to 4). In addition, samples were taken at the end of the durum wheat test (Day 14), silver sagebrush test (Day 28), collembola test (Day 28), and from samples prepared with the collembola test but analyzed on Day 63 (duration of the earthworm test), to quantify the loss of tebuthiuron over time (Tables 2 to 4). Soil samples were sent to Access Laboratories (Calgary, AB) for analyses.

1.1.3 Negative Control Soil

The experimental negative control soil used for the toxicity assessment was a formulated artificial soil (AS) and recommended by Environment Canada for toxicity testing (EC, 2004; 2005a and 2007). It was formulated from natural ingredients of silica sand, kaolinite clay, and *Sphagnum* peat, and was buffered to a neutral pH range (6.0 – 7.5) with calcium carbonate. This negative control soil served as an experimental QA/QC soil to check test organism health, technician proficiency, experimental conditions, and testing procedures. The formulation of this artificial soil (AS) is described in Appendix A and the soil characteristics are described in Table B.5, C.5, D.6, E.6, F.6, and G.6. (Appendices B to G, respectively.)

Table 2 Concentration of tebuthiuron in site soil (0.03 mg tebuthiuron/kg soil dry wt.) amended with Spike 80DF. Soils were amended at Day -1 and soils were submitted for analyses on Days 0, 30 and 63.

Nominal Concentration (mg tebuthiuron/kg soil dry wt.)	Measured Concentration (mg tebuthiuron/kg soil dry wt.)									
	Replicate	**Day 0**	**Day 0 corrected for % recovery**	**% of Nominal**	**Day 30 (28)***	**Day 30 (28) corrected for % recovery**	**% of Nominal**	**Day 63**	**Day 63 corrected for % recovery**	**% of Nominal**
63	1	53.5	55.5	88	44.3	54.6	87	35.2	34.4	55
	2	52.4	54.4	86	46.9	57.8	92	40.2	39.3	62
	3	53.7	55.7	88	43.9	54.1	86	38.9	38.0	60
	Mean	**53**	**55**	**88**	**45**	**56**	**88**	**38**	**37**	**59**
	Std Dev	**1**	**1**	**1**	**2**	**2**	**3**	**3**	**3**	**4**
	% CV	**1**	**1**	**1**	**4**	**4**	**4**	**7**	**7**	**7**
500	1	422	437.8	88	420	517.9	104	497	485.4	97
	2	390	404.6	81	390	480.9	96	516	503.9	101
	3	422	437.8	88	396	488.3	98	497	485.4	97
	Mean	**411**	**427**	**85**	**402**	**496**	**99**	**503**	**492**	**98**
	Std Dev	**18**	**19**	**4**	**16**	**20**	**4**	**11**	**11**	**2**
	% CV	**4**	**4**	**4**	**4**	**4**	**4**	**2**	**2**	**2**
4000	1	3550	3682.6	92	3280	4044.4	101	3990	3896.5	97
	2	2770	2873.4	72	2950	3637.5	91	3910	3818.4	95
	3	3850	3993.8	100	2720	3353.9	84	3970	3877.0	97
	Mean	**3390**	**3517**	**88**	**2983**	**3679**	**92**	**3957**	**3864**	**97**
	Std Dev	**557**	**578**	**14**	**281**	**347**	**9**	**42**	**41**	**1**
	% CV	**16**	**16**	**16**	**9**	**9**	**9**	**1**	**1**	**1**

* Soils were submitted on Day 30 rather than Day 28 due to timing; Day 28 fell upon Dec 24 2007 and Access Laboratories were closed Dec 25 and 26 2007. Courier service was also not available on Dec 25 and 26; therefore, the soils were sampled and shipped on the earliest possible date, Dec 27 2007 ("Day 30").

Table 3 Concentration of tebuthiuron in control and site soil amended with Spike 80DF at the beginning and end of the 14-d durum wheat test. Soils were amended at Day 0 and soils were submitted for analyses on Days 0 and 14.

Nominal Concentration (mg tebuthiuron/kg soil dry wt.)	Measured Concentration (mg tebuthiuron/kg soil dry wt.)						
	Replicate	Day 0	Day 0 corrected for % recovery	% of Nominal	Day 14	Day 14 corrected for % recovery	% of Nominal
0.0003	1	0.00034	0.00035	116	0.00320	0.00303	1010
0.003	1	0.00218	0.00223	74	0.00171	0.00162	54
0.03	1	0.0372	0.0380	127	0.03654	0.03460	115
0.3	1	0.345	0.352	117	0.291	0.276	92
3	1	2.64	2.70	90	2.51	2.38	79
30	1	28.3	28.9	96	21.4	20.3	68

Table 4 Concentration of tebuthiuron in control and site soil amended with Spike 80DF at the beginning and end of the 28-d silver sagebrush test. Soils were amended at Day 0 and soils were submitted for analyses on Days 0 and 28.

Nominal Concentration (mg tebuthiuron/kg soil dry wt.)	Measured Concentration (mg tebuthiuron/kg soil dry wt.)						
	Replicate	Day 0	Day 0 corrected for % recovery	% of Nominal	Day 28	Day 28 corrected for % recovery	% of Nominal
0.0003	1	0.00044	0.00043	144	0.00151	0.00159	529
	2	0.00038	0.00037	125	0.00149	0.00157	522
	3	0.00038	0.00037	125	0.00157	0.00165	550
	Mean	**0.00040**	**0.00039**	**131**	**0.00152**	**0.00160**	**533**
	Std Dev	**0.00003**	**0.00003**	**11**	**0.00004**	**0.00004**	**15**
	% CV	**9**	**9**	**9**	**3**	**3**	**3**
0.003	1	0.00146	0.00144	48	0.00232	0.00244	81
	2	0.00164	0.00161	54	0.00160	0.00168	56
	3	0.00160	0.00158	53	0.00219	0.00230	77
	Mean	**0.0016**	**0.0015**	**51**	**0.0020**	**0.0021**	**71**
	Std Dev	**0.0001**	**0.0001**	**3**	**0.0004**	**0.0004**	**13**
	% CV	**6**	**6**	**6**	**19**	**19**	**19**
0.03	1	0.0419	0.0413	138	0.0390	0.0410	137
	2	0.0421	0.0415	138	0.0387	0.0407	136
	3	0.0459	0.0452	151	0.0372	0.0390	130
	Mean	**0.043**	**0.043**	**142**	**0.038**	**0.040**	**134**
	Std Dev	**0.002**	**0.002**	**7**	**0.001**	**0.001**	**3**
	% CV	**5**	**5**	**5**	**3**	**3**	**3**

Table 4 **Concentration of tebuthiuron in control and site soil amended with Spike 80DF at the beginning and end of the 28-d silver sagebrush test. Soils were amended at Day 0 and soils were submitted for analyses on Days 0 and 28.**

Nominal Concentration (mg tebuthiuron/kg soil dry wt.)	Measured Concentration (mg tebuthiuron/kg soil dry wt.)						
	Replicate	**Day 0**	**Day 0 corrected for % recovery**	**% of Nominal**	**Day 28**	**Day 28 corrected for % recovery**	**% of Nominal**
0.3	1	0.394	0.388	129	0.317	0.333	111
	2	0.369	0.363	121	0.399	0.419	140
	3	0.423	0.417	139	0.298	0.313	104
	Mean	**0.40**	**0.39**	**130**	**0.34**	**0.36**	**118**
	Std Dev	**0.03**	**0.03**	**9**	**0.05**	**0.06**	**19**
	% CV	**7**	**7**	**7**	**16**	**16**	**16**
3	1	2.67	2.63	88	2.56	2.69	90
	2	2.64	2.60	87	3.29	3.46	115
	3	2.26	2.23	74	2.94	3.09	103
	Mean	**2.5**	**2.5**	**83**	**2.9**	**3.1**	**103**
	Std Dev	**0.2**	**0.2**	**8**	**0.4**	**0.4**	**13**
	% CV	**9**	**9**	**9**	**12**	**12**	**12**
30	1	26.7	26.3	88	27.1	28.5	95
	2	30.9	30.4	101	29.2	30.7	102
	3	31.2	30.7	102	28.3	29.7	99
	Mean	**29.6**	**29.1**	**97**	**28.2**	**29.6**	**99**
	Std Dev	**2.5**	**2.5**	**8**	**1.1**	**1.1**	**4**
	% CV	**8**	**8**	**8**	**4**	**4**	**4**

1.2 TEST SPECIES

The test species are representative of two major groups of soil organisms; plants and soil invertebrates. The four plant species included three monocotyledonous (grass) species; blue gramma grass (*Bouteloua gracilis*), western wheatgrass (*Pascopyrum smithii*) and durum wheat (*Triticum durum*); and, one dicotyledonous (broadleaf) species, silver sagebush (*Artemisia cana* subsp. *cana*). The invertebrate species included an earthworm (*Eisenia andrei*) and a collembola (soil arthropod) species (*Folsomia candida*).

The invertebrate species were selected because:

- they have a relatively short life cycle that make it possible to conduct reproduction tests in the laboratory;
- they are commonly used invertebrate toxicity test species;
- performance criteria are available for both species;
- reliable cultures are available for both species;
- toxicity data generated from tests with these species are reproducible and sensitive; and
- standardized test methods exist for both test species (Environment Canada biological test method (EC, 2004 and 2007)).

The plant species were selected because:

- they include di- and monocotyledonous species;
- reliable seed sources are available;
- performance criteria are available or could be generated;
- they include native species and an economically important crop species (durum wheat);
- the three native species are ecologically significant to the site, and two of the three are site-specific (western wheatgrass and silver sagebrush); and,
- two of the four species (blue gramma grass and durum wheat) are recommended in the Environment Canada biological test method for plants (EC, 2005a).

1.2.1 New Plant Species Assessment

Although silver sagebrush and western wheatgrass were used in the ecotoxicity assessment, four site-specific native species not on the Environment Canada list of recommended species were evaluated for their potential as test organisms. The four plant species included silver sagebrush, western wheatgrass, needle-and-thread (*Hesperostipa comata*) and Junegrass (*Koeleria macrantha*). Emergence and growth trials were conducted with all four species under different environmental conditions.

Junegrass, western wheatgrass and needle-and-thread were the initial candidate species for the ecotoxicity assessment; silver sagebrush was to be a candidate species if and when a reliable source of seed from the then-current (2007) growth year could be obtained. Junegrass, western wheatgrass and needle-and-thread were obtained from Hannas Seeds (Lacombe, AB) and emergence and growth trials were conducted with these three species between September

2007 and March 2008. Reliable seed was found for silver sagebrush from Wind River Seed (Manderson, WY) and emergence and growth testing was conducted in April 2008.

Initial emergence and growth experiments were conducted in artificial soil (AS) using smaller volumes of soil (100 g wet wt.) in order to determine the most appropriate temperature and photoperiod for seedling emergence. Seed stratification was not required for any of the candidate species.

Only one of the three grass species (western wheatgrass, needle-and-thread and Junegrass) was to be selected as a test species in the ecotoxicity assessment. The criteria for the selection of the test species from the three candidates were based on the emergence and growth performance of each species. The selection criteria included:

- germination and emergence of a reasonably high percentage of planted seeds under standard Environment Canada test method (EC, 2005a) conditions;
- relatively short time to emergence for seedlings (e.g., within 7 days);
- consistent growth of emerged seedlings among and within test units;
- sufficient above- and below-ground biomass at the end of either 14- or 21-d tests (the standard duration of plant tests in the Environment Canada method) to measure growth response; and,
- reasonably easy removal of roots from the soil at the end of the test such that there is no excessive breakage or loss of root tissue.

Based on the criteria listed above, the initial 14-d emergence and growth trials indicated that western wheatgrass was the most promising candidate (Table 5). Junegrass emergence was acceptable (66%); however, shoot and root biomass was very low, and the roots of this species were delicate, making it difficult to remove the roots from the test soil. Needle-and-thread emergence was low (41%) and inconsistent, and although there was sufficient biomass at the end of the test, roots were delicate and difficult to remove from the soil. Western wheatgrass emergence was good (76%), and significant biomass was generated by the end of the 14-d test. Seedlings were robust and roots were easily removed from the test soil. All tests were conducted in artificial soil at 35% soil moisture content.

As a result of the success of western wheatgrass emergence and growth in the initial testing, further performance tests were conducted with larger volumes of soil (e.g., 500 g wet wt.) in order to generate a sufficient database for this species to develop performance criteria. Reference toxicity tests were also conducted to determine if the species was sensitive to a commonly used reference toxicant (boric acid) and if reliable and consistent toxicity data could be generated. Western wheatgrass emergence and growth performance data were generated (Table 5) and consistent toxicity data were obtained from reference toxicity tests (Appendix F).

Table 5 Results from emergence and early shoot and root growth trials for new plant test species: Junegrass (*Koeleria macrantha*), needle-and-thread (*Hesperostipa comata*), western wheatgrass (*Pascopyrum smithii*) and silver sagebrush (*Artemesia cana* subsp. *cana*). Values in brackets indicate one standard deviation of the mean.

Test	Test Species	Test Conditions: Soil Moisture (%)	Test Conditions: No. Seeds Planted	Test Conditions: Test Duration (d)	Emergence (%)	Shoot Length (mm)	Root Length (mm)	Shoot Dry Mass (mg)	Root Dry Mass (mg)
1	Junegrass	35	30	14	66 (10)	18.9 (1.2)	37.9 (7.2)	ND	ND
1	Needle-and-thread	35	15	14	41 (16)	54.5 (8.7)	47.5 (6.2)	ND	ND
2	Needle-and-thread	35	20	21	20 (12)	29.1 (5.6)	69.0 (22.6)	1.06 (0.52)	0.56 (0.19)
3	Needle-and-thread	20	20	21	13 (6)	45.28 (8.33)	140.14 (33.49)	3.74 (0.89)	1.58 (0.50)
				Mean of all tests*	**25**	**37.2**	**104.6**	**2.40**	**1.07**
				Standard deviation*	**15**	**11.5**	**50.3**	**1.89**	**0.72**
1	Western wheatgrass	35	15	14	76 (8)	91.7 (7.4)	56.8 (4.4)	ND	ND
2	Western wheatgrass	35	15	14	63 (11)	85.6 (12.6)	83.1 (16.5)	2.73 (0.57)	0.66 (0.14)
3	Western wheatgrass	35	15	14	63 (9)	99.9 (10.0)	95.5 (14.1)	3.68 (0.61)	0.94 (0.23)
4	Western wheatgrass	35	15	14	59 (9)	91.6 (13.8)	96.0 (11.8)	3.25 (0.68)	0.88 (0.19)
5	Western wheatgrass	35	15	14	60 (13)	91.6 (10.0)	71.2 (11.4)	2.17 (0.58)	0.45 (0.13)
6	Western wheatgrass	35	15	14	67 (19)	97.2 (10.0)	59.9 (5.5)	1.85 (0.20)	0.28 (0.05)
7	Western wheatgrass	35	15	14	54 (14)	69.4 (13.5)	79.4 (12.1)	1.92 (0.33)	0.58 (0.12)
				Mean of all tests	**63**	**89.6**	**77.4**	**2.60**	**0.63**
				Standard deviation	**7**	**10.0**	**15.7**	**0.75**	**0.25**
1	Silver sagebrush	35	+Broadcast: thinned to 10 seedlings	21	+10	26.8 (2.1)	115.2 (20.7)	4.1 (0.4)	1.1 (0.2)
2	Silver sagebrush	35		21	10	32.0 (1.1)	107.0 (2.1)	3.6 (0.6)	0.9 (0.2)
3	Silver sagebrush	35		21	10	29.9 (1.1)	106.9 (6.9)	4.5 (0.3)	1.3 (1.2)
4	Silver sagebrush	35**		21	Endpoints not measured as it was obvious that emergence and growth was much poorer in this test with drier conditions compared to the other three tests that were kept hydrated to a 35% moisture content throughout the test.				
				Mean of all tests	**NA**	**29.6**	**109.7**	**4.1**	**1.1**
				Standard deviation	**NA**	**2.6**	**4.7**	**0.5**	**0.2**

ND No data
* Mean and standard deviation of shoot and root length and mass data are calculated from the two 21-d tests only
** soil in this test was allowed to dry out for the first 4 days after seeds were planted
NA Not applicable because seedlings were thinned to a uniform 10 seedlings per test unit 7 days after the seeds were broadcast

Subsequent to this work, a meeting was held with Stantec, EBA and EnCana in March, 2008. During this meeting Stantec was requested to revisit needle-and-thread as a candidate species due to its particular ecological relevance to the site. It was suggested that the poor emergence of needle-and-thread performance tests was possibly due to test soils that were either too moist for successful germination and emergence of this species or to the removal of the awl on the seed. Needle-and-thread performance tests using greater volumes of artificial soil (e.g., 500 g wet wt.) were conducted under two different moisture regimes; 35% (as per the Environment Canada method specifications) and 20% to more closely mimic the natural conditions to which needle-and-thread is adapted. Poor germination in both treatments (20% and 13% emergence for 35% and 20% moisture content, respectively) was observed in subsequent tests (Table 5 needle-and-thread test 2 and 3).

The results from the subsequent tests (Table 5 needle-and-threat tests 2 and 3) indicated that needle-and-thread still had very poor emergence; needle-and-thread roots were also still difficult to extricate from the test soil. Therefore, it was concluded that western wheatgrass was the most suitable candidate species. This was confirmed with two more western wheatgrass performance tests (Table 5, western wheatgrass tests 6 and 7).

Silver sagebrush emergence and growth trials were conducted as soon as seed was received in April 2008. The batch of silver sagebrush seed contained a low percentage (34%) of pure live seed, and it was very difficult to distinguish between viable seeds and inert plant material (e.g., chaff). Therefore, to minimize bias in the test results due to inaccurate planting, silver sagebrush seeds were broadcast seeded on Day 0, rather than individually planted. Once the majority of seedlings had emerged, they were thinned to ten individuals per test unit. Seedling emergence was standardized by thinning the seedlings in a test unit to ten individuals of the most uniform size within a treatment, and within a test unit. Although this potentially introduced a bias towards the mean growth response of individuals, mean organism response is the desired measurement endpoint in the Environment Canada test methods. Therefore, introducing this potential bias was considered to be more acceptable than an introducing a bias due to inaccurate planting.

Silver sagebrush emergence and growth trials were conducted with tests using different moisture regimes. All four initial tests were conducted in AS at 35% moisture content; however, in silver sagebrush test number 4 (Table 5), the soil was allowed to dry out for the first four days after seeds were planted, in order to mimic natural conditions. Silver sagebrush emergence and growth was noticeably reduced in the “dry” test compared to the other tests. In the other three tests, the survival and vigour of the 10 thinned seedlings was excellent, and significant biomass was generated by the end of the 21-d test (a standard test duration in the Environment Canada method). Seedlings were robust, roots were easily removed from the test soil, and performance data were consistent. Reference toxicant testing with silver sagebrush was conducted, and this species responded in a concentration-dependent manner to the reference toxicant, boric acid (Appendix G). Therefore the results of the emergence and growth trials, and reference toxicant testing indicated that silver sagebrush would be an acceptable test species for the ecotoxicity assessment.

1.3 REFERENCE TOXICITY TESTS

Reference toxicity tests were conducted according to the recommendations in the Environment Canada test methods (EC 2004, 2005a, and 2007). They are a mandatory requirement for accreditation by the Canadian Association of Environmental Analytical Laboratories (CAEAL). The Stantec Soils Laboratory is CAEAL-accredited for the Environment Canada plant, earthworm and collembola test methods. The reference toxicant was boric acid and the reference test soil was the artificial experimental control soil described in Subsection 1.1.3. The purpose of conducting reference toxicity tests was to evaluate the health of the test organisms, precision and accuracy of laboratory techniques and technicians, and suitability of the experimental conditions. Organisms used in the reference toxicity tests were from the same batch as those used in the site soil testing. The results from the reference toxicity tests are reported in Appendices B to G.

2.0 Chronic Ecotoxicity Assessment of Tebuthiuron-Contaminated Site Soils

Chronic (earthworm and collembola) and definitive (plant) screening tests were conducted with artificial negative control soil, reference control soils, and contaminated reference and/or site soils. The measurement endpoints for the 63-day earthworm test included 35-d adult survival, and 63-d number of progeny produced and wet and dry mass of individual progeny. The measurement endpoints for the 28-day collembola test were adult survival and number of progeny produced. The measurement endpoints for each plant test included seedling emergence, shoot and root length, and shoot and root dry mass. Plant test durations were 14 days for western wheatgrass and durum wheat, 21 days for blue gramma grass and 28 days for silver sagebrush. Plant and invertebrate performance data in the reference control subsurface soil (CSS) were generated to provide a basis for comparison to plant and invertebrate performance in the tebuthiuron-contaminated reference and/or site soils. Rangefinding tests with invertebrate and plant test species were conducted prior to definitive tests in order to identify the appropriate tebuthiuron exposure series for the definitive tests. Artificial soil was included in the experimental design for QA/QC purposes only, to assess test organism health, technician proficiency, experimental conditions, testing procedures, and test validity. The purpose of the longer-term plant and invertebrate tests was to examine the effects of prolonged exposure to tebuthiuron-contaminated soils on the survival and reproduction of earthworms and collembola and the emergence and growth of plants relative to test organism performance in the uncontaminated reference soil.

The earthworm and collembola tests commenced on November 9, 2007 and November 26, 2007, and were completed on January 11, 2008 and December 24, 2007, respectively. The blue gramma grass, durum wheat and western wheatgrass tests commenced on March 18, 2008 and were completed on April 8, April 1 and April 1, 2008, respectively. The silver sagebush plant test was conducted between June 6 and July 4, 2008.

2.1 MATERIAL AND METHODS

2.1.1 Test Methods

All tests were conducted following the Environment Canada test methods (EC 2004, EC 2005a, and 2007) and details of the experimental procedures followed can be found in these documents.

The experimental design and test conditions for each test species are summarized in Table A.1 (Appendix A) and in the test reports in Appendices B, C, D, E, F and G. The test reports also contain the results of the definitive and chronic tests and any modifications to, or deviations from, the procedures and conditions recommended in the test methods. The data for the rangefinding tests were provided to EBA and EnCana prior to commencing the chronic and definitive tests.

2.1.2 Statistical Analyses

Test organism performance in the tebuthiuron-amended or contaminated treatments was assessed relative to test organism performance in the CSS treatment so that point estimates (e.g., ICp, LCx, ECx) of tebuthiuron toxicity to plants and invertebrates could be generated. Point estimates of toxicity for plant growth and invertebrate reproduction (e.g., ICp) were determined using linear regression, non-linear regression, or linear interpolation. Point estimates of toxicity for invertebrate chronic survival (e.g., LCx) and plant emergence (e.g., ECx) were determined using the Spearman-Kärber method. All statistical analyses were conducted according to guidance provided by the Environment Canada test methods (EC, 2004, 2005a and 2007) as well as according to the recommendations provided in the Environment Canada guidance document on statistical methods for environmental toxicity tests (EC, 2005b).

Plant emergence and invertebrate survival metrics are quantal data. Therefore, the most appropriate statistical procedure to apply to these data sets is probit regression (EC, 2005b). However, none of the quantal data sets from this ecotoxicity assessment were amenable to analyses by probit regression and the next suitable method (the Spearman-Kärber method) was used instead (EC, 2005b).

Plant growth and invertebrate reproduction metrics are quantitative and as a result linear, non-linear regression, and linear interpolation were the most appropriate procedures to use to generate ICp data (EC, 2005b). The Environment Canada methods and guidance provides detailed direction on the selection and use of four non-linear models and one linear model for regression analyses. The analyses consisted of using a linear (i.e., $y = ((-m*0.5)/x)c+b$) or four nonlinear regression models (i.e., logistic $y = y^{o}/(1+(c/x)^{b})$; gompertz $y = y^{o}*\exp((\log(0.5))*(c/x)^{b})$; exponential $y = y^{o}*\exp(\log((y^{o}-y^{o}*0.5-b*0.5)/a)*(c/x))+b$; and logistic with hormesis $y = (y^{o}*(1+h*c))/(1+((0.5+h*c)/0.5)*(c/x)^{b})$; where c = tebuthiuron concentration) that had been re-parameterized to include the ICp and the associated 95% confidence limits. The ICp is the inhibiting concentration (IC) resulting in a specified percentage (p) effect. The guidance provided by the Environment Canada methods for selecting the most appropriate model (linear or non-linear) was followed (EC, 2004, 2005a, 2005b, 2007). Model selection was primarily based on the mean square error of the residual (the model with the lowest mean square error was usually selected). However, other factors were considered when selecting a model and included:

- the fit of the regression line (did the line seem to reasonably fit the data points?)
- magnitude of the 95% confidence intervals (were they excessively large or small?)
- the point estimates themselves (did they make sense?)
- the simplicity of the model (since models with fewer parameters often have greater predictive power than models with more parameters)

- was the assumption of a normal distribution of the residuals met? (if not, then regression procedures cannot be applied)
- was the assumption of homogeneity of variance met? (if not, then weighting the data was an option)

The assumptions that the data were normally distributed and that the variance was homogenous were tested in the analysis of every quantitative endpoint using the Shapiro-Wilks test for normality and the Levene's test for homogeneity (EC, 2005b). If the assumption of normality was not met for any of the quantitative endpoints, then linear or non-linear regression was not used. Instead, linear interpolation was applied to the data to generate IC50 and IC25 estimates, according to guidance provided by Environment Canada (EC, 2004, 2005a, 2005b, 2007).

If the assumption of homogeneity of the variance was not met, but that for normality was, then data were weighted with the inverse of the variance of each treatment. The weighted regression was then compared to the unweighted regression analysis. The weighted regression was chosen if weighting reduced the standard error for the ICp by 10% relative to the unweighted regression analysis (EC, 2004, 2005a, 2005b, 2007). If weighting did not reduce the standard error of the ICp by 10% then linear interpolation was applied to the data to generate IC50 and IC25 estimates, according to guidance provided by Environment Canada (EC, 2004, 2005a, 2005b, 2007).

Linear and non-linear regression analyses were performed with SYSTAT 12 (SSI, 2007). Linear interpolation was performed using the United States Environmental Protection Agency (US EPA) Linear Interpolation Program ICPIN Version 2.0. Trimmed Spearman-Kärber analyses were conducted using the US EPA program http://www.epa.gov/nerleerd/stat2.htm and Spearman-Kärber (Stephan, 1977).

2.2 RESULTS

2.2.1 Earthworm

Detailed descriptions of the experimental design, conditions, and test results are provided in the test report for the earthworm *Eisenia andrei* in Appendix B.

A concentration-dependent effect on earthworm survival and reproduction was observed following exposure to the field-collected contaminated site soil (SS) amended with tebuthiuron (Table B.2, Figure B.1; Appendix B). Earthworm survival and reproduction in the control topsoil reference soil (CTS) was excellent (100% survival and 34 progeny per 2 adults) (Table B.2; Appendix B) indicating that this soil is suitable habitat for this species. Survival in the CSS was good (95%) and reproduction was acceptable (6 per 2 adults), though greatly decreased compared to the CTS indicating that soil characteristics strongly influenced earthworm reproduction (Table B.2; Figure B.1; Appendix B). However, the pH, electrical conductivity and moisture content of all the soil treatments were well within the range tolerated by this species (Table B.4; Appendix B). The soil characteristics of the CSS and SS are reasonably well matched (Tables B.4 and B.5; Appendix B and Tables 1 and 2; Appendix H) and therefore, it is likely that any reduction observed in the site soil treatments can be reasonably attributed to the presence of tebuthiuron.

The LC50, IC50 and IC25 estimates for earthworm survival and reproduction are listed in Table B.3; Appendix B). Survival was predictably the least sensitive endpoint (LC50 = 759 mg/kg) and the number of progeny the most sensitive endpoint (IC50 = 81 mg/kg). Linear interpolation was applied to all reproduction metrics as the data were not amenable to regression analyses. Progeny wet and dry mass metrics did not display a monotonic response to tebuthiuron concentrations (e.g., mass did not decrease in sequence with increasing tebuthiuron concentration). The reason for this is unclear but might be an artifact of the variability of the data from treatments with low tebuthiuron concentrations (Figure B.1; Appendix B).

All performance criteria for test acceptability were met for the artificial soil treatment (EC 2004), indicating that the test procedures, conditions, organism health and technical proficiency were acceptable (Table B.1; Appendix B). Reference toxicity QA/QC data were also within the historical warning limits (Appendix B).

2.2.2 Collembola

Detailed descriptions of the experimental design, conditions, and test results are provided in the test report for *Folsomia candida* in Appendix C.

Though not monotonic, a concentration-dependent effect on collembola survival and reproduction was observed following exposure to SS amended with tebuthiuron (Table C.2, Figure C.1; Appendix C). Collembola survival and reproduction in the CTS was excellent (90% survival and 869 progeny per 10 adults) (Table C.2) indicating that this soil is suitable habitat for this species. Survival in the CSS was also good (72%) and reproduction was excellent (1500 per 10 adults) indicating that the CSS was also a suitable habitat for this species (Table C.2; Figure C.1; Appendix C). As mentioned in Subsection 2.2.1, the soil characteristics of the CSS and SS are reasonably well matched (Tables C.4 and C.5; Appendix C and Tables 1 and 2; Appendix H); therefore, it is likely that any significant reduction observed in the site soil treatments can be attributed to the presence of tebuthiuron. The pH, electrical conductivity and moisture content of all the soil treatments were well within the range tolerated by this species (Table C.4; Appendix C). Collembola reproduction in the SS treatment was lower than in the CSS treatment (Table C.2, Figure C.1; Appendix C) but was higher in treatments with greater tebuthiuron concentrations up to 500 mg/kg. The reason for the reduced reproduction in the SS treatment is unclear and an examination of the soil properties and experimental conditions did not provide any explanation. Survival was somewhat reduced in the SS treatment and the 63 mg/kg treatment, and that might have led to slightly reduced reproduction in the SS treatment. It was concluded that the slight decrease in reproduction and survival of *F. candida* in the SS treatment could not be explained; however, what is clear is that there was a concentration-dependent decrease in collembola survival and reproduction with increasing tebuthiuron starting at concentrations 4 to 5 orders of magnitude greater than tebuthiuron concentrations in the SS (Table C.2, Figure C.1; Appendix C).

The LC50, IC50 and IC25 estimates for collembola survival and reproduction are provided in Table C.3; Appendix C). Survival was the less sensitive endpoint (LC50 = 1236 mg/kg) and the number of progeny the more sensitive endpoint (IC50 = 254 mg/kg). Linear interpolation was applied to all reproduction metrics as the data were not amenable to regression analyses. The number of progeny produced did not display a monotonic response to tebuthiuron concentrations (e.g., mass did not decrease in sequence with increasing tebuthiuron concentration). The reason for this is unclear but might be an artifact of the variability of the data from treatments with low tebuthiuron concentrations (Figure C.1; Appendix C).

All performance criteria for test acceptability were met for the artificial soil treatment (EC 2007), indicating that the test procedures, conditions, organism health and technical proficiency were acceptable (Table C.1; Appendix C). Reference toxicity QA/QC data were also within the historical warning limits (Appendix C).

2.2.3 Blue gramma grass

Detailed descriptions of the experimental design, conditions, and test results are provided in the test report for blue gramma grass in Appendix D.

A concentration-dependent effect on blue gramma grass emergence was not observed following exposure to the field-collected reference soils and field-collected contaminated site soils amended with tebuthiuron (Tables D.2, D.3 and D.4, Figures D.1 and D.2; Appendix D). A concentration-dependent effect was observed; however, for seedling growth metrics (Tables D.3 and D.4, Figures D.1 and D.2; Appendix D). Seedling growth in the CTS and CSS reference control soils was reduced relative to that in the AS, but was still acceptable. The pH, electrical conductivity and moisture content of all the soil treatments were well within the range tolerated by this species (Table D.5; Appendix D). As with the results from the collembola and earthworm tests, the soil characteristics of the CSS and SS are reasonably well matched (Tables D.5 and D.6; Appendix D and Tables 1 and 2; Appendix H); therefore, it is likely that any significant reduction observed in the site soil treatments can be attributed to the presence of tebuthiuron.

The IC50 and IC25 estimates for seedling growth are provided in Table D.4 and Figure D.2; Appendix D). Shoot length was the least sensitive growth endpoint (IC50 = 13 mg/kg) and root dry mass the most sensitive growth endpoint (IC50 = 0.5 mg/kg). Linear interpolation was applied to root dry mass metrics as the data were not amenable to regression analyses.

Four of the five validity criteria were met for this test (Table D.1; Appendix D) (EC, 2005a). The four criteria that were met were percent seedling emergence, percent survival of emerged seedlings, percent of emerged control seedlings exhibiting phytotoxicity or developmental anomalies and seedling shoot length. Seedlings that emerged in the negative control soil were uniformly healthy and vigourous; however, they did not quite meet the validity criteria for root length. However, in performance tests with the same batch of seeds, the validity criterion for root length was easily met in all tests conducted. Seedling emergence was excellent and plants appeared vigourous and healthy with no signs of stress and it is unclear why the root length validity criterion was not met in this test. We reviewed the test procedures and conditions and concluded that healthy blue gramma grass seed stock was used and that the experimental conditions were acceptable, including the slightly lower than recommended nightly temperature. Reference toxicity QA/QC data were within the historical warning limits (Appendix D).

2.2.4 Durum wheat

Detailed descriptions of the experimental design, conditions, and test results are provided in the test report for durum wheat in Appendix E.

A concentration-dependent effect on durum wheat emergence was not observed following exposure to the field-collected reference soils and field-collected contaminated site soils amended with tebuthiuron (Tables E.2., E.3. and E.4., Figures E.1 and E.2; Appendix E). A concentration-dependent effect was observed; however, for seedling growth metrics (Tables E.3 and E.4, Figures E.1 and E.2.; Appendix E). Seedling growth in both the CTS and CSS reference control soils was acceptable (Table E. 3; Appendix E). The pH, electrical conductivity and moisture content of all the soil treatments were well within the range tolerated by this species (Table E.5; Appendix E). As described in Subsection 2.2.1 to 2.2.3, the soil characteristics of the CSS and SS are reasonably well matched (Tables E.5 and E.6; Appendix E and Tables 1 and 2; Appendix H); therefore, it is likely that any reduction observed in the site soil treatments can be reasonably attributed to the presence of tebuthiuron.

The IC50 and IC25 estimates for seedling growth are provided in Table E.4. and Figure E.2.; Appendix E). Shoot length was the least sensitive growth endpoint (IC50 = 1479 mg/kg) and shoot dry mass the most sensitive growth endpoint (IC50 = 0.75 mg/kg).

All performance criteria for test acceptability were met for the artificial soil treatment (EC 2005a), indicating that the test procedures, conditions, organism health and technical proficiency were acceptable (Table E.1; Appendix E). Reference toxicity QA/QC data were also within the historical warning limits (Appendix E).

2.2.5 Western wheatgrass

Detailed descriptions of the experimental design, conditions, and test results are provided in the test report for western wheatgrass in Appendix F.

A concentration-dependent effect on western wheatgrass was not observed, despite the fact that an EC50 estimate was determined for this species using the Spearman-Kärber method (Tables F.2, F.3 and F.4, Figures F.1 and F.2; Appendix F). The EC50 value was only generated after almost 50% of the points were trimmed from the data set (e.g., trim = 45%). The reason for this is evident in Figure F.1 (Appendix F) where emergence in the treatment with the highest concentration (3000 mg/kg) was greater than emergence in treatments at concentrations between 0.3 and 300 mg/kg.

A concentration-dependent effect was observed for seedling growth metrics (Tables F.3 and F.4, Figures F.1 and F.2.; Appendix F). Seedling growth in both the CTS and CSS reference control soils was acceptable (Table F. 3; Appendix F). The pH, electrical conductivity and moisture content of all the soil treatments were well within the range tolerated by this species

(Table F.5; Appendix F). As described in Subsection 2.2.1 to 2.2.4, the soil characteristics of the CSS and SS are reasonably well matched (Tables F.5 and F.6; Appendix F and Tables 1 and 2; Appendix H); therefore, it is likely that any reduction observed in the site soil treatments can be reasonably attributed to the presence of tebuthiuron.

The IC50 and IC25 estimates for seedling growth are provided in Table F.4. and Figure F.2.; Appendix F). Root length was the least sensitive growth endpoint (IC50 = 13 mg/kg) and shoot dry mass the most sensitive growth endpoint (IC50 = 0.12 mg/kg).

Western wheatgrass is not one of the 12 species specified for use in the Environment Canada method; therefore, test acceptability criteria do not exist. However, performance of seedlings in the negative control treatment (AS) was comparable to the mean performance in AS in performance tests conducted with this species (Table F.1; Appendix F). Reference toxicity QA/QC data were also within the historical warning limits (Appendix F).

2.2.6 Silver sagebrush

Detailed descriptions of the experimental design, conditions, and test results are provided in the test report for silver sagebrush in Appendix G.

A concentration-dependent effect on silver sagebrush emergence was observed following exposure to the field-collected reference soils and field-collected contaminated site soils amended with tebuthiuron (Tables G.2, G.3 and G.4, Figures G.1 and G.2; Appendix G). As the concentration of tebuthiuron in soil increased, the mean percent emergence for silver sagebrush decreased, with no seedlings emerging in the 300 and 3000 mg/kg soil dry wt. treatments. An EC50 estimate of 0.385 mg/kg soil dry wt. was determined for this species using the Spearman-Kärber method, with 0.625 % trim (Tables G.2, G.3 and G.4, Figures G.1 and G.2; Appendix G). A concentration-dependent effect was also observed for seedling growth metrics (Tables G.3 and G.4, Figures G.1 and G.2; Appendix G). The soil characteristics of the CSS and SS are reasonably well matched (Tables G.5 and G.6; Appendix G and Tables 1 and 2; Appendix H). Therefore, it is likely that any significant reduction of growth metrics observed in the site soil treatments can be attributed to the presence of tebuthiuron. In general, as the concentration of tebuthiuron increased, the growth of the silver sagebrush seedlings decreased. The pH, electrical conductivity and moisture content of all the soil treatments were well within the range tolerated by this species (Table G.5; Appendix G).

The IC50 and IC25 estimates for seedling growth are provided in Table G.4 and Figure G.2; Appendix G). Shoot length was the least sensitive growth endpoint (IC50 = 120.504 mg/kg) and root length the most sensitive growth endpoint (IC50 = 0.018 mg/kg). Linear interpolation was applied to root length metrics as the data were not amenable to regression analyses.

Silver sagebrush is not one of the 12 species specified for use in the Environment Canada method; therefore, test acceptability criteria do not exist. However, performance of seedlings in the negative control treatment (AS) was compared to the mean performance in AS in the performance test conducted with this species (Table G.1; Appendix G). Four of the five validity criteria were met for this test (Table G.1; Appendix G). The results for percent survival of emerged seedlings, percent of emerged control seedlings exhibiting phytotoxicity or developmental anomalies, percent seeding emergence and mean shoot length were deemed acceptable. The result for mean root length was below the result from the performance test. While the seedlings did not meet the mean root length criteria achieved in the performance test, the seedlings that emerged in the negative control treatment (AS) were healthy. Test procedures and conditions were reviewed and it was concluded that healthy silver sagebrush seed stock was used and that the experimental conditions were acceptable, except for one temporary temperature deviation (one minimum temperature was recorded as 7.3 °C, which is outside of the acceptable night time temperature range of 15 ± 3 °C); no adverse effects on the silver sagebrush test were noted. The performance test results for this species were based on a single performance test and the differences in mean root length could represent the natural variability of this species.

A reference toxicity test was performed with the silver sagebrush species (Appendix G), to determine the response of this species to an acceptable reference toxicant. All emerged seedlings in the negative control soil were healthy with no phytotoxicity symptoms. Results could not be compared to historical warning limits as this was the first reference toxicant test performed with this species.

3.0 Summary and Discussion

3.1 QUANTIFICATION OF TEBUTHIURON CONCENTRATIONS IN TEST SOILS

Concentrations of tebuthiuron were analyzed in the historically contaminated site soil (SS), the CSS amended with tebuthiuron, and the SS amended with tebuthiuron at the beginning and end of the collembola, durum wheat and silver sagebrush tests (Tables 2, 3 and 4 and Table 2; Appendix I) to quantify the initial tebuthiuron exposure concentrations in the soils, to determine the relationship between nominal and measured tebuthiuron concentrations and to quantify the loss of tebuthiuron over time. In addition, subsamples were taken from samples prepared for the collembola test but analyzed on Day 63 (duration of the earthworm test).

In general there was good agreement between the nominal and measured concentrations of tebuthiuron among all concentrations and soil types and percent of nominal ranged between 51 to 142 % with the majority greater than 80% (Tables 2 to 4). There were two exceptions. The measured value for the Day 14 0.0003 mg/kg sample from the durum wheat test was an order of magnitude greater than the nominal value (1010 % of nominal) (Table 3). Although procedures and reporting in both the Stantec and Access Laboratories were carefully scrutinized, the cause of this disparity could not be determined, especially as the measured and nominal values for Day 0 were very similar. The measured value of the Day 28 0.0003 mg/kg sample from the silver sagebrush was 533 % of nominal (Table 4).

In most cases, triplicate samples were collected from a batch of soil representing a tebuthiuron treatment. The variability within a soil batch (and therefore among treatment test units) was low and percent coefficients of variability (%CV) ranged from 1 to 19% (Tables 2 and 4).

Tebuthiuron concentrations were stable over time, and minimal loss was noted between the beginning and end of the 14-, 28-, and 63-d tests (Tables 2 to 4).

3.2 TOXICITY TEST RESULTS

It was evident from the results of the toxicity tests with the four plant and two invertebrate species that plant species were more sensitive to the soil sterilant than were invertebrate species. This was expected as tebuthiuron is a non-selective phenylurea herbicide that inhibits photosynthesis (Tomlin, 1997).

The LC50, IC50 and IC25 point toxicity estimates of tebuthiuron, and their 95% confidence limits, for both earthworm and collembola were at least two orders of magnitude above the concentration of tebuthiuron in the site soil (0.03 mg/kg (Table 2; Appendix I)) (Tables B.3 and C.3; Appendices B and C).

Among the plants, many of the IC50 estimates and their 95% confidence limits for the endpoints were at least one order of magnitude greater than the concentration of tebuthiuron in the site soil. The exceptions were silver sagebrush (three endpoints) and western wheatgrass (one endpoint), where endpoints were at or below the concentration of tebuthiuron in the site soil. However, the IC25 estimates and/or their 95% confidence limits for western wheatgrass shoot length, root length, shoot dry mass and root dry mass were similar to or less than the concentration of tebuthiuron in the site soil. The same was true for blue gramma grass shoot and root dry mass, durum wheat shoot dry mass, and silver sagebrush root length and root dry mass (Tables D.4, E.4, F.4 and G.4; Appendices D, E, F and G).

Based on the toxicity test results, *Folsomia candida*, *Eisenia andrei* and durum wheat were the least sensitive species to tebuthiuron in the site soils. Of the other three species, western wheatgrass was the most sensitive.

The ecotoxicity assessment conducted generated four types of point estimates of toxicity (LC50, EC50, IC50 and IC25) for six different species with a total of 26 different measurement endpoints. Following statistical analyses, a total of 24 IC50/EC50/LC50 and 19 IC25 point estimates of toxicity are available for the generation of a species-sensitivity distribution based on data from site-specific species exposed in site-specific soils that can be used for the derivation of a Tier 2 benchmark for tebuthiuron.

STANTEC CONSULTING LTD

Kelly Olaveson, Laboratory Manager

Gladys Stephenson, Project Director

4.0 References

Environment Canada (EC). 2004. Biological Test Method: Tests for Toxicity of Contaminated Soil to Earthworms (*Eisenia andrei*, *Eisenia fetida*, or *Lumbricus terrestris*). Report EPS 1/RM/43, June 2004. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Environment Canada (EC). 2005a. Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Report EPS 1/RM/45, February 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Environment Canada (EC). 2005b. Guidance Document on Statistical Methods for Environmental Toxicity Tests. Report EPS 1/RM/46, March 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Environment Canada (EC). 2007. Biological Test Method: Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil. Report EPS 1/RM/47 September 2007. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Hendershot W.H., H. Lalonde, and M. Duquette. 1993. Soil reaction and exchangeable acidity. P 141-145 in: Soil Sampling and Methods of Analysis, M.R. Carter, ed., Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida.

McKeague, J.A. ed. 1978. Manual on Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Ottawa, Ontario.

Microsoft Excel. 2002. Version 10.6501.6735 SP3. Copyright 1985-2001 Microsoft Corporation.

Soil Analysis Handbook. 1992. Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc., Georgia University Station, Athens, Georgia, 202 p.

Stephan, C.E. 1977. Methods for Calculating an LC50, p 65-84, In: Aquatic Toxicology and Hazard Evaluation, F.L. Mayer and J.L. Hamelink (eds). ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA.

Systat Software Inc. (SSI). 2007. SYSTAT© 12 for Windows. Version 12.00.08. Systat Software Inc., USA.

Tomlin, CDS. 1997. The Pesticide Manual: A World Compendium, 11th Edition. British Crop Protection Council, Surrey, UK, pp. 1606.

Appendix A

Test Design, Procedures and Conditions

Table A.1. Experimental design and conditions of definitive plant and chronic invertebrate toxicity tests.

Test	Plant	Earthworm	Collembola
Test type	Definitive Screening	Chronic Screening	Chronic Screening
Test duration (d)	14, 21 or 28	63 (35-d adult survival)	28
Test unit (chamber)	1-L polypropylene container	Glass 500-mL mason jar	Glass 125-mL mason jar
Amount of soil	425 (silver sagebrush) or 500 g wet wt.	270 g wet wt.	30 g wet wt.
Temperature (day/night)	24/15 ± 3°C	20 ± 2°C	20 ± 2°C
Photoperiod (h)	16 light : 8 dark	16 light : 8 dark	16 light : 8 dark
Treatments	Artificial soil (AS) Control Top Soil (CTS) Control Subsoil (CSS) Site soil (SS) (0.03 mg/kg) Tebuthiuron-amended CSS diluted with clean CCS to 0.00003, 0.0003 and 0.003 mg/kg Tebuthiuron-amended SS to 0.3, 3, 30, 300 and 3000 mg/kg	Artificial soil (AS) Control Top Soil (CTS) Control Subsoil (CSS) Site soil (SS) (0.03 mg/kg) Tebuthiuron-amended SS to 31, 63, 125, 250, 500, 1000, 2000, and 4000 mg/kg	Artificial soil (AS) Control Top Soil (CTS) Control Subsoil (CSS) Site soil (SS) (0.03 mg/kg) Tebuthiuron-amended SS to 31, 63, 125, 250, 500, 1000, 2000, and 4000 mg/kg
Number of replicate test units per treatment	6 (AS, CTS, CSS) 4 (0.00003 to 3 mg/kg) 3 (30 to 3000 mg/kg)	10	5 (AS, CTS, CSS) 3 (0.03 to 4000 mg/kg)
Number of organisms per test unit	5 (durum wheat) 10 (silver sagebrush, blue gramma grass) 15 (western wheatgrass)	2	10
Lighting (Type & Intensity)	Full spectrum Durotest or Vita Lights 200-400 μmoles/($m^2 \cdot s$)	Fluorescent 400-800 Lux	Fluorescent 400-800 Lux
Physicochemical measurements	Conductivity, pH, % moisture	Conductivity, pH, % moisture	Conductivity, pH, % moisture
Biological endpoint measurements	Emergence, shoot and root length and shoot and root dry mass	Adult survival, no. progeny produced, progeny wet and dry mass	Adult survival, no. progeny produced
Statistical endpoints	EC50 (emergence) IC50, IC25 (shoot and root length and dry mass)	LC50 (adult survival) IC50, IC25 (no. progeny, progeny wet and dry mass)	LC50 (adult survival) IC50, IC25 (no. progeny)
Description of methods	EC 2005	EC 2004	EC 2007

A.1. FORMULATION OF ARTIFICIAL SOIL

The artificial control soil (AS) was formulated in the laboratory by mixing the ingredients in their dry form, then gradually hydrating with de-ionized water, and mixing further until the soil was visibly uniform in colour and texture. The ingredients of AS were 70% silica sand (No. 200, Barco 71; Optima Minerals, Waterdown, ON), 20% kaolinite clay (Tuckers Pottery Supplies, Richmond Hill, ON), 10% *Sphagnum* spp. peat (Canadian HydroGardens Ltd., Ancaster, ON), and calcium carbonate (10-30 g per 1 kg peat). A 12 kg batch of AS was formulated on a dry weight basis by adding 7 kg of sand, 2 kg of kaolinite clay, 1 kg of ground (approximately 2 mm) peat, approximately 30 g of $CaCO_3$ (sieved), and 2 L of de-ionized water. The amount of calcium carbonate required to adjust the soil pH to 6.0-7.5, depended on the nature (i.e., acidity) of the *Sphagnum* peat and the silica sand. Each time a new batch of either of these ingredients was used, it was necessary to adjust the amount of $CaCO_3$ used in each batch of formulated soil.

A.2. DETERMINATION OF SOIL MOISTURE CONTENT

Prior to the day of test soil formulation, a 3 to 5 g sample of control soil wet weight (wet wt.) was placed into a pre-weighed aluminum weigh boat (1 or 2.5 g) and the wet mass recorded. Each weigh boat was then placed into a drying oven at 105°C for a minimum of 24 hours. The dry weight of each soil was then determined. Percent moisture content was calculated by expressing the dry mass as a percentage of the wet mass:

$$\text{Percent Moisture} = \frac{\text{wet mass (g wet wt.)} - \text{dry mass (g dry wt.)}}{\text{wet mass (g wet wt.)}} \times 100$$

The initial moisture content of the soils was needed in order to standardize the moisture content in the test soils.

A.3. DETERMINATION OF WATER-HOLDING CAPACITY

The water-holding capacity of a soil was determined by placing ~130 g wet weight of soil sample into a large aluminum container and drying the sample at 105°C to a constant weight. Subsequent to drying, the sample was removed from the oven and cooled in a desiccator for at least 20 minutes. 100 g of the dried soil sample were placed into a 250-mL glass beaker and 100 mL of de-ionized water were added to the sample and mixed thoroughly with a stainless steel spoon to ensure that the sample was wetted and that a slurry of soil and water existed. A circle of filter paper was folded into quarters and placed into a glass funnel; the folded filter paper was level with the top of the funnel. 7 mL of de-ionized water were slowly added, using a pipette, to the filter paper to wet the entire surface. The combined weight of the funnel and hydrated filter paper was measured. The weight of the dried soil, funnel and hydrated filter paper was recorded as the initial weight. The funnel was placed into an Erlenmeyer flask and the slurry of soil and water was slowly poured onto the hydrated filter paper held in the funnel. Any soil remaining on the beaker and stir rod was rinsed into the funnel with minimal amounts of de-ionized water to ensure that all of the solid material had been washed onto the filter. The funnel was covered tightly with aluminum foil and allowed to drain for 3 hours at room temperature. After the 3-hours, the funnel, hydrated filter paper, and soil were weighed and recorded as the final weight. The water-holding capacity, expressed as mL water/100 g soil, was equal to the difference between the final and initial weights of the funnel, filter, and sample.

A.4. MEASURING SOIL PH AND CONDUCTIVITY (WATER SLURRY) (MODIFIED FROM THE SOIL ANALYSIS HANDBOOK, 1992).

Approximately 25 g (wet wt.) of test soil and 50 mL of de-ionized water were placed into a glass beaker and stirred with a glass rod for two minutes. The beakers sat at room temperature in the laboratory for a minimum of 20 minutes. Immediately prior to measuring pH and conductivity, the soil slurry was mixed again. Soil pH was measured with a pH and ATC probe submersed in the soil slurry that was gently agitated until the readings were constant. Conductivity was measured with a conductivity and ATC probe submersed in the freshly mixed slurry, and was recorded once the readings were constant. The slurry was not agitated while conductivity measurements were taken. The soil pH and conductivity were measured using an Accumet® Meter (Fisher Scientific Model 20) that had been calibrated before use with three (pH 4, 7 and 10) external buffers and an external conductivity standard. The probes were washed between samples.

A.5. NUTRIENT SOLUTION PREPARATION (FOR PLANTS GROWN IN ARTIFICIAL SOIL)

Artificial soil is low in the nutrients required by some plants for definitive seedling growth. For testing purposes, plant test units containing artificial soil are formulated and irrigated with a dilute nutrient solution. The nutrient solution used is a 20-8-20 (N:P:K) formulation (Plant Products Company Ltd., 314 Orenda Road, Brampton, ON L6T 1G1) recommended by the Department of Plant Agriculture, University of Guelph. At the Stantec Laboratory, a nutrient solution is made out of a powdered formulation to a concentration of 1 g/L. When preparing artificial soil for testing on Day 0 the soil is hydrated to a standard moisture content with nutrient solution at 1 g/L. A half-strength nutrient solution is used (0.5 g/L) to irrigate plant test units containing artificial soil for the duration of the test, as necessary.

Appendix B

Test Conditions, Experimental Design, Data Summaries, and Results of the Earthworm Chronic Reproduction Test

Stantec Consulting Ltd.
361 Southgate Drive
Guelph, ON N1G 3M5
Tel: (519) 836-6050 Fax: (519) 836-2493
stantec.com

Earthwork Test Report

Survival, Reproduction and Growth

Multi-concentration chemical-amended soil

Sample Identification

Client:	EnCana
Sample(s) description:	Reference soil, site soil contaminated with weathered tebuthiuron, and site and reference soil amended with formulated tebuthiuron
Sample(s) identification:	AS 2007-10-1, 0757-1-CTS, 0756-1-CSS, 0755-1-SS
Date collected/formulated:	2007-08-02
Method of soil collection:	Composite samples
Date sample(s) received:	2007-08-16
Time sample(s) received:	10:00 am
Temperature on arrival:	22-23^{o}C
Soil storage temperature:	20.9 ± 0.2 ^{o}C
Date sample(s) tested:	November 9, 2007 – January 11, 2008
Technicians:	Kelly Olaveson, Carolyn Brown
Analyst:	Natalie Feisthauer
QA/QC:	Gladys Stephenson

Test Organism

Test Organism:	*Eisenia andrei*
Organism Source:	In house culture Ea 07-1, 07-3, 07-4, 07-5, 07-6, 07-7, 07-8, 07-13, 07-14
Initial mean adult wet weight (g):	0.487 ± 0.081

Test Conditions and Procedures

Test type:	Static, chronic
Test duration:	63 days
Number of treatments:	12, including 1 negative control (AS) and 2 experimental controls (CTS and CSS)
Temperature:	20.2 ± 0.3 ^{o}C
Light intensity:	426 ± 143 lux
Photoperiod:	16 h light; 8 h dark
Watering regime:	De-ionized water, misted every 14 days, as required
Feeding regime:	Cooked oatmeal, fed at test initiation and every 14 days, as required
Test unit description:	500-mL glass wide-mouthed mason jar
Soil volume/test unit:	270 mL (3/4 of volume of test unit)
No. organisms per test unit:	2
No. field samples/treatment:	1
No. replicate test units/treatment:	10 replicates
Measured soil chemistry parameters:	Initial and final soil pH, electrical conductivity, and percent moisture content
Measured endpoint(s):	Day 35 adult survival, number of progeny produced at Day 63, and wet and dry mass of individual progeny at Day 63
Test Protocol:	Biological Test Method: Tests for Toxicity of Contaminated Soil to Earthworms (*Eisenia andrei*, *Eisenia fetida*, or *Lumbricus terrestris*). Report EPS 1/RM/43, June 2004. Method Development

and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Statistical Analyses: Mean, SD – Microsoft Excel (2002)
Survival LC50 - Trimmed Spearman-Kärber U.S. EPA program (http://www.epa.gov/nerleerd/stat2.htm) and Spearman-Kärber (Stephan, 1977)
Progeny number and growth – Linear interpolation (ICPIN, U.S. EPA ICPIN program Version 2.0)
Nominal ☒ measured ☐ concentrations analysed

Test acceptability criteria met? See Table B.1

Table B.1. Performance of earthworms in negative control soil treatment relative to test method validity criteria.

Criterion in Negative Control Soil	Negative Control Soil	Criteria Met?	Positive Control Soil	Solvent Control Soil
28- or 35-d adult survival ≥ 90%	100%	Yes	N/A	N/A
Mean # live progeny/adult ≥ 3	15	Yes	N/A	N/A
Mean dry wt of individual progeny ≥ 2.0 mg	6	Yes	N/A	N/A

Boric Acid Reference Toxicant Data for Artificial Soil

Type of Test: Acute lethality
Test Duration 7 days
Date Tested: 2007-11-13
Organism Lab Code: Laboratory Code No.
Ea 07-1, 07-3, 07-4, 07-5, 07-6, 07-7, 07-8, 07-13, 07-14

LC50 Survival: 5768 mg/kg
95% CL: 5297 to 6281 mg/kg
Statistical Analyses: Spearman-Kärber (Stephan, 1977)
Historical Mean LC50: 4347 mg/kg
Warning Limits (± 2 SD): 2682 to 6175 mg/kg
Technician(s): Kelly Olaveson and Natalie Feisthauer
Analyst(s): Natalie Feisthauer

Results

Table B.2. Effect of exposure to artificial soil (AS), reference topsoil (CTS), reference subsoil (CSS), site subsoil contaminated with 0.03 mg/kg tebuthiuron (SS) and SS-amended soils on earthworm (*E. andrei*) survival (Day 35), growth (Day 63), and reproduction (Day 63). Results are reported as treatment means and the values in brackets indicate one standard deviation of the mean.

Soil Treatment	Mean Percent 35-d Adult Survival (n = 2 adults)	Mean Number of Progeny	Mean Individual Wet Mass of Progeny (mg)	Mean Individual Dry Mass of Progeny (mg)
AS	100 (0)	30.4 (12.7)	29.29 (9.92)	5.82 (2.19)
CTS	100 (0)	34.0 (16.2)	41.75 (17.78)	9.45 (4.43)
CSS (0 mg/kg)	95 (16)	5.7 (5.0)	36.86 (24.38)	8.58 (6.42)
SS (0.03 mg/kg)	100 (0)	5.0 (5.7)	79.76 (58.22)	13.89 (9.26)
31 mg/kg*	100 (0)	3.4 (3.6)	46.75 (55.39)	8.73 (10.95)
63 mg/kg *	100 (0)	4.4 (6.2)	35.07 (18.31)	6.61 (4.27)
125 mg/kg *	100 (0)	1.1 (2.8)	35.25 (5.02)	5.59 (0.58)
250 mg/kg *	95 (16)	0.4 (1.0)	8.48 (10.16)	1.63 (1.89)
500 mg/kg *	100 (0)	0.0 (0.0)	-	-
1000 mg/kg *	15 (34)	0.0 (0.0)	-	-
2000 mg/kg *	0 (0)	0.0 (0.0)	-	-
4000 mg/kg *	0 (0)	0.0 (0.0)	-	-

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.
- No data for these endpoints as there were no progeny produced in this treatment

Table B.3. Effect of tebuthiuron-contaminated site soil (0.03 mg/kg) further amended with fresh tebuthiuron on earthworm (*E. andrei*) adult survival (Day 35), growth (Day 63), and reproduction (Day 63) expressed as nominal concentrations that inhibit survival, by 50% (LC50), and reproduction, by 25 and 50% (i.e., IC50s and IC25s), of that of the control treatment, respectively, along with their upper and lower confidence limits (UCL and LCL, respectively).

Parameter	Model	L/IC50 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	IC25 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	T(%) W?
Adult 35-d Survival	Spearman-Kärber	758.6	676.1	851.1	NA	NA	NA	0
Number of Progeny	Linear interpolation	81.4	1.1	116.9	9.8	1.4	84.3	NA
Wet Mass of Individual Progeny	Linear interpolation	148.7	16.5	190.1	39.7	3.0	170.6	NA
Dry Mass of Individual Progeny	Linear interpolation	126.4	15.6	157.1	35.9	1.1	112.2	NA

LCL lower confidence limit
UCL upper confidence limit
T indicates if emergence data have been trimmed and to what percent
W? indicates if data has been weighted (only applicable if non-linear or linear regression procedures have been applied to the data)
NA not applicable

The results reported relate only to the sample(s) tested

Date: 2008-07-23 Approved by: Gladys L. Stephenson
Laboratory Director

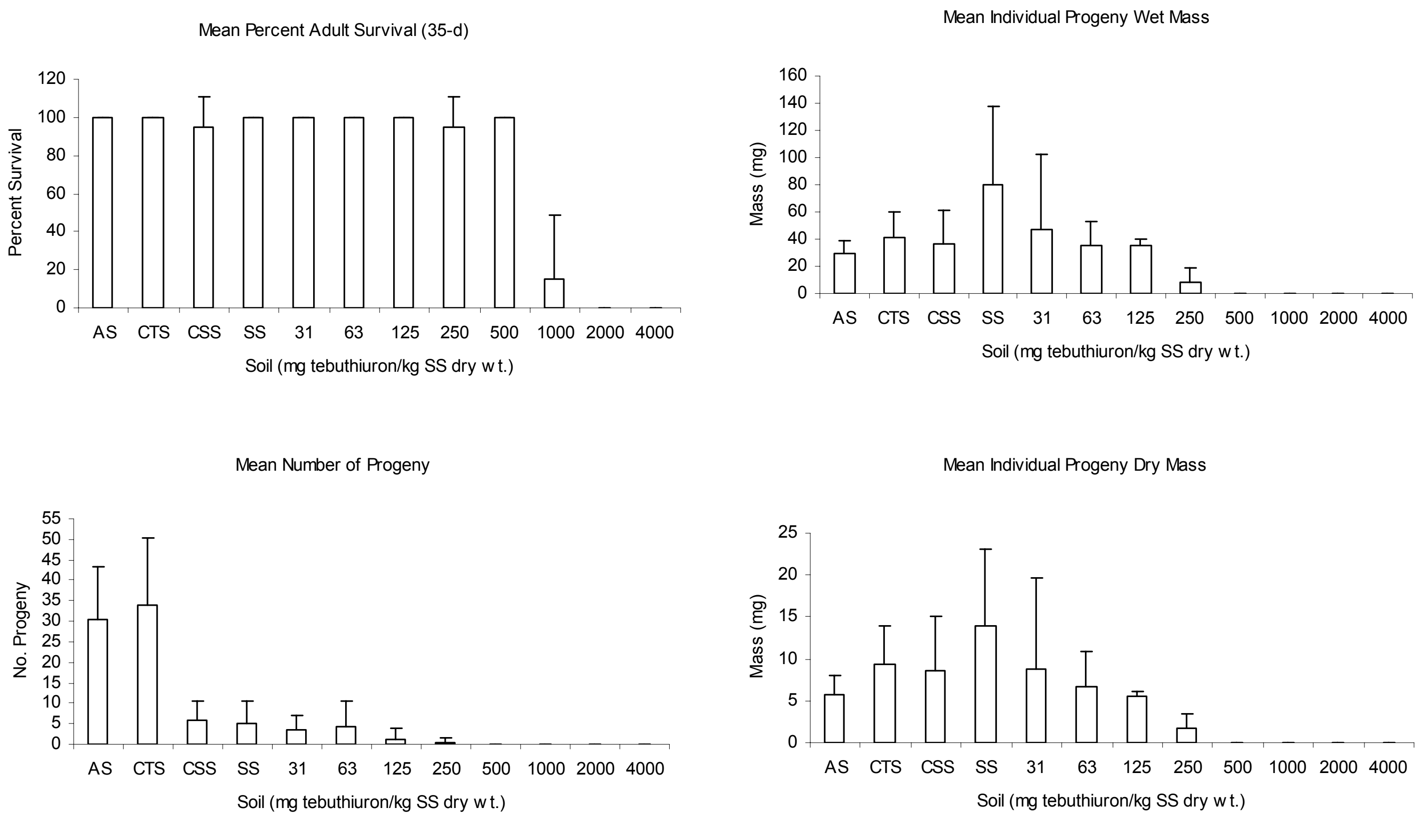


Figure B.1. Earthworm (*E. andrei*) adult survival (Day 35), growth (Day 63), and progeny production (Day 63) following exposure to artificial soil (AS), reference topsoil (CTS), reference subsoil (CSS), site subsoil contaminated with 0.03 mg/kg tebuthiuron (SS) and SS-amended soils. The concentration of tebuthiuron (mg/kg) in the amended subsoil is indicated on the x-axis. Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean.

Soil Characteristics

Table B.4. Moisture content, conductivity and pH of test soils at the beginning (Day 0) and end (Day 63) of the test.

Soil Treatment	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial Soil Moisture			Final Soil Moisture		
					(%) (ww-dw/ww)	(%) (ww-dw/dw)	(% WHC[2])	(%) (ww-dw/ww)	(%) (ww-dw/dw)	(% WHC[2])
AS	6.71	6.53	169	155	38	60	82	41	69	93
CTS	7.53	7.03	78	208	23	30	52	28	39	68
CSS (0 mg/kg)	7.35	7.00	101	203	16	19	40	20	25	53
SS (0.03 mg/kg)	8.37	7.95	213	360	14	17	37	21	26	58
31 mg/kg*	8.23	7.90	253	355	15	18	39	19	24	53
63 mg/kg *	8.33	7.97	217	354	14	16	35	19	23	51
125 mg/kg *	8.35	7.95	211	386	15	17	39	22	28	61
250 mg/kg *	8.35	8.03	241	286	13	15	33	22	28	63
500 mg/kg *	8.33	7.94	228	382	15	17	39	18	22	48
1000 mg/kg *	8.28	7.80	209	495	16	18	41	21	27	59
2000 mg/kg *	8.38	8.29	215	317	16	19	41	20	24	54
4000 mg/kg *	8.32	8.19	243	343	16	19	43	21	27	60

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.
[1] pH and conductivity were measured using a 2:1 water:soil slurry
[2] % WHC - percent of water-holding capacity of the soil

Table B.5. Texture, organic matter content, carbon content and fertility of test soils (prior to testing).

Parameter	Artificial Soil	Control Topsoil (CTS)	Control Subsoil (CSS)	Site Soil (SS)
Soil Texture	Fine Sandy Loam	Sandy Clay Loam	Sandy Clay-Clay	Sandy Clay Loam
Sand (%)	79	66	48	60
Silt (%)	9	3	10	16
Clay (%)	13	30	42	25
Organic Matter (%)	7.9	3.1*	1.4*	1.5*
Total Carbon (%)	3.85	NA	NA	NA
Total Inorganic Carbon (%)	0.05	NA	NA	NA
Total Organic Carbon (%)	3.80	1.77	0.82	0.84
Total Nitrogen (%)	0.11	0.20	0.09	0.04
Total Phosphorus (mg/kg)	NA	473.6	312.5	368.3
Plant Available Phosphorus (mg/kg)	14	39	25	9

* Organic matter content (%) for these soils was calculated by multiplying total organic carbon (%) by 1.73

Comments

No organisms exhibiting unusual appearance, behaviour or undergoing unusual treatment were used in this test.

Test Method Modifications

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by the method for pH. This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the different pHs (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al*., 1993).

Test Method Deviations

1. Percent organic matter content was not measured in the site soils. However, total organic carbon was measured, and this parameter provides data of the same value and use as information provided by percent organic matter.

References

Hendershot W.H., H. Lalonde, and M. Duquette. 1993. Soil reaction and exchangeable acidity. P 141-145 in: Soil Sampling and Methods of Analysis, M.R. Carter, ed., Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida.

McKeague, J.A. ed. 1978. *Manual on Soil Sampling and Methods of Analysis*. Canadian Society of Soil Science, Ottawa, Ontario.

Microsoft Excel. 2002. Version 10.6501.6735 SP3. Copyright 1985-2001 Microsoft Corporation.

Soil Analysis Handbook. 1992. Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc., Georgia University Station, Athens, Georgia, 202 p.

Stephan, C.E. 1977. Methods for Calculating an LC50, p 65-84, In: Aquatic Toxicology and Hazard Evaluation, F.L. Mayer and J.L. Hamelink (eds). ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA.

Systat Software Inc. (SSI). 2007. SYSTAT© 12 for Windows. Version 12.00.08. Systat Software Inc., USA.

Appendix C

Test Conditions, Experimental Design, Data Summaries, and Results of the Collembola Chronic Reproduction Test

Stantec Consulting Ltd.
361 Southgate Drive
Guelph, ON N1G 3M5
Tel: (519) 836-6050 Fax: (519) 836-2493
stantec.com

Sample Identification

Client:	EnCana
Sample(s) description:	Reference soil, site soil contaminated with weathered tebuthiuron, and site and reference soil amended with formulated tebuthiuron
Sample(s) identification:	AS 2007-10-1, 0757-1-CTS, 0756-1-CSS, 0755-1-SS
Date collected/formulated:	2007-08-02
Method of soil collection:	Composite samples
Date sample(s) received:	2007-08-16
Time sample(s) received:	10:00 am
Temperature on arrival:	22-23^{o}C
Soil storage temperature:	20.8 ± 0.1 ^{o}C
Date sample(s) tested:	November 26, 2007 – December 24, 2007
Technicians:	Kelly Olaveson, Carolyn Brown
Analyst:	Natalie Feisthauer
QA/QC:	Gladys Stephenson

Test Organism

Test Organism:	*Folsomia candida*
Organism Source:	In house culture (Laboratory Code No. Fc 07-5)
Age range at start of test (d):	9-11 d

Test Conditions and Procedures

Test type:	Static, chronic
Test duration:	28 days
Number of treatments:	12, including 1 negative control (AS) and 2 experimental controls (CTS and CSS)
Temperature:	Mean 20.3 ± 0.2^{o}C
Light intensity:	526 ± 78 lux
Photoperiod:	16 h light; 8 h dark
Watering regime:	De-ionized water, misted at test initiation and every 7 days, as required
Feeding regime:	Activated yeast, fed at test initiation and every 14 days, as required
Test unit description:	125-mL glass wide-mouthed mason jar
Soil volume/test unit:	30 g soil wet weight
No. organisms per test unit:	10
No. field samples/treatment:	1
No. replicate test units/treatment:	5 (controls) 3 (contaminated soils)
Measured soil chemistry parameters:	Initial and final soil pH, electrical conductivity, percent moisture content; and, tebuthiuron soil concentration
Measured endpoint(s):	Adult survival, number of progeny produced at Day 28
Test Protocol:	Biological Test Method: Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil. Report EPS 1/RM/47 September 2007. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Statistical Analyses: Mean, SD – Microsoft Excel (2002)
Survival LC50 - Trimmed Spearman-Kärber U.S. EPA program (http://www.epa.gov/nerleerd/stat2.htm) and Spearman-Kärber (Stephan, 1977)
Progeny number – Linear interpolation (ICPIN, U.S. EPA ICPIN program Version 2.0)
Nominal ☒ measured ☐ concentrations analysed

Test acceptability criteria met? See Table C.1

Table C.1. Performance of collembola in negative control soil treatment relative to test method validity criteria.

Criterion in Negative Control Soil		Negative Control Soil	Criteria Met?	Positive Control Soil	Solvent Control Soil
Measurement	Criterion				
Adult survival	≥ 70%	90%	Yes	N/A	N/A
Mean number of progeny per vessel	≥ 100	934	Yes	N/A	N/A

Boric Acid Reference Toxicant Data for Artificial Soil

Type of Test:	Acute lethality
Test Duration	14 days
Date Tested:	2007-11-13
Organism Lab Code:	Laboratory Code No. Fc 07-5
LC50 Survival:	2553 mg/kg
95% CL:	2173 to 3006 mg/kg
Statistical Analyses:	Spearman-Kärber (Stephan, 1977)
Historical Mean LC50:	2311 mg/kg
Warning Limits (± 2 SD):	1623 to 3052 mg/kg
Technician(s):	Kelly Olaveson
Analyst(s):	Natalie Feisthauer

Results

Table C.2. Effect of exposure to artificial soil (AS), reference topsoil (CTS), reference subsoil (CSS), site subsoil contaminated with 0.03 mg/kg tebuthiuron (SS) and SS-amended soils on collembola (*F. candida*) survival and reproduction (Day 28). Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Treatment	Mean Percent 28-d Adult Survival (n =10 adults)	Mean Number of Progeny
AS	90 (7)	934.0 (427.5)
CTS	90 (10)	869.2 (152.9)
CSS (0 mg/kg)	72 (16)	1499.8 (387.1)
SS (0.03 mg/kg)	53 (25)	636.0 (104.4)
31 mg/kg*	47 (25)	940.0 (389.2)
63 mg/kg *	93 (40)	883.3 (5.8)
125 mg/kg *	80 (10)	757.3 (283.5)
250 mg/kg *	87 (21)	376.3 (270.5)
500 mg/kg *	87 (15)	65.7 (55.3)
1000 mg/kg *	3 (6)	1.3 (1.5)
2000 mg/kg *	0 (0)	0.0 (0.0)
4000 mg/kg *	0 (0)	0.0 (0.0)

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.

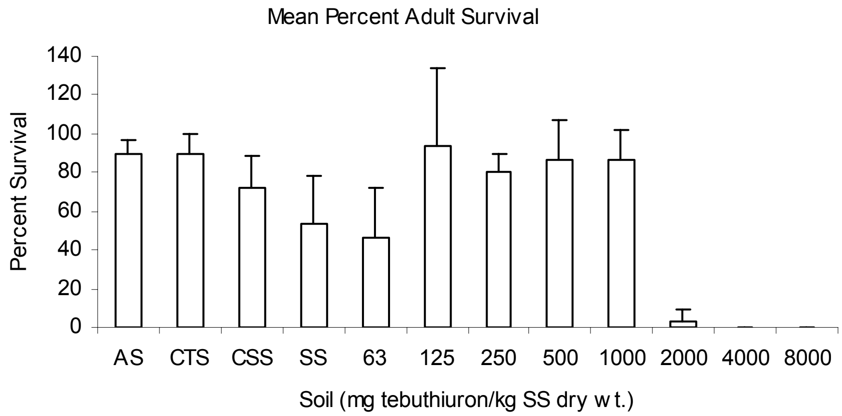
Table C.3. Effect of tebuthiuron-contaminated site soil (0.03 mg/kg) further amended with fresh tebuthiuron on collembola (*F. candida*) adult survival and reproduction (Day 28) expressed as nominal concentrations that inhibit survival, by 50% (LC50), and reproduction, by 25 and 50% (i.e., IC50s and IC25s), of that of the control treatment, respectively, along with their upper and lower confidence limits (UCL and LCL, respectively).

Parameter	Model	L/IC50 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	IC25 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	T(%) W?
Adult 35-d Survival	Spearman-Kärber	1235.9	1104.1	1386.8	NA	NA	NA	28
Number of Progeny	Linear interpolation	253.5	1.1	356.6	1.0	1.0	1.1	NA

LCL lower confidence limit
UCL upper confidence limit
T indicates if emergence data have been trimmed and to what percent
W? indicates if data has been weighted (only applicable if non-linear or linear regression procedures have been applied to the data)
NA not applicable

The results reported relate only to the sample(s) tested

Date: 2008-07-23 Approved by: [signature]
Laboratory Director



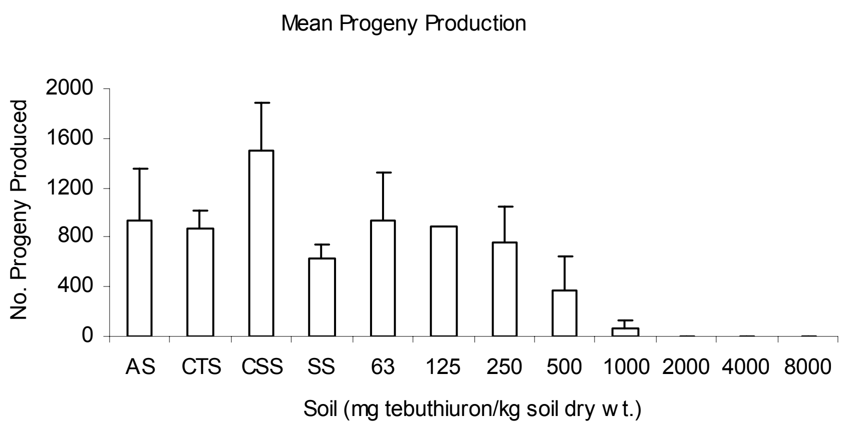


Figure C.1. Collembola (*F. candida*) adult survival (and progeny production (Day 28) following exposure to artificial soil (AS), reference topsoil (CTS), reference subsoil (CSS), site subsoil contaminated with 0.03 mg/kg tebuthiuron (SS) and SS-amended soils. The concentration of tebuthiuron (mg/kg) in the amended subsoil is indicated on the x-axis. Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean.

Soil Characteristics

Table C.4. Moisture content, conductivity and pH of test soils at the beginning (Day 0) and end (Day 28) of the test.

Soil Treatment	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial Soil Moisture (%) (ww-dw/ww)	Initial Soil Moisture (%) (ww-dw/dw)	Initial Soil Moisture (% WHC[2])	Final Soil Moisture (%) (ww-dw/ww)	Final Soil Moisture (%) (ww-dw/dw)	Final Soil Moisture (% WHC[2])
AS	6.54	6.65	194	124	35	55	74	41	70	94
CTS	7.39	7.60	96	103	24	31	53	25	33	58
CSS (0 mg/kg)	7.25	7.32	115	127	16	19	39	19	24	50
SS (0.03 mg/kg)	8.25	8.25	225	270	13	15	33	20	24	54
63 mg/kg *	8.27	8.27	226	257	13	15	34	20	25	55
125 mg/kg *	8.27	8.31	221	252	16	19	42	14	16	37
250 mg/kg *	8.30	8.27	234	257	14	17	38	20	25	57
500 mg/kg *	8.29	8.25	236	282	15	18	39	24	32	72
1000 mg/kg *	8.29	8.30	240	275	15	17	38	18	22	48
2000 mg/kg *	8.28	8.28	256	265	14	17	37	23	30	67
4000 mg/kg *	8.32	8.26	269	295	16	19	43	23	29	65
8000 mg/kg *	8.27	8.29	330	337	17	20	44	21	27	59

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.
[1] pH and conductivity were measured using a 2:1 water:soil slurry
[2] % WHC - percent of water-holding capacity of the soil

Table C.5. Texture, organic matter content, carbon content and fertility of test soils (prior to testing).

Parameter	Artificial Soil	Control Topsoil (CTS)	Control Subsoil (CSS)	Site Soil (SS)
Soil Texture	Fine Sandy Loam	Sandy Clay Loam	Sandy Clay-Clay	Sandy Clay Loam
Sand (%)	79	66	48	60
Silt (%)	9	3	10	16
Clay (%)	13	30	42	25
Organic Matter (%)	7.9	3.1*	1.4*	1.5*
Total Carbon (%)	3.85	NA	NA	NA
Total Inorganic Carbon (%)	0.05	NA	NA	NA
Total Organic Carbon (%)	3.80	1.77	0.82	0.84
Total Nitrogen (%)	0.11	0.20	0.09	0.04
Total Phosphorus (mg/kg)	NA	473.6	312.5	368.3
Plant Available Phosphorus (mg/kg)	14	39	25	9

* Organic matter content (%) for these soils was calculated by multiplying total organic carbon (%) by 1.73

Comments

No organisms exhibiting unusual appearance, behaviour or undergoing unusual treatment were used in this test; however some organisms were younger than recommended by the method (see Test Method Deviation No. 2).

Test Method Modifications

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a CaCl2 slurry, as recommended by the method for pH. This had no impact on the results of the test. The method of using CaCl2 was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the different pHs (McKeague, 1978). As a result, the CaCl2 method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot et al., 1993).

Test Method Deviations

1. Percent organic matter content was not measured in the site soils. However, total organic carbon was measured, and this parameter provides data of the same value as information provided by percent organic matter.
2. The method stipulates that 10 to 12-day old collembola should be used for *F. candida* reproduction tests. We had planned to use 10- and 11-day old collembola for the test; however, by the end of the test set up, we were unexpectedly short of a sufficient number of 10-to 11-day old juveniles for 9 test units and there were no 12-day old juveniles available. Therefore, 9-day old collembola were placed into these test units instead. 12-day old juveniles were not available because of scheduling difficulties surrounding the process of the test as originally planned on December 25 (Christmas Day). Care was taken to insure that only 1 replicate of a treatment contained 9-day old juveniles, and at least one was a negative control treatment. Tests containing 9-day old collembola have been identified, and the results of the test were scrutinized. No obvious effect of this deviation on the results was observed on the test results.

References

Hendershot W.H., H. Lalonde, and M. Duquette. 1993. Soil reaction and exchangeable acidity. P 141-145 in: Soil Sampling and Methods of Analysis, M.R. Carter, ed., Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida.

McKeague, J.A. ed. 1978. *Manual on Soil Sampling and Methods of Analysis*. Canadian Society of Soil Science, Ottawa, Ontario.

Microsoft Excel. 2002. Version 10.6501.6735 SP3. Copyright 1985-2001 Microsoft Corporation.

Soil Analysis Handbook. 1992. Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc., Georgia University Station, Athens, Georgia, 202 p.

Stephan, C.E. 1977. Methods for Calculating an LC50, p 65-84, In: Aquatic Toxicology and Hazard Evaluation, F.L. Mayer and J.L. Hamelink (eds). ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA.

Systat Software Inc. (SSI). 2007. SYSTAT© 12 for Windows. Version 12.00.08. Systat Software Inc., USA.

Appendix D

Test Conditions, Experimental Design, Data Summaries, and Results of the Blue Gramma Grass Definitive Plant Test

Stantec Consulting Ltd.
361 Southgate Drive
Guelph, ON N1G 3M5
Tel: (519) 836-6050 Fax: (519) 836-2493
stantec.com

Plant Test Report
Definitive Emergence and Seedling Growth
Site soil screening with blue gramma grass

Sample Identification

Client:	EnCana
Sample(s) description:	Reference soil, site soil contaminated with weathered tebuthiuron, and site and reference soil amended with formulated tebuthiuron; amended reference soil diluted with clean reference soil
Sample(s) identification:	Mixed AS 2008-02-26-1, 0757-2-CTS, 0756-2-CSS, 0755-4-SS
Date collected/formulated:	2007-08-02
Method of soil collection:	Composite samples
Date sample(s) received:	2007-08-16
Time sample(s) received:	10:00 am
Temperature on arrival:	22-23°C
Soil storage temperature:	20.8 ± 0.3 °C
Date sample(s) tested:	March 18, 2008 – April 8, 2008
Technicians:	Kelly Olaveson, Carolyn Brown, Emma Shrive
Analyst:	Natalie Feisthauer
QA/QC:	Gladys Stephenson

Test Organism

Test Organism:	Blue gramma grass (*Bouteloua gracilis*)
Organism Source:	Hanna Seeds, Lacombe, Alberta
Seed Lot Number:	BGG_2007

Test Conditions and Procedures

Test type:	Static, chronic
Test duration:	21 days
Number of treatments:	12, including 1 negative control (AS) and 2 experimental controls (CTS and CSS)
Temperature:	21.9 ± 0.3°C (day), 12.1 ± 0.4°C (night)
Light intensity:	279 ± 31 µmol/m^2•s
Photoperiod:	16 h light; 8 h dark
Watering regime:	Artificial soil treatments watered with nutrient solution, site soil treatments watered alternately with dechlorinated municipal tap water and deionised water, as required
Test unit description:	1-L clear polypropylene container
Soil volume/test unit:	500 g wet weight
No. organisms per test unit:	10
No. field samples/treatment:	1
No. replicate test units/treatment:	6 replicates for 3 controls, 4 for lower 6 concentrations, 3 for upper 3 concentrations
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 21: Seedling emergence, shoot and root lengths, shoot and root dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Report EPS 1/RM/45, February

2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Statistical Analyses: Mean, SD – Microsoft Excel (2002)
Emergence EC50 - Trimmed Spearman-Kärber U.S. EPA program (http://www.epa.gov/nerleerd/stat2.htm) and Spearman-Kärber (Stephan, 1977)
Shoot and root length and shoot dry mass – Non-linear regression (Systat Version 12.0, SSI, 2007)
Root dry mass – Linear interpolation (ICPIN, U.S. EPA ICPIN program Version 2.0)
Nominal ☒ measured ☐ concentrations analysed

Test acceptability criteria met? See Table D.1

Table D.1. Performance of plants in negative control soil treatment relative to test method validity criteria.

Criterion in Negative Control Soil		Negative Control Soil	Criteria Met?	Positive Control Soil	Solvent Control Soil
Measurement	Criterion				
% survival of emerged seedlings	≥ 90%	100	Yes	N/A	N/A
% seedlings with phytotoxicity symptoms	< 10%	0	Yes	N/A	N/A
Mean % emergence	≥ 70	93	Yes	N/A	N/A
Mean shoot length (mm)	≥ 50	75	Yes	N/A	N/A
Mean root length (mm)	≥ 70	65	No	N/A	N/A

Boric Acid Reference Toxicant Data for Artificial Soil

Type of Test: Seedling emergence and shoot growth
Test Duration 10 days
Date Tested: 2008-03-24
Seed Lot: BGG_2007
EC50 (emergence): 650 mg/kg
95% CL: 505 to 845 mg/kg
ICp (shoot length): 454 mg/kg
95% CL: 390 to 528 mg/kg
Statistical Analyses: Emergence - Spearman-Kärber (Stephen, 1977)
ICp, 95% CL - Linear and non-linear regression (SSI, 2007)
Historical Mean EC50: 648 mg/kg
Warning Limits (± 2 SD): 359 to 973 mg/kg
Historical Mean ICp: 515 mg/kg
Warning Limits (± 2 SD): 321 to 725 mg/kg
Technician(s): Emma Shrive, Carolyn Brown
Analyst(s): Natalie Feisthauer

Results

Table D.2. Effects on seedling (blue gramma grass) emergence and condition following exposure for 21 days to test soils. Results reported are number of seedlings and seedling condition in each test unit, as observed at the end of the test.

Soil Treatment	Number of Seedlings (Day 21)						Seedling Condition[1] (Day 21)					
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
AS	9	10	10	10	9	8	N	N	N	N	N	N
CTS	10	10	10	10	8	10	N	N	N	N	N	N
CSS (0 mg/kg)	9	9	10	9	9	8	N	N	N	C/N	N	N
0.00003 mg/kg †	9	8	8	9			N	N	N	N		
0.0003 mg/kg †	10	5	8	4			N	N	N	N		
0.003 mg/kg †	8	10	9	9			N	N	N	N/C		
SS (0.03 mg/kg)	9	9	10	10			N	N	N	N		
0.3 mg/kg *	9	9	9	9			N	N	N			
3 mg/kg *	9	9	9	8			N	N	N			
30 mg/kg *	5	4	7				C/Nc	C	C			
300 mg/kg *	5	6	7				C	C	C			
3000 mg/kg *	8	10	6				C/Di	C	C/Nc			

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated.
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.
[1]Condition of seedlings indicates a visual assessment of seedling health and vigour, relative to those in negative control soil. Normal seedlings are green, robust and without deformities or discolouration. “Abnormal” seedlings are seedlings that exhibit symptoms of suboptimal health such as chlorosis or necrosis, or those that are wilted, desiccated, discoloured, etc. These signs can result from the phytotoxic effect of the contaminant. Explanations of codes are provided below.

N	Normal	Wi	Wilting	C	Chlorotic
Di	Discoloured	Nc	Necrotic	Dd	Dead

Table D.3. Effects on seedling (blue gramma grass) emergence and growth following exposure for 21 days to the test soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Treatment	Mean Percent Emergence (n = 10 seeds)	Mean Shoot Length (mm)	Mean Root Length (mm)	Mean Individual Shoot Dry Mass (mg)	Mean Individual Root Dry Mass (mg)
AS	93 (8)	75.4 (6.0)	64.7 (5.1)	4.15 (0.45)	0.71 (0.09)
CTS	100 (11)	48.1 (9.3)	83.2 (10.3)	1.72 (0.35)	0.89 (0.21)
CSS (0 mg/kg)	90 (6)	37.4 (2.4)	48.4 (6.5)	1.35 (0.19)	0.39 (0.04)
0.00003 mg/kg †	88 (10)	35.5 (4.4)	54.4 (13.1)	1.17 (0.14)	0.36 (0.09)
0.0003 mg/kg †	68 (28)	29.7 (7.9)	43.8 (12.8)	0.86 (0.41)	0.24 (0.12)
0.003 mg/kg †	85 (10)	31.3 (2.7)	56.1 (5.3)	0.92 (0.22)	0.40 (0.13)
SS (0.03 mg/kg)	95 (6)	30.1 (3.3)	63.4 (18.5)	0.84 (0.19)	0.30 (0.10)
0.3 mg/kg *	90 (0)	32.1 (2.7)	51.5 (13.2)	0.74 (0.14)	0.24 (0.05)
3 mg/kg *	85 (10)	22.9 (2.5)	25.3 (5.1)	0.27 (0.03)	0.03 (0.02)
30 mg/kg *	53 (6)	9.2 (2.0)	9.0 (6.0)	0.03 (0.01)	0.05 (0.03)
300 mg/kg *	60 (26)	9.8 (0.6)	6.0 (1.4)	0.03 (0.01)	0.02 (0.01)
3000 mg/kg *	50 (10)	6.9 (0.7)	3.1 (1.8)	0.03 (0.01)	0.04 (0.02)

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated.
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.

Table D.4. Effect of tebuthiuron-amended reference and site soil on seedling emergence and growth of blue gramma grass (Day 21) expressed as concentrations that effect seedling emergence by 50% of those in the control treatment (EC50) and concentrations that inhibit seedling growth by 25 and 50% of those of the control treatment (i.e., IC25 and IC50) along with the EC50, IC25 and IC50 upper and lower confidence limits (UCL and LCL, respectively).

Parameter	Model	E/IC50 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	IC25 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	T (%) W?
Emergence	Effect not calculable							
Shoot Length	Logistic	12.76	4.98	32.81	1.14	0.34	3.78	No
Root Length	Hormesis	3.80	1.61	8.97	1.35	0.59	3.08	No
Shoot Dry Mass	Logistic	0.54	0.30	0.99	0.14	0.04	0.48	Yes
Root Dry Mass	Linear interpolation	0.52	0.32	0.69	0.13	0.02	0.21	NA

LCL lower confidence limit
UCL upper confidence limit
T indicates if emergence data have been trimmed and to what percent
W? indicates if data has been weighted (only applicable if non-linear or linear regression procedures have been applied to the data)
NA not applicable

The results reported relate only to the sample(s) tested

Date: 2008-07-23 Approved by: Gladys L. Stephenson
Laboratory Director

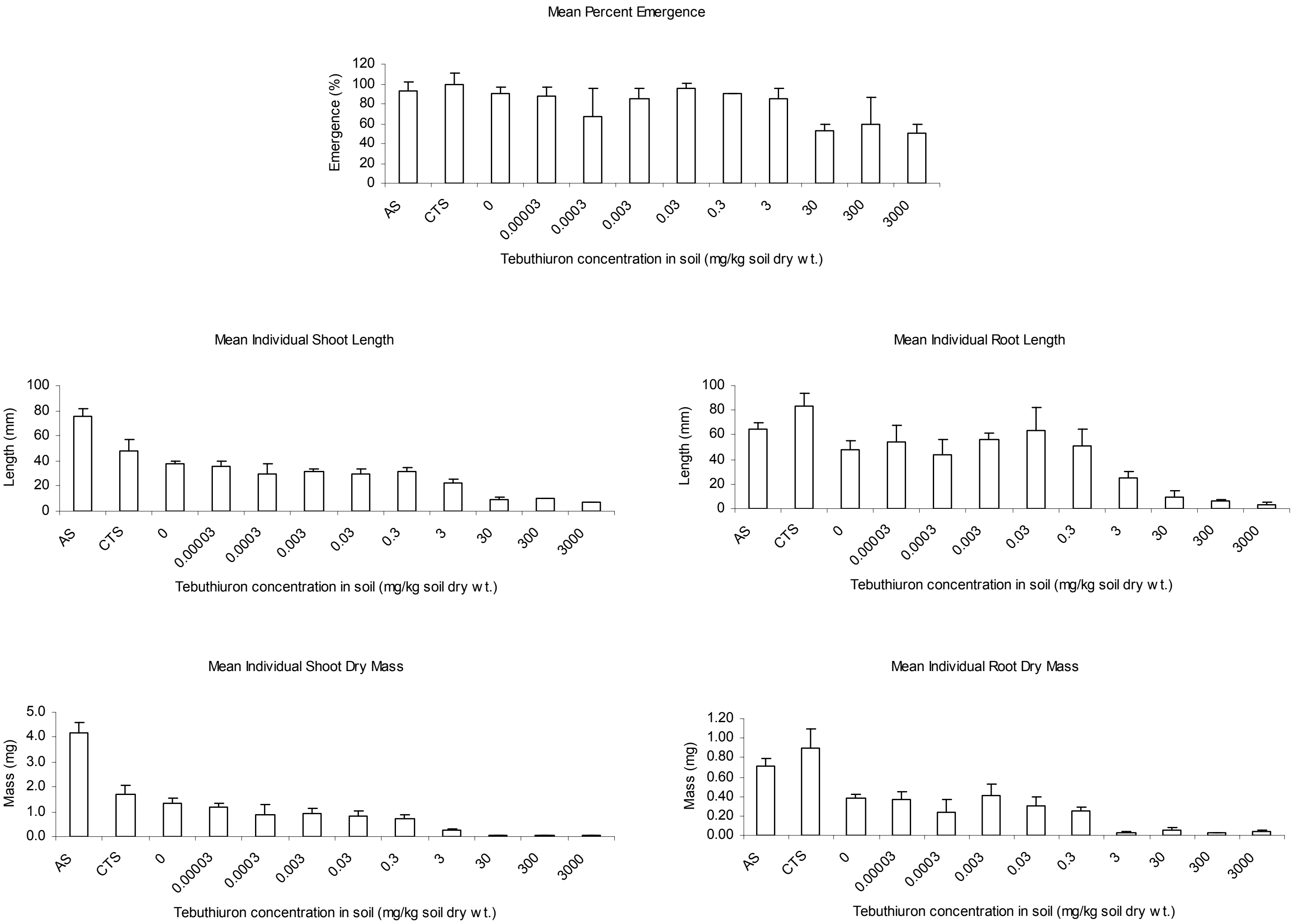


Figure D.1. Seedling (blue gramma grass) emergence and growth following 21 days of exposure to artificial soil (AS), reference topsoil (CTS), reference subsoil (CSS, as 0 mg tebuthiuron/kg soil dry wt.), CSS-amended soils (0.00003 to 0.003 mg tebuthiuron/kg soil dry wt.), site subsoil contaminated with 0.03 mg tebuthiuron/kg soil dry wt. (SS) and SS-amended soils (0.3 to 3000 mg tebuthiuron/kg soil dry wt.). Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean.

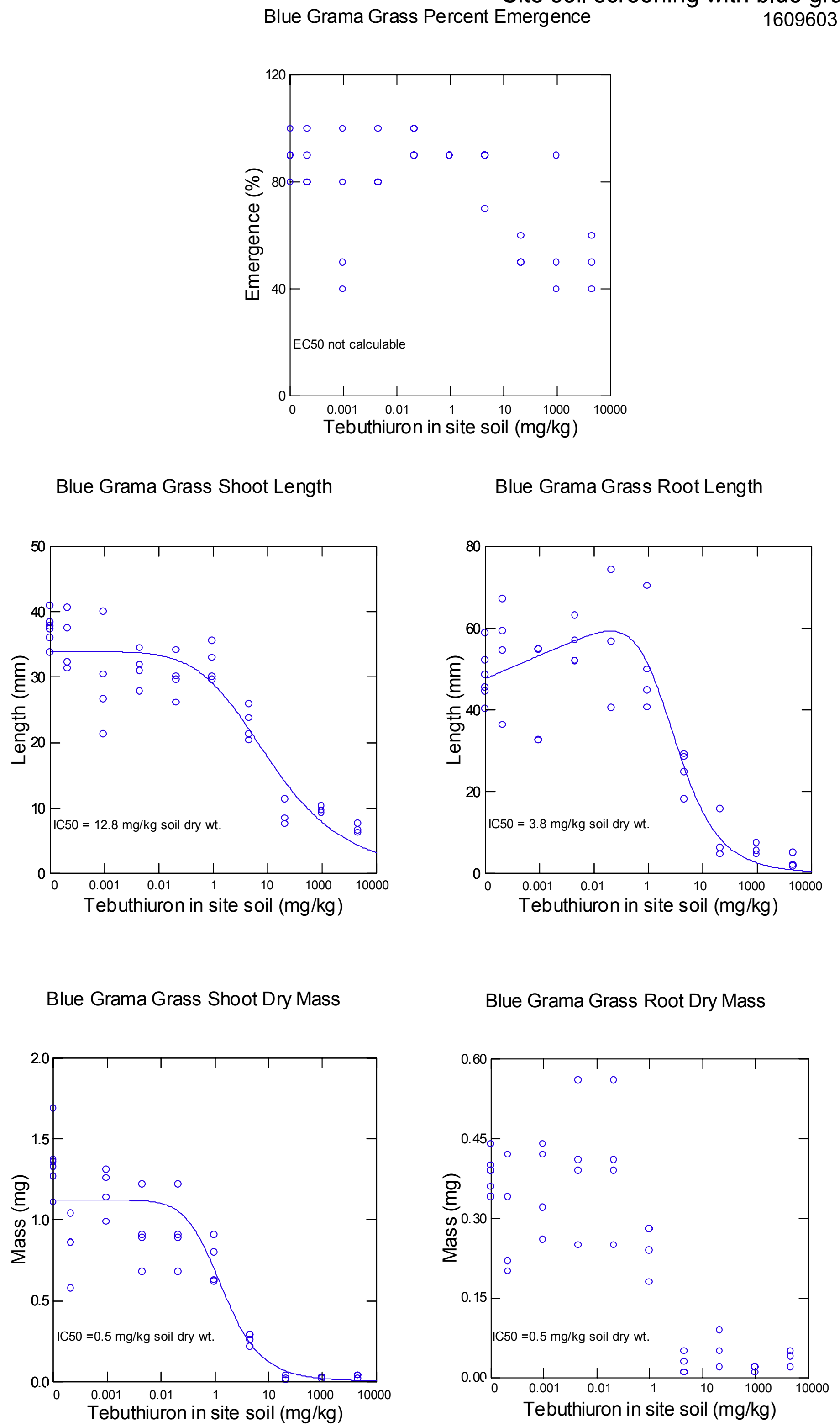


Figure D.2. Blue gramma grass seedling growth following 21 days of exposure to tebuthiuron-amended site soil. Open circles indicate data points and the solid line, where present, is the fitted regression line.

Soil Characteristics

Table D.5. Moisture content, conductivity and pH of test soils at the beginning (Day 0) and end (Day 21) of the test.

Soil Treatment	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial[2] Soil Moisture (%) (ww-dw/ww)	Initial[2] Soil Moisture (%) (ww-dw/dw)	Initial[2] Soil Moisture (% WHC[3])
AS	7.02	7.38	192	408	35	55	74
CTS	7.45	7.52	93	202	24	31	53
CSS (0 mg/kg)	7.33	7.26	113	251	14	16	33
0.00003 mg/kg †	7.31	7.24	111	278	15	18	38
0.0003 mg/kg †	7.29	7.24	115	242	16	19	39
0.003 mg/kg †	7.31	7.18	108	346	16	19	39
SS (0.03 mg/kg)	8.38	8.18	234	657	16	18	41
0.3 mg/kg *	8.38	8.24	236	707	16	19	43
3 mg/kg *	8.42	8.23	228	675	14	17	38
30 mg/kg *	8.42	8.27	225	579	13	15	33
300 mg/kg *	8.40	8.28	234	573	15	18	40
3000 mg/kg *	8.37	8.24	273	659	15	17	38

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated
[1] pH and conductivity were measured using a 2:1 water:soil slurry
[2] Moisture content measured at start of test only for plants
[3] % WHC - percent of water-holding capacity of the soil

Table D.6. Texture, organic matter content, carbon content and fertility of test soils (prior to testing).

Parameter	Artificial Soil	Control Topsoil (CTS)	Control Subsoil (CSS)	Site Soil (SS)
Soil Texture	Fine Sandy Loam	Sandy Clay Loam	Sandy Clay-Clay	Sandy Clay Loam
Sand (%)	79	66	48	60
Silt (%)	9	3	10	16
Clay (%)	13	30	42	25
Organic Matter (%)	7.9	3.1*	1.4*	1.5*
Total Carbon (%)	3.85	NA	NA	NA
Total Inorganic Carbon (%)	0.05	NA	NA	NA
Total Organic Carbon (%)	3.80	1.77	0.82	0.84
Total Nitrogen (%)	0.11	0.20	0.09	0.04
Total Phosphorus (mg/kg)	NA	473.6	312.5	368.3
Plant Available Phosphorus (mg/kg)	14	39	25	9

* Organic matter content (%) for these soils was calculated by multiplying total organic carbon (%) by 1.73

Comments

No organisms exhibiting unusual appearance, behaviour or undergoing unusual treatment were used in this test.

Test Method Modifications

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by the method for pH. This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the different pHs (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al.*, 1993).

Test Method Deviations

1. Percent organic matter content was not measured in the site soils. However, total organic carbon was measured, and this parameter provides data of the same value and use as information provided by percent organic matter.
2. The Environment Canada test method requires the average nightly temperature for a plant test to be 15 $\pm$ 3^{o}C. The mean nightly temperature during the test was 12.1 $\pm$ 0.4^{o}C, which was lower by 0.3^{o}C than the recommended temperature. However, the plants showed no signs of stress in the experimental negative control treatment at the experimental temperature range. Therefore, the effect on the results of the toxicity test was considered negligible.
3. Four of the five validity criteria were met for this test. The four criteria that were met were percent seedling emergence, percent survival of emerged seedlings, percent of emerged control seedlings exhibiting phytotoxicity or developmental anomalies and seedling shoot length. Seedlings that emerged in the negative control soil were uniformly healthy and vigourous; however they did not quite meet the validity criteria for root length. However, in performance tests with the same batch of seeds, the validity criterion for root length was easily met in all tests conducted. Seedling emergence was excellent and plants appeared vigourous and healthy with no signs of stress and it is unclear why the root length validity criterion was not met in this test. We reviewed the test procedures and conditions, we concluded that healthy blue gramma grass seed stock was used and that the experimental conditions were acceptable, including the slightly lower than recommended nightly temperature.

References

Hendershot W.H., H. Lalonde, and M. Duquette. 1993. Soil reaction and exchangeable acidity. P 141-145 in: Soil Sampling and Methods of Analysis, M.R. Carter, ed., Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida.

McKeague, J.A. ed. 1978. *Manual on Soil Sampling and Methods of Analysis*. Canadian Society of Soil Science, Ottawa, Ontario.

Microsoft Excel. 2002. Version 10.6501.6735 SP3. Copyright 1985-2001 Microsoft Corporation.

Soil Analysis Handbook. 1992. Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc., Georgia University Station, Athens, Georgia, 202 p.

Stephan, C.E. 1977. Methods for Calculating an LC50, p 65-84, In: Aquatic Toxicology and Hazard Evaluation, F.L. Mayer and J.L. Hamelink (eds). ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA.

Systat Software Inc. (SSI). 2007. SYSTAT© 12 for Windows. Version 12.00.08. Systat Software Inc., USA.

Appendix E

Test Conditions, Experimental Design, Data Summaries, and Results of the Durum Wheat Definitive Plant Test

Stantec Consulting Ltd.
361 Southgate Drive
Guelph, ON N1G 3M5
Tel: (519) 836-6050 Fax: (519) 836-2493
stantec.com

Plant Test Report
Definitive Emergence and Seedling Growth
Site soil screening with durum wheat

Sample Identification

Client:	EnCana
Sample(s) description:	Reference soil, site soil contaminated with weathered tebuthiuron, and site and reference soil amended with formulated tebuthiuron; amended reference soil diluted with clean reference soil
Sample(s) identification:	Mixed AS 2008-02-26-1, 0757-2-CTS, 0756-2-CSS, 0755-4-SS
Date collected/formulated:	2007-08-02
Method of soil collection:	Composite samples
Date sample(s) received:	2007-08-16
Time sample(s) received:	10:00 am
Temperature on arrival:	22-23^{o}C
Soil storage temperature:	20.8 ± 0.3 ^{o}C
Date sample(s) tested:	March 18, 2008 – April 1, 2008
Technicians:	Kelly Olaveson, Carolyn Brown, Emma Shrive, Yvonne Busby
Analyst:	Natalie Feisthauer
QA/QC:	Gladys Stephenson

Test Organism

Test Organism:	Durum Wheat (*Triticum durum*)
Organism Source:	C&M Seeds, Palmerston, Ontario
Seed Lot Number:	DW_2007

Test Conditions and Procedures

Test type:	Static, chronic
Test duration:	14 days
Number of treatments:	12, including 1 negative control (AS) and 2 experimental controls (CTS and CSS)
Temperature:	22.7 ± 0.2^{o}C (day), 14.6 ± 0.3^{o}C (night)
Light intensity:	285 ± 31 µmol/m^{2}•s
Photoperiod:	16 h light; 8 h dark
Watering regime:	Artificial soil treatments watered with nutrient solution, site soil treatments watered alternately with dechlorinated municipal tap water and deionised water, as required
Test unit description:	1-L clear polypropylene container
Soil volume/test unit:	500 g wet weight
No. organisms per test unit:	5
No. field samples/treatment:	1
No. replicate test units/treatment:	6 replicates for 3 controls, 4 for lower 6 concentrations, 3 for upper 3 concentrations
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 14: Seedling emergence, shoot and root lengths, shoot and root dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to

Contaminants in Soil. Report EPS 1/RM/45, February 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Statistical Analyses: Mean, SD – Microsoft Excel (2002)
Emergence EC50 - Trimmed Spearman-Kärber U.S. EPA program (http://www.epa.gov/nerleerd/stat2.htm) and Spearman-Kärber (Stephan, 1977)
Shoot and root length and root and shoot dry mass – Non-linear regression (Systat Version 12.0, SSI, 2007)
Nominal ☒ measured ☐ concentrations analysed

Test acceptability criteria met? See Table E.1

Table E.1. Performance of plants in negative control soil treatment relative to test method validity criteria.

Criterion in Negative Control Soil		Negative Control Soil	Criteria Met?	Positive Control Soil	Solvent Control Soil
Measurement	Criterion				
% survival of emerged seedlings	≥ 90%	100	Yes	N/A	N/A
% seedlings with phytotoxicity symptoms	< 10%	0	Yes	N/A	N/A
Mean % emergence	≥ 80	90	Yes	N/A	N/A
Mean shoot length (mm)	≥ 160	190	Yes	N/A	N/A
Mean root length (mm)	≥ 200	233	Yes	N/A	N/A

Boric Acid Reference Toxicant Data for Artificial Soil

Type of Test:	Seedling emergence and shoot growth
Test Duration	7 days
Date Tested:	2008-03-24
Seed Lot:	DW_2007
EC50 (emergence):	1208 mg/kg
95% CL:	993 to 1479 mg/kg
ICp (shoot length):	322 mg/kg
95% CL:	285 to 365 mg/kg
Statistical Analyses:	Emergence - Spearman-Kärber (Stephen, 1977) ICp, 95% CL - Linear and non-linear regression (SSI, 2007)
Historical Mean EC50:	1671 mg/kg
Warning Limits (± 2 SD):	764 to 2673 mg/kg
Historical Mean ICp:	456 mg/kg
Warning Limits (± 2 SD):	68 to 899 mg/kg
Technician(s):	Carolyn Brown, Emma Shrive, Kelly Olaveson
Analyst(s):	Natalie Feisthauer

Results

Table E.2. Effects on seedling (durum wheat) emergence and condition following exposure for 14 days to test soils. Results reported are number of seedlings and seedling condition in each test unit, as observed at the end of the test.

Soil Treatment	Number of Seedlings (Day 14)						Seedling Condition[1] (Day 14)					
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
AS	5	5	4	4	5	4	N	N	N	N	N	N
CTS	5	5	5	4	5	5	N	N	N	N	N	N
CSS (0 mg/kg)	3	4	5	3	3	5	N/C	N/C	N/C	N/C	N/C	N/C
0.00003 mg/kg †	4	5	5	4			N	N	N	N		
0.0003 mg/kg †	4	4	5	5			N	N	N	N/C		
0.003 mg/kg †	4	5	5	5			N	N	N	N		
SS (0.03 mg/kg)	5	5	5	5			N	N	N	N		
0.3 mg/kg *	5	5	5	3			N	N	N	N		
3 mg/kg *	5	4	3	4			N/Nc	N	N/Nc	N		
30 mg/kg *	5	4	5				N	N	N			
300 mg/kg *	3	5	5				N/Nc	N	N/Nc			
3000 mg/kg *	5	4	5				N	N	N			

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated.
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.
[1]Condition of seedlings indicates a visual assessment of seedling health and vigour, relative to those in negative control soil. Normal seedlings are green, robust and without deformities or discolouration. “Abnormal” seedlings are seedlings that exhibit symptoms of suboptimal health such as chlorosis or necrosis, or those that are wilted, desiccated, discolourated, etc. These signs can result from the phytotoxic effect of the contaminant. Explanations of codes are provided below.

N	Normal	Wi	Wilting	C	Chlorotic
Di	Discoloured	Nc	Necrotic	Dd	Dead

Table E.3. Effects on seedling (durum wheat) emergence and growth following exposure for 14 days to the test soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Treatment	Mean Percent Emergence (n = 5 seeds)	Mean Shoot Length (mm)	Mean Root Length (mm)	Mean Individual Shoot Dry Mass (mg)	Mean Individual Root Dry Mass (mg)
AS	90 (11)	190.2 (9.7)	232.8 (28.9)	56.24 (60.6)	15.42 (2.64)
CTS	100 (0)	172.7 (5.7)	226.8 (23.0)	40.99 (2.64)	29.57 (4.66)
CSS (0 mg/kg)	80 (18)	154.1 (12.9)	151.0 (30.8)	31.97 (4.32)	19.33 (3.10)
0.00003 mg/kg †	100 (0)	156.0 (5.6)	178.1 (31.5)	31.94 (1.30)	21.13 (2.28)
0.0003 mg/kg †	90 (12)	145.9 (8.4)	158.6 (19.1)	27.96 (5.52)	19.30 (3.09)
0.003 mg/kg †	95 (10)	152.9 (9.3)	170.8 (18.7)	30.23 (4.96)	20.40 (3.74)
SS (0.03 mg/kg)	100 (0)	128.5 (13.8)	188.5 (22.8)	19.86 (2.74)	20.87 (3.79)
0.3 mg/kg *	90 (20)	131.8 (11.1)	179.8 (11.4)	16.16 (2.31)	15.87 (1.24)
3 mg/kg *	90 (12)	132.3 (14.3)	186.3 (11.5)	14.63 (2.12)	13.96 (0.78)
30 mg/kg *	93 (12)	109.5 (10.5)	141.6 (18.5)	11.17 (1.84)	9.14 (0.75)
300 mg/kg *	93 (12)	108.5 (6.4)	133.7 (9.5)	10.76 (0.23)	9.37 (0.34)
3000 mg/kg *	93 (12)	54.8 (13.0)	21.2 (10.8)	5.04 (1.60)	3.28 (0.22)

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated.
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.

Table E.4. Effect of tebuthiuron-amended reference and site soil on seedling emergence and growth of durum wheat (Day 14) expressed as concentrations that effect seedling emergence by 50% of those in the control treatment (EC50) and concentrations that inhibit seedling growth by 25 and 50% of those of the control treatment (i.e., IC25 and IC50) along with the EC50, IC25 and IC50 upper and lower confidence limits (UCL and LCL, respectively).

Parameter	Model	E/IC50 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	IC25 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	T (%) W?
Emergence	No effect							
Shoot Length	Gompertz	1479.11	429.54	4742.42	30.27	7.82	117.22	No
Root Length	Hormesis	737.90	404.58	1348.96	328.10	174.18	618.02	No
Shoot Dry Mass	Logistic	0.75	0.20	2.82	0.01	0.003	0.05	No
Root Dry Mass	Logistic	31.62	9.93	100.69	1.26	0.30	5.33	No

LCL lower confidence limit
UCL upper confidence limit
T indicates if emergence data have been trimmed and to what percent
W? indicates if data has been weighted (only applicable if non-linear or linear regression procedures have been applied to the data)

The results reported relate only to the sample(s) tested

Date: 2008-07-23 Approved by: Gladys L. Stephenson
Laboratory Director

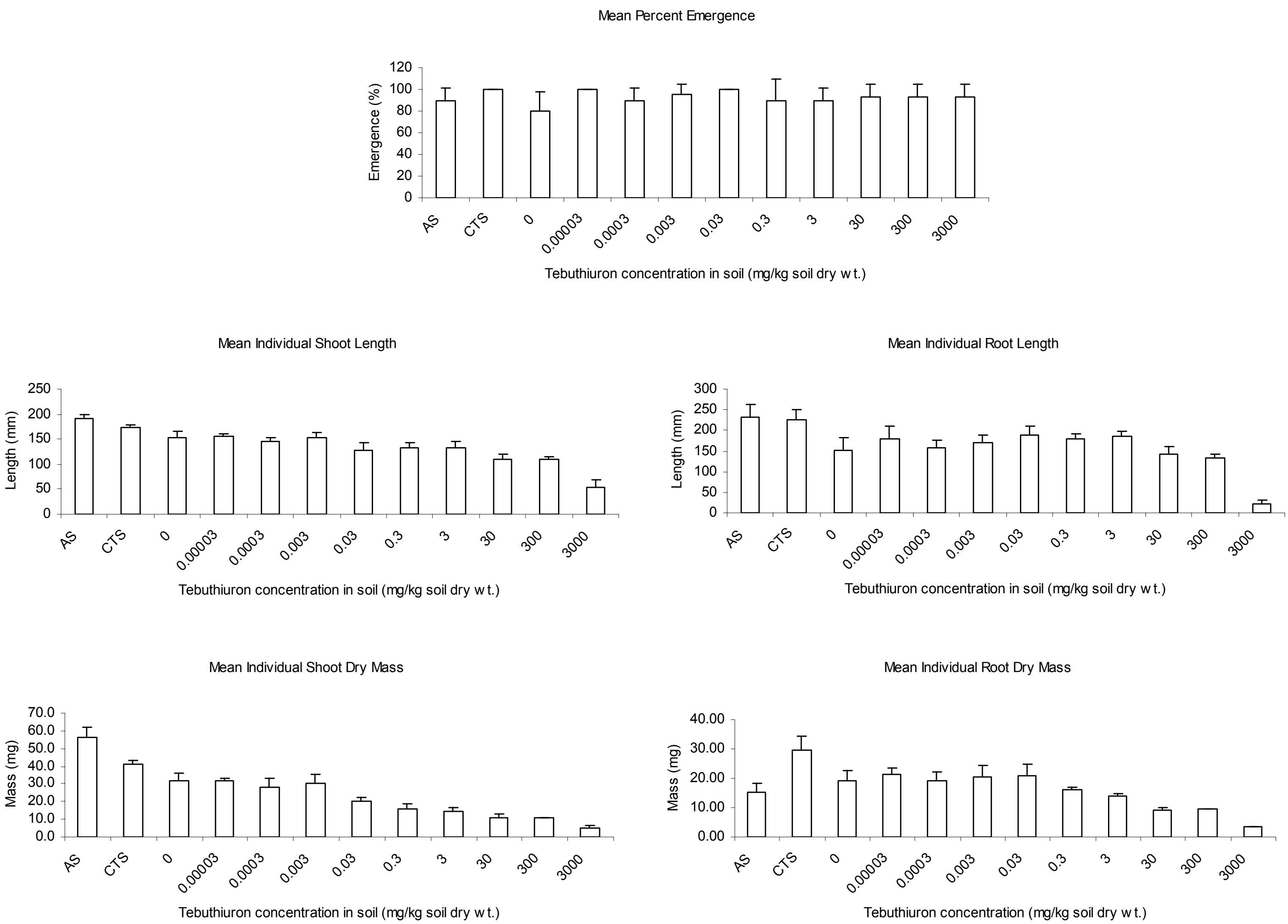


Figure E.1. Seedling (durum wheat) emergence and growth following 14 days of exposure to artificial soil (AS), reference topsoil (CTS), reference subsoil (CSS, as 0 mg tebuthiuron/kg soil dry wt.), CSS-amended soils (0.00003 to 0.003 mg tebuthiuron/kg soil dry wt.), site subsoil contaminated with 0.03 mg tebuthiuron/kg soil dry wt. (SS) and SS-amended soils (0.3 to 3000 mg tebuthiuron/kg soil dry wt.). Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean.

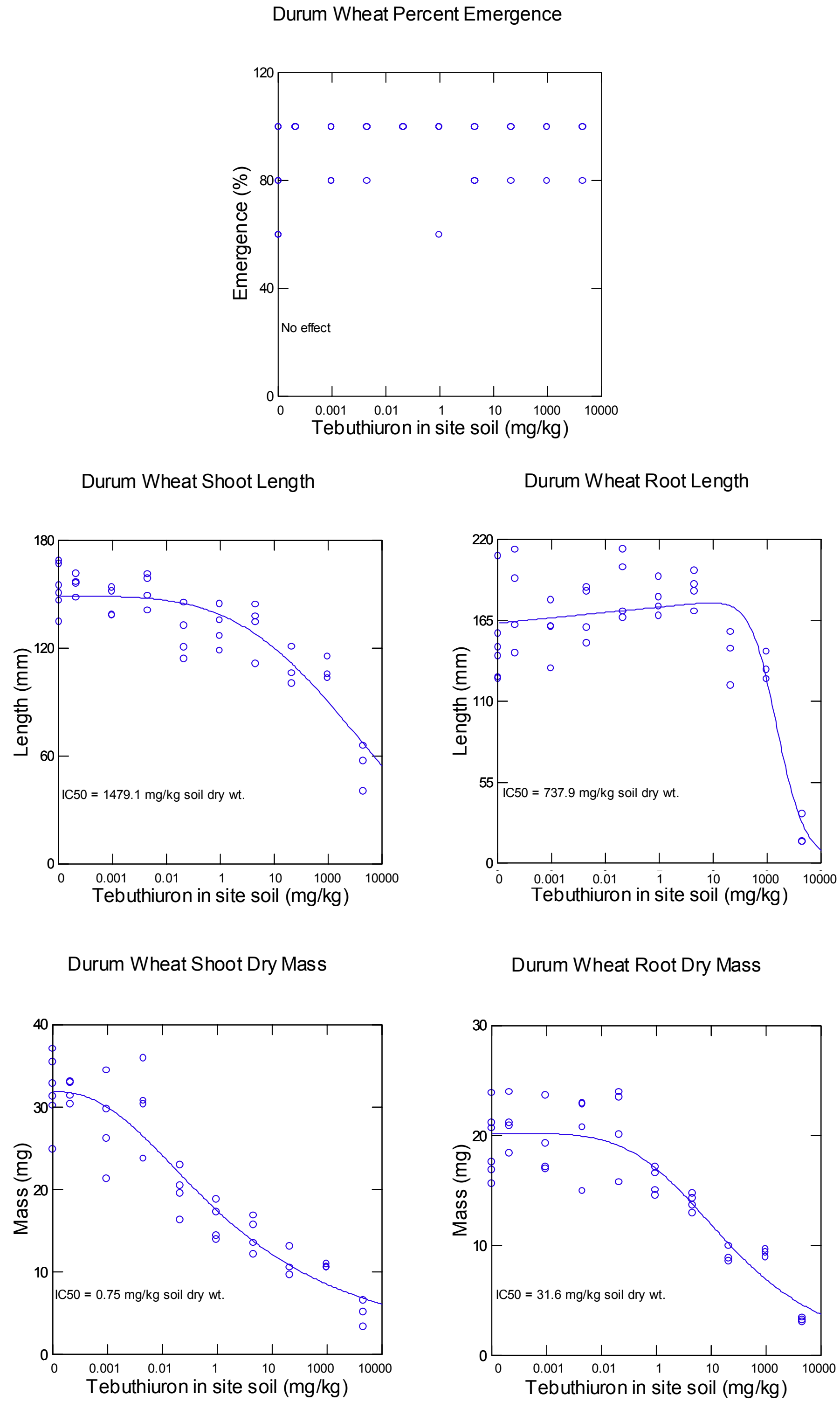


Figure E.2. Durum wheat seedling growth following 14 days of exposure to tebuthiuron-amended site soil. Open circles indicate data points and the solid line, where present, is the fitted regression line.

Soil Characteristics

Table E.5. Moisture content, conductivity and pH of test soils at the beginning (Day 0) and end (Day 14) of the test.

Soil Treatment	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial[2] Soil Moisture (%) (ww-dw/ww)	Initial[2] Soil Moisture (%) (ww-dw/dw)	Initial[2] Soil Moisture (% WHC[3])
AS	7.02	6.94	192	347	35	55	74
CTS	7.45	7.58	93	259	24	31	53
CSS (0 mg/kg)	7.33	7.26	113	429	14	16	33
0.00003 mg/kg †	7.31	7.17	111	408	15	18	38
0.0003 mg/kg †	7.29	7.18	115	385	16	19	39
0.003 mg/kg †	7.31	7.22	108	349	16	19	39
SS (0.03 mg/kg)	8.38	8.26	234	576	16	18	41
0.3 mg/kg *	8.38	8.29	236	624	16	19	43
3 mg/kg *	8.42	8.28	228	672	14	17	38
30 mg/kg *	8.42	8.35	225	533	13	15	33
300 mg/kg *	8.40	8.35	234	545	15	18	40
3000 mg/kg *	8.37	8.26	273	634	15	17	38

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated
[1] pH and conductivity were measured using a 2:1 water:soil slurry
[2] Moisture content measured at start of test only for plants
[3] % WHC - percent of water-holding capacity of the soil

Table E.6. Texture, organic matter content, carbon content and fertility of test soils (prior to testing).

Parameter	Artificial Soil	Control Topsoil (CTS)	Control Subsoil (CSS)	Site Soil (SS)
Soil Texture	Fine Sandy Loam	Sandy Clay Loam	Sandy Clay-Clay	Sandy Clay Loam
Sand (%)	79	66	48	60
Silt (%)	9	3	10	16
Clay (%)	13	30	42	25
Organic Matter (%)	7.9	3.1*	1.4*	1.5*
Total Carbon (%)	3.85	NA	NA	NA
Total Inorganic Carbon (%)	0.05	NA	NA	NA
Total Organic Carbon (%)	3.80	1.77	0.82	0.84
Total Nitrogen (%)	0.11	0.20	0.09	0.04
Total Phosphorus (mg/kg)	NA	473.6	312.5	368.3
Plant Available Phosphorus (mg/kg)	14	39	25	9

* Organic matter content (%) for these soils was calculated by multiplying total organic carbon (%) by 1.73

Comments

No organisms exhibiting unusual appearance, behaviour or undergoing unusual treatment were used in this test.

Test Method Modifications

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by the method for pH. This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the different pHs (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al*., 1993).

Test Method Deviations

1. Percent organic matter content was not measured in the site soils. However, total organic carbon was measured, and this parameter provides data of the same value and use as information provided by percent organic matter.

References

Hendershot W.H., H. Lalonde, and M. Duquette. 1993. Soil reaction and exchangeable acidity. P 141-145 in: Soil Sampling and Methods of Analysis, M.R. Carter, ed., Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida.

McKeague, J.A. ed. 1978. *Manual on Soil Sampling and Methods of Analysis*. Canadian Society of Soil Science, Ottawa, Ontario.

Microsoft Excel. 2002. Version 10.6501.6735 SP3. Copyright 1985-2001 Microsoft Corporation.

Soil Analysis Handbook. 1992. Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc., Georgia University Station, Athens, Georgia, 202 p.

Stephan, C.E. 1977. Methods for Calculating an LC50, p 65-84, In: Aquatic Toxicology and Hazard Evaluation, F.L. Mayer and J.L. Hamelink (eds). ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA.

Systat Software Inc. (SSI). 2007. SYSTAT© 12 for Windows. Version 12.00.08. Systat Software Inc., USA.

Appendix F

Test Conditions, Experimental Design, Data Summaries, and Results of the Western Wheatgrass Definitive Plant Test

Stantec

Stantec Consulting Ltd.
361 Southgate Drive
Guelph, ON N1G 3M5
Tel: (519) 836-6050 Fax: (519) 836-2493
stantec.com

Plant Test Report
Definitive Emergence and Seedling Growth
Site soil screening with western wheatgrass

Sample Identification

Client:	EnCana
Sample(s) description:	Reference soil, site soil contaminated with weathered tebuthiuron, and site and reference soil amended with formulated tebuthiuron; amended reference soil diluted with clean reference soil
Sample(s) identification:	Mixed AS 2008-02-26-1, 0757-2-CTS, 0756-2-CSS, 0755-4-SS
Date collected/formulated:	2007-08-02
Method of soil collection:	Composite samples
Date sample(s) received:	2007-08-16
Time sample(s) received:	10:00 am
Temperature on arrival:	22-23^{o}C
Soil storage temperature:	20.8 ± 0.3 ^{o}C
Date sample(s) tested:	March 18, 2008 – April 1, 2008
Technicians:	Kelly Olaveson, Carolyn Brown, Emma Shrive, Jessica Sosa Campos
Analyst:	Natalie Feisthauer
QA/QC:	Gladys Stephenson

Test Organism

Test Organism:	Western wheatgrass (*Pascopyrum smithii)*
Organism Source:	Hanna Seeds, Lacombe, Alberta
Seed Lot Number:	WW_2007A

Test Conditions and Procedures

Test type:	Static, chronic
Test duration:	14 days
Number of treatments:	12, including 1 negative control (AS) and 2 experimental controls (CTS and CSS)
Temperature:	22.7 ± 0.2^{o}C (day), 14.6 ± 0.3^{o}C (night)
Light intensity:	285 ± 31 µmol/m^{2}•s
Photoperiod:	16 h light; 8 h dark
Watering regime:	Artificial soil treatments watered with nutrient solution, site soil treatments watered alternately with dechlorinated municipal tap water and deionised water, as required
Test unit description:	1-L clear polypropylene container
Soil volume/test unit:	500 g wet weight
No. organisms per test unit:	15
No. field samples/treatment:	1
No. replicate test units/treatment:	6 replicates for 3 controls, 4 for lower 6 concentrations, 3 for upper 3 concentrations
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 14: Seedling emergence, shoot and root lengths, shoot and root dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to

Contaminants in Soil. Report EPS 1/RM/45, February 2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Statistical Analyses:

Mean, SD, Binomial – Microsoft Excel (2002)
Emergence EC50 - Trimmed Spearman-Kärber U.S. EPA program (http://www.epa.gov/nerleerd/stat2.htm) and Spearman-Kärber (Stephan, 1977)
Root length– Non-linear regression (Systat Version 12.0, SSI, 2007)
Shoot length and shoot and root dry – Linear interpolation (ICPIN, U.S. EPA ICPIN program Version 2.0)
Nominal ☒ measured ☐ concentrations analysed

Test acceptability criteria met?
Western wheatgrass is not one of the 12 species specified for use in the Environment Canada method; therefore, test acceptability criteria do not exist. However, performance of seedlings in the negative control treatment (AS) was compared to the mean performance in AS in performance tests conducted with this species (Table F.1).

Table F.1. Performance of plants in negative control soil treatment relative to mean performance test data.

Mean Performance Data (n = 5 tests)			Negative Control Soil	Negative Control Performance Acceptable?*
Measurement	Mean Value	Std Dev.		
% survival of emerged seedlings	100	0	100	Yes
% seedlings with phytotoxicity symptoms	0	0	0	Yes
Mean % emergence	62	12	61	Yes
Mean shoot length (mm)	93	12	86	Yes
Mean root length (mm)	81	12	93	Yes

*Performance considered acceptable if values in the negative control soil are within one standard deviation of the mean performance values.

Boric Acid Reference Toxicant Data for Artificial Soil

Type of Test:	Seedling emergence and shoot growth
Test Duration	10 days
Date Tested:	2008-03-24
Seed Lot:	WW_2007A
EC50 (emergence):	905 mg/kg
95% CL:	640 to 1280 mg/kg
ICp (shoot length):	443 mg/kg
95% CL:	282 to 693 mg/kg
Statistical Analyses:	Emergence - Binomial (Microsoft Excel, 2002) ICp, 95% CL - Linear and non-linear regression (SSI, 2007)
Historical Mean EC50:	665 mg/kg
Warning Limits (± 2 SD):	99 to 1344 mg/kg
Historical Mean ICp:	612 mg/kg
Warning Limits (± 2 SD):	261 to 1002 mg/kg
Technician(s):	Carolyn Brown, Emma Shrive, Kelly Olaveson
Analyst(s):	Natalie Feisthauer

Results

Table F.2. Effects on seedling (western wheatgrass) emergence and condition following exposure for 14 days to test soils. Results reported are number of seedlings and seedling condition in each test unit, as observed at the end of the test.

Soil Treatment	Number of Seedlings (Day 14)						Seedling Condition[1] (Day 14)					
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
AS	10	9	9	10	8	9	N	N	N	N	N	N
CTS	5	9	8	13	7	8	N	N	N	N	N	N
CSS (0 mg/kg)	10	6	12	12	8	8	N	N	N	N	N	N
0.00003 mg/kg †	7	9	8	8			N	N	N	N		
0.0003 mg/kg †	10	6	9	9			N	N	N	N		
0.003 mg/kg †	6	5	8	8			N	N	N	N		
SS (0.03 mg/kg)	10	7	8	11			N	N	N	N		
0.3 mg/kg *	5	2	7	4			Di	N	N	Di		
3 mg/kg *	3	2	3	4			N	N	N	N		
30 mg/kg *	3	2	5	3			Di	Di	Di	N		
300 mg/kg *	1	4	4				N	N	N			
3000 mg/kg *	4	6	7				N	N	N			

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated.
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.
[1]Condition of seedlings indicates a visual assessment of seedling health and vigour, relative to those in negative control soil. Normal seedlings are green, robust and without deformities or discolouration. "Abnormal" seedlings are seedlings that exhibit symptoms of suboptimal health such as chlorosis or necrosis, or those that are wilted, desiccated, discolourated, etc. These signs can result from the phytotoxic effect of the contaminant. Explanations of codes are provided below.

N	Normal	Wi	Wilting	C	Chlorotic
Di	Discoloured	Nc	Necrotic	Dd	Dead

Table F.3. Effects on seedling (western wheatgrass) emergence and growth following exposure for 14 days to the test soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Treatment	Mean Percent Emergence (n = 15 seeds)	Mean Shoot Length (mm)	Mean Root Length (mm)	Mean Individual Shoot Dry Mass (mg)	Mean Individual Root Dry Mass (mg)
AS	61 (5)	86.4 (11.8)	93.2 (8.3)	2.96 (0.70)	0.78 (0.21)
CTS	58 (17)	76.3 (13.1)	58.8 (13.5)	2.16 (0.64)	0.72 (0.35)
CSS (0 mg/kg)	62 (16)	64.5 (7.5)	58.0 (4.8)	1.44 (0.30)	0.49 (0.10)
0.00003 mg/kg †	55 (6)	64.5 (7.5)	58.0 (4.8)	1.78 (0.24)	0.74 (0.08)
0.0003 mg/kg †	57 (12)	54.4 (7.7)	49.9 (8.6)	1.14 (0.22)	0.43 (0.05)
0.003 mg/kg †	45 (10)	60.6 (5.6)	47.4 (9.6)	1.23 (0.17)	0.43 (0.05)
SS (0.03 mg/kg)	62 (10)	43.9 (8.3)	43.2 (8.7)	0.86 (0.19)	0.37 (0.25)
0.3 mg/kg *	30 (14)	20.6 (6.4)	31.2 (7.4)	0.31 (0.20)	0.12 (0.08)
3 mg/kg *	18 (3)	34.7 (11.2)	32.4 (10.9)	0.50 (0.13)	0.28 (0.07)
30 mg/kg *	22 (10)	26.4 (6.0)	36.3 (6.2)	0.26 (0.19)	0.13 (0.01)
300 mg/kg *	18 (10)	26.6 (9.5)	25.7 (9.5)	0.37 (0.08)	0.22 (0.08)
3000 mg/kg *	38 (10)	32.0 (0.2)	4.4 (0.5)	0.56 (0.13)	0.09 (0.08)

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated.
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.

Table F.4. Effect of tebuthiuron-amended reference and site soil on seedling emergence and growth of western wheatgrass (Day 14) expressed as concentrations that affect seedling emergence by 50% of those in the control treatment (EC50) and concentrations that inhibit seedling growth by 25 and 50% of those of the control treatment (i.e., IC25 and IC50) along with the EC50, IC25 and IC50 upper and lower confidence limits (UCL and LCL, respectively).

Parameter	Model	E/IC50 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	IC25 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	T (%) W?
Emergence	Spearman-Kärber	0.01	0.00	20.70	NA	NA	NA	45%
Shoot Length	Linear interpolation	0.22	0.17	13.46	0.06	0.05	0.08	NA
Root Length	Gompertz	12.88	1.06	155.96	0.13	0.005	3.35	No
Shoot Dry Mass	Linear interpolation	0.12	0.08	0.17	0.05	0.001	0.06	NA
Root Dry Mass	Linear interpolation	0.16	0.10	0.26	0.04	0.002	0.08	NA

LCL lower confidence limit
UCL upper confidence limit
T indicates if emergence data have been trimmed and to what percent
W? indicates if data has been weighted (only applicable if non-linear or linear regression procedures have been applied to the data)
NA not applicable

The results reported relate only to the sample(s) tested

Date: 2008-07-23 Approved by: Gladys L. Stephenson
Laboratory Director

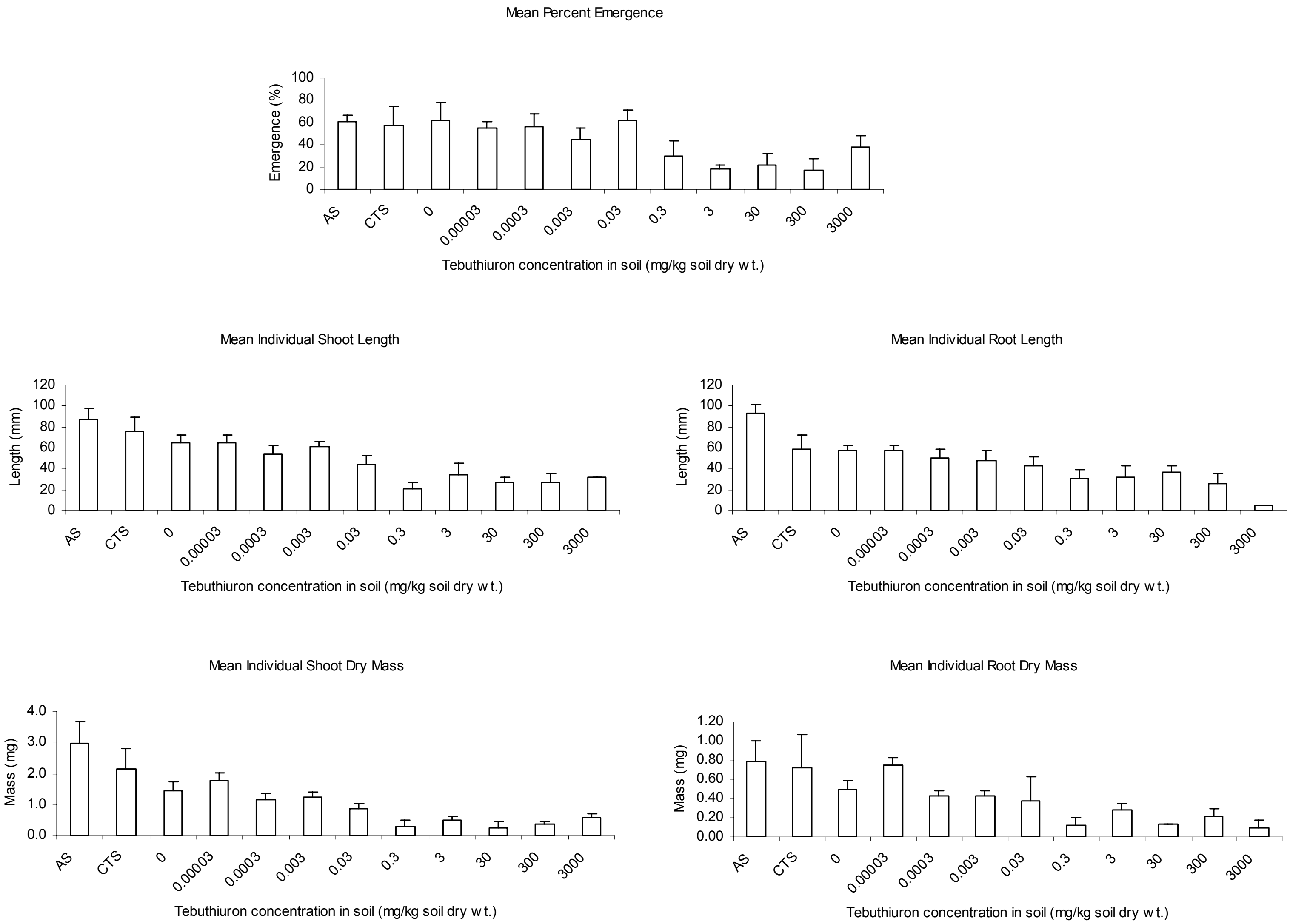


Figure F.1. Seedling (western wheatgrass) emergence and growth following 14 days of exposure to artificial soil (AS), reference topsoil (CTS), reference subsoil (CSS, as 0 mg tebuthiuron/kg soil dry wt.), CSS-amended soils (0.00003 to 0.003 mg tebuthiuron/kg soil dry wt.), site subsoil contaminated with 0.03 mg tebuthiuron/kg soil dry wt. (SS) and SS-amended soils (0.3 to 3000 mg tebuthiuron/kg soil dry wt.). Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean.

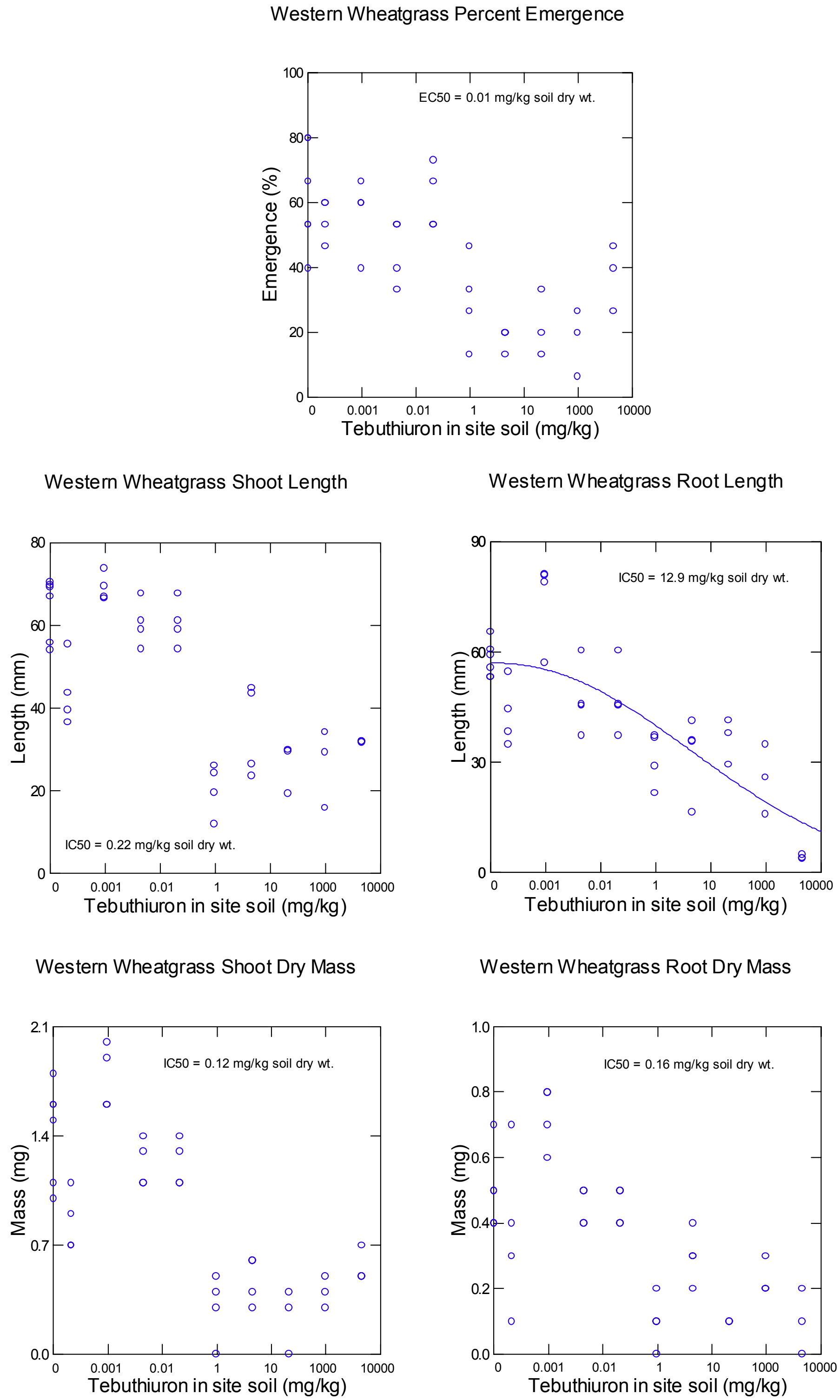


Figure F.2. Western wheatgrass seedling growth following 14 days of exposure to tebuthiuron-amended site soil. Open circles indicate data points and the solid line, where present, is the fitted regression line.

Soil Characteristics

Table F.5. Moisture content, conductivity and pH of test soils at the beginning (Day 0) and end (Day 14) of the test.

Soil Treatment	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial[2] Soil Moisture (%) (ww-dw/ww)	Initial[2] Soil Moisture (%) (ww-dw/dw)	Initial[2] Soil Moisture (% WHC[3])
AS	7.02	7.03	192	337	35	55	74
CTS	7.45	7.61	93	174	24	31	53
CSS (0 mg/kg)	7.33	7.29	113	273	14	16	33
0.00003 mg/kg †	7.31	7.26	111	228	15	18	38
0.0003 mg/kg †	7.29	7.26	115	252	16	19	39
0.003 mg/kg †	7.31	7.26	108	237	16	19	39
SS (0.03 mg/kg)	8.38	8.29	234	627	16	18	41
0.3 mg/kg *	8.38	8.30	236	584	16	19	43
3 mg/kg *	8.42	8.32	228	546	14	17	38
30 mg/kg *	8.42	8.30	225	582	13	15	33
300 mg/kg *	8.40	8.32	234	544	15	18	40
3000 mg/kg *	8.37	8.30	273	653	15	17	38

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated
[1] pH and conductivity were measured using a 2:1 water:soil slurry
[2] Moisture content measured at start of test only for plants
[3] % WHC - percent of water-holding capacity of the soil

Table F.6. Texture, organic matter content, carbon content and fertility of test soils (prior to testing).

Parameter	Artificial Soil	Control Topsoil (CTS)	Control Subsoil (CSS)	Site Soil (SS)
Soil Texture	Fine Sandy Loam	Sandy Clay Loam	Sandy Clay-Clay	Sandy Clay Loam
Sand (%)	79	66	48	60
Silt (%)	9	3	10	16
Clay (%)	13	30	42	25
Organic Matter (%)	7.9	3.1*	1.4*	1.5*
Total Carbon (%)	3.85	NA	NA	NA
Total Inorganic Carbon (%)	0.05	NA	NA	NA
Total Organic Carbon (%)	3.80	1.77	0.82	0.84
Total Nitrogen (%)	0.11	0.20	0.09	0.04
Total Phosphorus (mg/kg)	NA	473.6	312.5	368.3
Plant Available Phosphorus (mg/kg)	14	39	25	9

* Organic matter content (%) for these soils was calculated by multiplying total organic carbon (%) by 1.73

Comments

No organisms exhibiting unusual appearance, behaviour or undergoing unusual treatment were used in this test.

Test Method Modifications

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by the method for pH. This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the different pHs (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al.*, 1993).

Test Method Deviations

1. Percent organic matter content was not measured in the site soils. However, total organic carbon was measured, and this parameter provides data of the same value and use as information provided by percent organic matter.

References

Hendershot W.H., H. Lalonde, and M. Duquette. 1993. Soil reaction and exchangeable acidity. P 141-145 in: Soil Sampling and Methods of Analysis, M.R. Carter, ed., Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida.

McKeague, J.A. ed. 1978. *Manual on Soil Sampling and Methods of Analysis*. Canadian Society of Soil Science, Ottawa, Ontario.

Microsoft Excel. 2002. Version 10.6501.6735 SP3. Copyright 1985-2001 Microsoft Corporation.

Soil Analysis Handbook. 1992. Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc., Georgia University Station, Athens, Georgia, 202 p.

Stephan, C.E. 1977. Methods for Calculating an LC50, p 65-84, In: Aquatic Toxicology and Hazard Evaluation, F.L. Mayer and J.L. Hamelink (eds). ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA.

Systat Software Inc. (SSI). 2007. SYSTAT© 12 for Windows. Version 12.00.08. Systat Software Inc., USA.

Appendix G

Test Conditions, Experimental Design, Data Summaries, and Results of the Silver Sagebrush Definitive Plant Test

Stantec Consulting Ltd.
361 Southgate Drive
Guelph, ON N1G 3M5
Tel: (519) 836-6050 Fax: (519) 836-2493
stantec.com

Plant Test Report
Definitive Emergence and Seedling Growth
Site soil screening with silver sagebrush

Sample Identification

Client:	EnCana
Sample(s) description:	Reference soil, site soil contaminated with weathered tebuthiuron, and site and reference soil amended with formulated tebuthiuron; amended reference soil diluted with clean reference soil
Sample(s) identification:	Mixed AS 2008-03-04, 0757-1-CTS, 0756-1-CSS, 0755-5-SS
Date collected/formulated:	2007-08-02
Method of soil collection:	Composite samples
Date sample(s) received:	2007-08-16
Time sample(s) received:	10:00 am
Temperature on arrival:	22-23°C
Soil storage temperature:	20.6 ± 0.4 °C
Date sample(s) tested:	June 6, 2008 – July 4, 2008
Technicians:	Natalie Feisthauer, Emma Shrive, Kelly Olaveson
Analyst:	Kelly Olaveson
QA/QC:	Gladys Stephenson

Test Organism

Test Organism:	Silver sagebrush (*Artemisia cana* subsp. *cana)*
Organism Source:	Wind River Seed, Manderson, Wyoming
Seed Lot Number:	SS_2008

Test Conditions and Procedures

Test type:	Static, chronic
Test duration:	28 days
Number of treatments:	12, including 1 negative control (AS) and 2 experimental controls (CTS and CSS)
Temperature:	22.1 ± 0.4°C (day), 14.7 ± 0.1°C (night)
Light intensity:	374 ± 9 µmol/m^2•s
Photoperiod:	16 h light; 8 h dark
Watering regime:	Artificial soil treatments watered with nutrient solution, site soil treatments watered with dechlorinated municipal tap water, as required
Test unit description:	1-L clear polypropylene container
Soil volume/test unit:	425 g wet weight
No. organisms per test unit:	10 (broadcast seeded; thinned to 10 seedlings on Day 10 and weeded on Day 17)
No. field samples/treatment:	1
No. replicate test units/treatment:	6 replicates for 3 controls, 4 for lower 6 concentrations, 3 for upper 3 concentrations
Measured soil chemistry parameters:	Initial soil pH, electrical conductivity, and percent moisture content, final soil pH and electrical conductivity
Measured endpoint(s):	Day 28: Seedling emergence, shoot and root lengths, shoot and root dry masses
Test Protocol:	Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Report EPS 1/RM/45, February

2005. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario.

Statistical Analyses: Mean, SD – Microsoft Excel (2002)
Emergence EC50 - Trimmed Spearman-Kärber U.S. EPA program (http://www.epa.gov/nerleerd/stat2.htm) and Spearman-Kärber (Stephan, 1977)
Shoot length and shoot and root dry mass – Non-linear regression (Systat Version 12.0, SSI, 2007)
Root length – Linear interpolation (ICPIN, U.S. EPA ICPIN program Version 2.0)
Nominal ☒ measured ☐ concentrations analysed

Test acceptability criteria met?
Silver sagebrush is not one of the 12 species specified for use in the Environment Canada method; therefore, test acceptability criteria do not exist.

Table G.1. Performance of plants in negative control soil treatment relative to performance test data.

Mean Performance Data (n = 4 reps)			Negative Control Soil	Negative Control Performance Acceptable?*
Measurement	Mean Value	Std Dev.		
% survival of emerged seedlings	100	0	100	NA
% seedlings with phytotoxicity symptoms	0	0	0	NA
Mean % emergence	100	0	100	NA (thinned seedlings)
Mean shoot length (mm)	30 (28.2-31.6)*	1	29.4	NA
Mean root length (mm)	107 (96.0-117.8)*	7	63.6	NA

* values in bracket are mean +/- confidence level for mean (95%)
NA – insufficient data available to assess performance at this time.

Boric Acid Reference Toxicant Data for Artificial Soil

Type of Test: Seedling emergence and shoot growth
Test Duration: 17 days
Date Tested: 2008-06-23
Seed Lot: SS_2008
EC50 (emergence): 933.25 mg/kg
95% CL: 891.25 to 1000.00 mg/kg
IC50 (shoot length): 767.4 mg/kg
95% CL: 666.8 to 885.1 mg/kg
Statistical Analyses: Emergence – Spearman-Kärber (EPA program (http://www.epa.gov/nerleerd/stat2.htm)
ICp, 95% CL - Linear and non-linear regression (SSI, 2007)
Technician(s): Emma Shrive, Kelly Olaveson
Analyst(s): Kelly Olaveson

Seedling emergence was not considered a valid endpoint because seedlings were thinned to ten individuals per test unit on Day 8 for the concentrations below 640 mg/kg. On Day 14, seedlings were thinned to 10 individuals in the higher concentrations and weeded (to 10 individuals) in the lower concentrations as needed, after broadcast seeding at the beginning of the test.

Results

Table G.2. Effects on seedling (silver sagebrush) emergence and condition following exposure for 28 days to test soils. Results reported are number of seedlings and seedling condition in each test unit, as observed at the end of the test.

Soil Treatment	Number of Seedlings (Day 28)						Seedling Condition[1] (Day 28)					
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
AS	10	10	10	10	10	10	N	N	N	N	N	N
CTS	10	10	10	10	10	10	N/C	N	N	N/C	N	N/C
CSS (0 mg/kg)	10	10	10	10	10	10	N	N	N	N	N	N
0.00003 mg/kg †	10	10	9	10			N/C	N/C	N	N		
0.0003 mg/kg †	10	10	10	10			N/Nc/C	N/Nc/C	N/C	N/C/Nc		
0.003 mg/kg †	10	10	10	10			N	N	N	N		
SS (0.03 mg/kg)	10	10	10	10			N	N	N	N		
0.3 mg/kg *	5	5	5	6			Nc/C/Wi/N	Nc/Di	N/Di	N/Di		
3 mg/kg *	0	0	3	3					N	N		
30 mg/kg *	1	0	0				N					
300 mg/kg *	0	0	0									
3000 mg/kg *	0	0	0									

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated.
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.
[1]Condition of seedlings indicates a visual assessment of seedling health and vigour, relative to those in negative control soil. Normal seedlings are green, robust and without deformities or discolouration. “Abnormal” seedlings are seedlings that exhibit symptoms of suboptimal health such as chlorosis or necrosis, or those that are wilted, desiccated, discolourated, etc. These signs can result from the phytotoxic effect of the contaminant. Explanations of codes are provided below.

N	Normal	Wi	Wilting	C	Chlorotic
Di	Discoloured	Nc	Necrotic	Dd	Dead

Table G.3. Effects on seedling (silver sagebrush) emergence and growth following exposure for 28 days to the test soils. Results are reported as treatment means, and the values in brackets indicate one standard deviation of the mean.

Soil Treatment	Mean Percent Emergence (broadcast and thinned to 10 seedlings)	Mean Shoot Length (mm)	Mean Root Length (mm)	Mean Individual Shoot Dry Mass (mg)	Mean Individual Root Dry Mass (mg)
AS	100 (0)	29.4 (1.5)	63.6 (6.6)	5.85 (0.50)	0.93 (0.12)
CTS	100 (0)	27.5 (1.0)	109.9 (12.9)	6.44 (0.70)	2.55 (0.33)
CSS (0 mg/kg)	100 (0)	18.2 (2.5)	38.0 (10.1)	2.32 (0.19)	0.64 (0.17)
0.00003 mg/kg †	98 (5)	19.9 (1.4)	92.9 (23.1)	2.09 (0.41)	1.06 (0.28)
0.0003 mg/kg †	100 (0)	22.1 (1.9)	29.3 (3.7)	2.42 (0.32)	0.43 (0.06)
0.003 mg/kg †	100 (0)	19.4 (0.5)	60.2 (14.7)	2.25 (0.30)	0.71 (0.13)
SS (0.03 mg/kg)	100 (0)	19.7 (0.9)	25.9 (8.3)	2.10 (0.41)	0.26 (0.08)
0.3 mg/kg *	53 (5)	14.8 (3.4)	9.4 (3.8)	0.42 (0.12)	0.06 (0.03)
3 mg/kg *	15 (17)	10.2 (0.7)	9.8 (2.6)	0.23 (0.05)	0.07 (0.00)
30 mg/kg *	3 (6)	13.0 (N/A)	18.0 (N/A)	0.40 (N/A)	0.30 (N/A)
300 mg/kg *	0 (0)	N/A	N/A	N/A	N/A
3000 mg/kg *	0 (0)	N/A	N/A	N/A	N/A

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated.
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated.
N/A indicates that either 1 or no plants emerged in the treatment so values could not be determined.

Table G.4. Effect of tebuthiuron-amended reference and site soil on seedling emergence and growth of silver sagebrush (Day 28) expressed as concentrations that affect seedling emergence by 50% of those of the control treatment (EC50) and concentrations that inhibit seedling growth by 25 and 50% of those of the control treatment (i.e., IC25 and IC50) along with the EC50, IC25 and IC50 upper and lower confidence limits (UCL and LCL, respectively).

Parameter	Model	E/IC50 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	IC25 (mg/kg)	LCL (mg/kg)	UCL (mg/kg)	T (%) W?
Emergence	Spearman-Karber	0.385	0.245	0.614	NA	NA	NA	0.625
Shoot Length	Hormesis	120.504	1.130	12823.306	0.973	0.159	5.957	N
Root Length	Linear Interpolation	0.018	0.009	0.048	0.000	0.000	0.007	N
Shoot Dry Mass	Hormesis	0.140	0.066	0.296	NC	NC	NC	N
Root Dry Mass	Hormesis	0.221	0.005	10.116	0.044	0.003	0.646	N

LCL lower confidence limit
UCL upper confidence limit
T indicates if emergence data have been trimmed and to what percent
W? indicates if data has been weighted (only applicable if non-linear or linear regression procedures have been applied to the data)
NA not applicable NC not calculable

The results reported relate only to the sample(s) tested

Date: 2008-07-23 Approved by: Gladys L. Stephenson
Laboratory Director

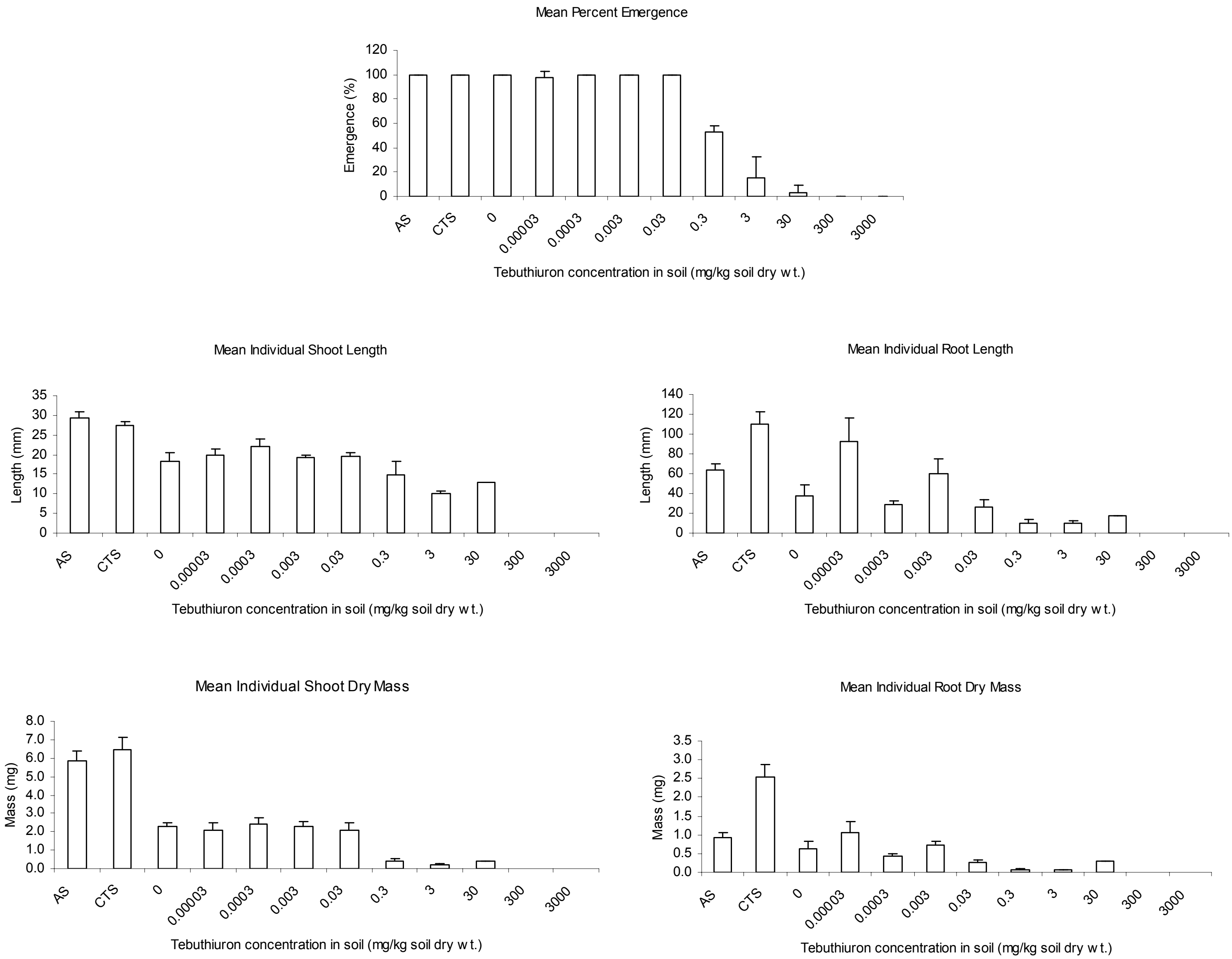


Figure G.1. Seedling (silver sagebrush) emergence and growth following 28 days of exposure to artificial soil (AS), reference topsoil (CTS), reference subsoil (CSS, as 0 mg tebuthiuron/kg soil dry wt.), CSS-amended soils (0.00003 to 0.003 mg tebuthiuron/kg soil dry wt.), site subsoil contaminated with 0.03 mg tebuthiuron/kg soil dry wt. (SS) and SS-amended soils (0.3 to 3000 mg tebuthiuron/kg soil dry wt.). Columns indicate treatment means. Bars above the columns represent one standard deviation of the mean.

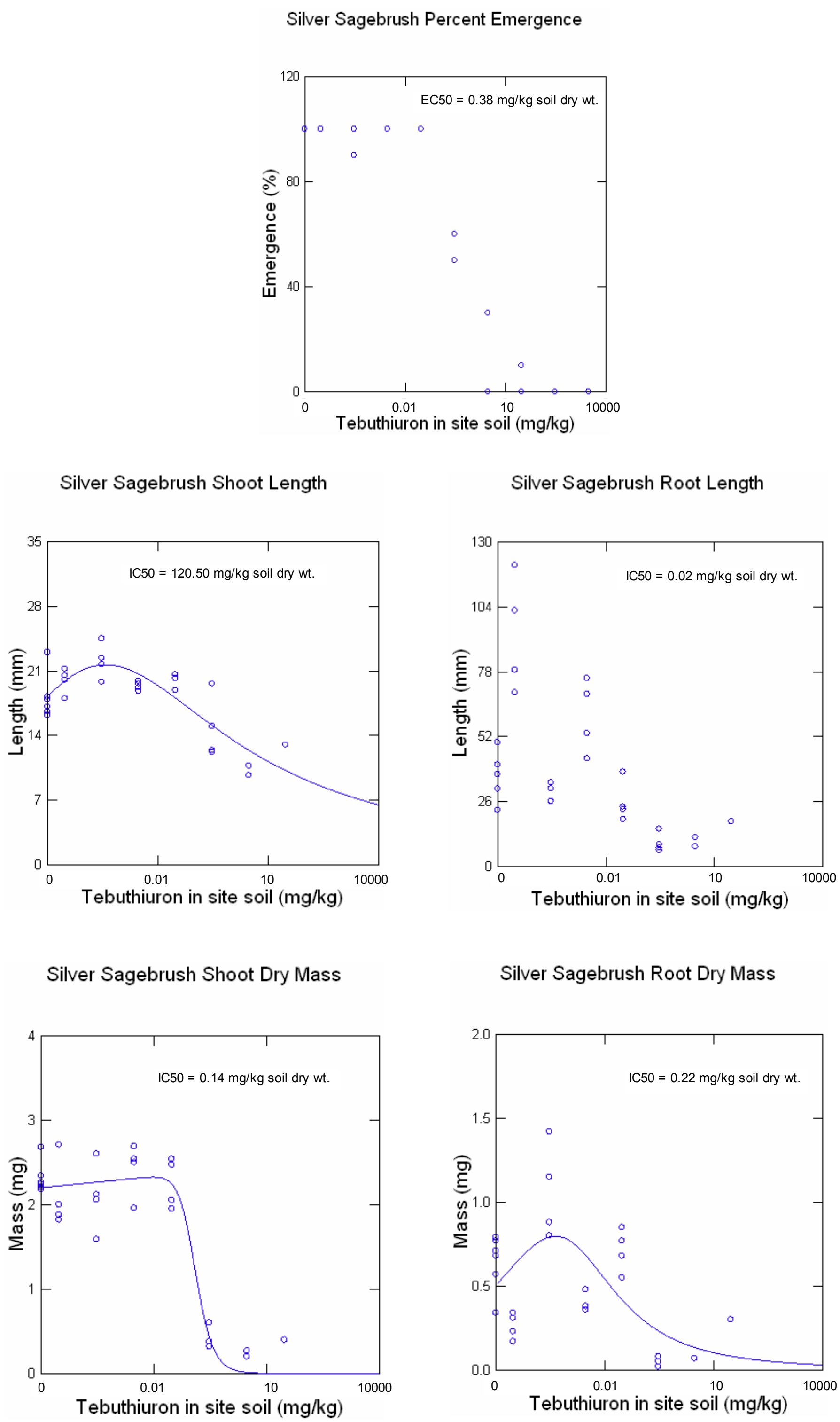


Figure G.2. Silver sagebrush seedling growth following 28 days of exposure to tebuthiuron-amended site soil. Open circles indicate data points and the solid line, where present, is the fitted regression line.

Soil Characteristics

Table G.5. Moisture content, conductivity and pH of test soils at the beginning (Day 0) and end (Day 28) of the test.

Soil Treatment	Initial pH[1]	Final pH[1]	Initial Conductivity[1] (µS/cm)	Final Conductivity[1] (µS/cm)	Initial[2] Soil Moisture (%) (ww-dw/ww)	Initial[2] Soil Moisture (%) (ww-dw/dw)	Initial[2] Soil Moisture (% WHC[3])
AS	7.17	7.58	224	626	36	56	75
CTS	7.24	7.95	113	320	25	34	58
CSS (0 mg/kg)	7.24	7.73	114	291	17	20	42
0.00003 mg/kg †	7.23	7.57	111	341	16	20	41
0.0003 mg/kg †	7.22	7.58	116	405	16	19	39
0.003 mg/kg †	7.23	7.55	118	480	16	20	41
SS (0.03 mg/kg)	8.26	8.43	242	508	14	16	37
0.3 mg/kg *	8.30	8.41	223	701	14	16	35
3 mg/kg *	8.29	8.29	246	928	15	17	39
30 mg/kg *	8.30	8.43	236	600	15	18	39
300 mg/kg *	8.30	8.45	246	594	15	17	38
3000 mg/kg *	8.27	8.32	253	829	18	21	48

AS Artificial soil
CTS Control top soil (uncontaminated reference soil)
CSS Control subsoil (uncontaminated reference soil used as the negative control soil for the SS-exposure treatments)
SS Site soil (soil contaminated with weathered tebuthiuron (0.03 mg/kg))
† These treatments are CSS amended with tebuthiuron and diluted with clean CSS to the nominal concentration indicated
* These treatments are SS amended with tebuthiuron to the nominal concentration indicated
[1] pH and conductivity were measured using a 2:1 water:soil slurry
[2] Moisture content measured at start of test only for plants
[3] % WHC - percent of water-holding capacity of the soil

Table G.6. Texture, organic matter content, carbon content and fertility of test soils (prior to testing).

Parameter	Artificial Soil	Control Topsoil (CTS)	Control Subsoil (CSS)	Site Soil (SS)
Soil Texture	Fine Sandy Loam	Sandy Clay Loam	Sandy Clay-Clay	Sandy Clay Loam
Sand (%)	79	66	48	60
Silt (%)	9	3	10	16
Clay (%)	13	30	42	25
Organic Matter (%)	7.9	3.1*	1.4*	1.5*
Total Carbon (%)	3.85	NA	NA	NA
Total Inorganic Carbon (%)	0.05	NA	NA	NA
Total Organic Carbon (%)	3.80	1.77	0.82	0.84
Total Nitrogen (%)	0.11	0.20	0.09	0.04
Total Phosphorus (mg/kg)	NA	473.6	312.5	368.3
Plant Available Phosphorus (mg/kg)	14	39	25	9

* Organic matter content (%) for these soils was calculated by multiplying total organic carbon (%) by 1.73

Comments

No organisms exhibiting unusual appearance, behaviour or undergoing unusual treatment were used in this test.

Test Method Modifications

1. Soil pH was measured using a soil-water slurry, which represents our normal practices and is a method modified from the Soil Analysis Handbook (1992), instead of using a $CaCl_2$ slurry, as recommended by the method for pH. This had no impact on the results of the test. The method of using $CaCl_2$ was developed for soil scientists who were comparing the pH of different soils, and wished to minimize the variability of the different pHs (McKeague, 1978). As a result, the $CaCl_2$ method will, by design, minimize the variability of the soil pH among soil samples, and will be less sensitive to differences in pH. In addition, soil pH measured in water is considered to be the pH closest to the pH of soil solution in the field (Hendershot *et al*., 1993).
2. Silver sagebrush seeds were broadcast seeded on Day 0, rather than individually planted; once the majority of seedlings had emerged they were thinned to ten individuals per test unit. Seeds were broadcast seeded and then thinned because the seed source contained a low percentage (34%) of pure live seed, and it was very difficult to distinguish between viable seeds and inert plant material (e.g., chaff). Therefore, to minimize bias in the test results due to inaccurate planting, seedling emergence was standardized by thinning the seedlings in a test unit to ten individuals of the most uniform size within a treatment and within a test unit.
3. Each test unit contained 425 g wet wt. of soil instead of 500 g wet wt., as recommended in the Environment Canada method. The amount of soil placed in each test unit was reduced because of the limited amount of control subsoil (CSS) remaining for use in this test. The reduced volume (425 g wet wt.) filled approximately half of a test unit, and therefore provided adequate soil volume for silver sagebrush seedlings to grow. In addition the volume provided by 425 g wet wt. was similar to that provided by 500 g wet wt. in other plant tests with these soils.

Test Method Deviations

1. Percent organic matter content was not measured in the site soils. However, total organic carbon was measured, and this parameter provides data of the same value and use as information provided by percent organic matter.
2. The datalogger used to monitor the temperature of the growth room for the silver sagebrush test was set to record temperature starting at 21:00 on Day 0 of the test. Four temperature readings were recorded per day, including 03:00, 09:00, 15:00, and 21:00. This temperature schedule resulted in 1 night time (03:00) temperature and 3 daytime temperatures (09:00, 15:00 and 21:00) being recorded. This schedule deviates from our SOP on the use of dataloggers, which states that the proper temperature recording schedule is 0:00, 06:00, 12:00 and 18:00 (2 daytime and 2 night time temperatures recorded per day). This deviation did not affect the results of the silver sagebrush test.
3. A temperature deviation was recorded by the min/max thermometer located with the silver sagebrush test in the growth room at the University of Guelph. On 2008-07-01, the min temperature was recorded as 7.3 °C, which is outside of the acceptable night time range of 15 ± 3 °C. The temperature deviation was not recorded by the datalogger, therefore was transient. No adverse effects on the silver sagebrush test were noted.

References

Hendershot W.H., H. Lalonde, and M. Duquette. 1993. Soil reaction and exchangeable acidity. P 141-145 in: Soil Sampling and Methods of Analysis, M.R. Carter, ed., Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida.

McKeague, J.A. ed. 1978. *Manual on Soil Sampling and Methods of Analysis*. Canadian Society of Soil Science, Ottawa, Ontario.

Microsoft Excel. 2002. Version 10.6501.6735 SP3. Copyright 1985-2001 Microsoft Corporation.

Soil Analysis Handbook. 1992. Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc., Georgia University Station, Athens, Georgia, 202 p.

Stephan, C.E. 1977. Methods for Calculating an LC50, p 65-84, In: Aquatic Toxicology and Hazard Evaluation, F.L. Mayer and J.L. Hamelink (eds). ASTM STP 634, American Society for Testing and Materials, Philadelphia, PA.

Systat Software Inc. (SSI). 2007. SYSTAT© 12 for Windows. Version 12.00.08. Systat Software Inc., USA.

Appendix H

Physical and Chemical Characteristics of Test Soils

TABLE 1: Physio-chemical Characteristics of Reference and Site Soils

Parameters	Units	Guideline[1] Agric., coarse surface soil	Reference Soils Topsoil (TS)	Reference Soils Subsoil (SS)	Site Subsoil (SS)
Physical Observations					
Material Type			Topsoil (Ah)	Subsoil (Bnjtj)	Fill
Field Texture			FSL	SCL	CL
Colour			Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.
Soil Staining			No	No	No
Hydrocarbons					
Benzene	mg/kg	0.073			<0.02
Toluene	mg/kg	0.49			<0.02
Ethylbenzene	mg/kg	0.21			<0.02
Xylenes	mg/kg	12			<0.02
F1 (C_6 to C_{10})	mg/kg	24			<0.1
F2 (C_{10} to C_{16})	mg/kg	130			<10
F3 (C_{16} to C_{34})	mg/kg	300			105
F4 (C_{34} to C_{50})	mg/kg	2,800			184
Soil Moisture Content	%	NG			5.6
Routine					
pH	pH-unit	6 to 8.5	6.93 ± 0.06	6.78 ± 0.06	7.75 ± 0.068
Electrical Conductivity (EC)	dS/m	2 (TS), 3 (SS)	0.675 ± 0.030	0.823 ± 0.038	1.128 ± 0.1701
Sodium Adsorption Ratio (SAR)	Ratio	4	0.30 ± 0.17	1.83 ± 0.059	2.20 ± 0.096
Exchangeable Sodium Percent	%	NG	<0.1 ± 0	1.40 ± 0.096	1.93 ± 0.148
Saturation	%	NG	70.8 ± 6.10	47.8 ± 1.13	45.5 ± 5.64
Soluble Salts					
Calcium (Ca)	mg/kg	NG	82.3 ± 5.29	49.8 ± 5.71	42.3 ± 11.30
Magnesium (Mg)	mg/kg	NG	15.8 ± 2.61	10.3 ± 1.13	16.3 ± 4.44
Sodium (Na)	mg/kg	NG	10.0 ± 6.72	1.9 ± 2.25	44.8 ± 9.62
Potassium (K)	mg/kg	NG	17.5 ± 2.04	0.6 ± 0.68	11.3 ± 2.01
Chloride (Cl)	mg/kg	NG	6.55 ± 2.132	0.50 ± 0.587	6.28 ± 1.634
Sulphate (SO_4)	mg/kg	NG	24.8 ± 5.47	47.3 ± 2.43	166.3 ± 49.14
Calcium (Ca)	meq/L	NG	5.838 ± 0.2381	5.270 ± 0.5932	4.640 ± 0.9282
Magnesium (Mg)	meq/L	NG	1.888 ± 0.1220	1.880 ± 0.1830	2.978 ± 0.5688
Sodium (Na)	meq/L	NG	0.62 ± 0.338	3.480 ± 0.2417	4.298 ± 0.6252
Potassium (K)	meq/L	NG	0.658 ± 0.0389	0.445 ± 0.0353	0.658 ± 0.0633
Chloride (Cl)	meq/L	NG	0.26 ± 0.075	0.408 ± 0.0364	0.388 ± 0.0777
Sulphate (SO_4)	meq/L	NG	0.738 ± 0.0967	2.073 ± 0.1182	7.518 ± 1.5198
Metals					
Arsenic (As)	mg/kg	17			6.0
Barium (Ba)	mg/kg	750			192.8
Beryllium (Be)	mg/kg	5			<0.6
Cadmium (Cd)	mg/kg	1.4			<0.1
Chromium (Cr), total	mg/kg	64			10.7
Cobalt (Co)	mg/kg	20			6.4
Copper (Cu)	mg/kg	63			12.1
Lead (Pb)	mg/kg	70			10.7
Mercury (Hg)	mg/kg	6.6			<0.5
Molybdenum (Mo)	mg/kg	4			0.4
Nickel (Ni)	mg/kg	50			14.8
Selenium (Se)	mg/kg	1			<0.5
Thallium (Tl)	mg/kg	1			<0.5
Vanadium (V)	mg/kg	130			12.7
Zinc (Zn)	mg/kg	200			48.7
Particle Size Analysis					
% Sand	%	NG	65.5 ± 2.96	47.8 ± 2.43	59.5 ± 3.53
% Silt	%	NG	3.3 ± 2.01	9.8 ± 1.76	15.8 ± 3.09
% Clay	%	NG	30.3 ± 1.48	42.0 ± 2.35	24.8 ± 1.13
CSSC Texture		NG	SCL	SC	SCL
Sterilants					
Tebuthiuron	mg/kg	0.11	<0.00016 ± 0	<0.00016 ± 0	0.03258 ± 0.001027
Gypsum Requirements					
Gypsum Requirements	tons/acre	NG	<0.1 ± 0	<0.1 ± 0	<0.1 ± 0
Available Nutrients					
Ammonia - N	mg/kg		8.0 ± 0.96	5.8 ± 0.59	3.8 ± 0.59
Nitrate-N	mg/kg	NG	<1 ± 0	<1 ± 0	4.0 ± 0.96
Phosphate-P	mg/kg	NG	39.3 ± 1.13	24.8 ± 1.13	9.3 ± 3.38
Potassium-K	mg/kg	NG	375.3 ± 21.23	359.0 ± 45.76	354.5 ± 36.64
Sulfur-S	mg/kg		11.8 ± 1.8	33.0 ± 2.15	120.0 ± 24.19
Total Nutrients					
Total Nitrogen	mg/kg	NG	0.203 ± 0.0294	0.088 ± 0.0201	0.048 ± 0.0201
Total Phosphorus	mg/kg	NG	473.6 ± 21.85	312.53 ± 38.164	368.325 ± 110.6422
Total Carbon					
Total Organic Carbon	%	NG	1.770 ± 0.3576	0.818 ± 0.1220	0.838 ± 0.1834

Data are **mean (n=4) ± 95% confidence interval; Calculated as Average X ±$t_{\alpha,d.f.}(s/\sqrt{n})$**, where α=0.05, d.f.=3, and $t_{0.05,3}$=2.353 (Scheaffer and McClave, 1990)

Hydrocarbon and metal results are based on single measurements.

Notes:

[1] Alberta Environment (AENV). June 2007. Alberta Tier I Soil and Groundwater Remediation Guidelines.

NG - No guidelines established.

Soil Texture Abbreviations - FSL=Fine Sandy Loam, SCL=Sandy Clay Loam, SC=Sandy Clay.

Soil Colour Abbreviations - Dk.=Dark, Gr.=Grey or Greyish, Br.=Brown.

TABLE 2: ANALYTICAL RESULTS FOR ECOTOXICITY SOIL SAMPLES

Control–Topsoil

Parameters	Units	Guideline[1]	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Minimum	Maximum	Mean	Standard Deviation
Physical Observations										
Material Type			Topsoil (Ah)	Topsoil (Ah)	Topsoil (Ah)	Topsoil (Ah)				
Field Texture			FSL	FSL	FSL	FSL				
Colour			Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.				
Measured Headspace Vapours	% LEL/ppm		NM	NM	NM	NM				
Soil Staining			No	No	No	No				
Hydrocarbons										
Benzene	mg/kg	0.073								
Toluene	mg/kg	0.49								
Ethylbenzene	mg/kg	0.21								
Xylenes	mg/kg	12								
F1 (C_6 to C_{10})	mg/kg	24								
F2 (C_{10} to C_{16})	mg/kg	130								
F3 (C_{16} to C_{34})	mg/kg	300								
F4 (C_{34} to C_{50})	mg/kg	2,800								
Soil Moisture Content	%	NG								
Routine										
pH	pH-unit	6 to 8.5	6.9	6.9	6.9	7.0	6.9	7.0	6.93	0.05
Electrical Conductivity (EC)	dS/m	**	0.68	0.70	0.64	0.68	0.64	0.70	0.675	0.025
Sodium Adsorption Ratio (SAR)	Ratio	**	0.2	0.3	0.2	0.5	0.2	0.5	0.30	0.14
Exchangeable Sodium Percent	%	NG	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
Saturation	%	NG	67	67	71	78	67	78	70.8	5.2
Soluble Salts										
Calcium (Ca)	mg/kg	NG	77	82	82	88	77	88	82.3	4.5
Magnesium (Mg)	mg/kg	NG	14	15	15	19	14	19	15.8	2.2
Sodium (Na)	mg/kg	NG	5	10	7	18	5	18	10.0	5.7
Potassium (K)	mg/kg	NG	16	17	17	20	16	20	17.5	1.7
Chloride (Cl)	mg/kg	NG	5.1	7.4	5.0	8.7	5.0	8.7	6.55	1.81
Sulphate (SO_4)	mg/kg	NG	20	25	23	31	20	31	24.8	4.6
Calcium (Ca)	meq/L	NG	5.81	6.12	5.78	5.64	5.64	6.12	5.838	0.202
Magnesium (Mg)	meq/L	NG	1.81	1.92	1.80	2.02	1.80	2.02	1.888	0.104
Sodium (Na)	meq/L	NG	0.38	0.66	0.43	1.01	0.38	1.01	0.620	0.287
Potassium (K)	meq/L	NG	0.62	0.69	0.64	0.68	0.62	0.69	0.658	0.033
Chloride (Cl)	meq/L	NG	0.21	0.31	0.20	0.32	0.20	0.32	0.260	0.064
Sulphate (SO_4)	meq/L	NG	0.65	0.78	0.69	0.83	0.65	0.83	0.738	0.082
Metals										
Arsenic (As)	mg/kg	17								
Barium (Ba)	mg/kg	750								
Beryllium (Be)	mg/kg	5								
Cadmium (Cd)	mg/kg	1.4								
Chromium (Cr), total	mg/kg	64								
Cobalt (Co)	mg/kg	20								
Copper (Cu)	mg/kg	63								
Lead (Pb)	mg/kg	70								
Mercury (Hg)	mg/kg	6.6								
Molybdenum (Mo)	mg/kg	4								
Nickel (Ni)	mg/kg	50								
Selenium (Se)	mg/kg	1								
Thallium (Tl)	mg/kg	1								
Vanadium (V)	mg/kg	130								
Zinc (Zn)	mg/kg	200								
Particle Size Analysis										
% Sand	%	NG	62	66	66	68	62	68	65.5	2.5
% Silt	%	NG	5	4	3	1	1	5	3.3	1.7
% Clay	%	NG	32	29	30	30	29	32	30.3	1.3
CSSC Texture		NG	SCL	SCL	SCL	SCL			SCL	
Sterilants										
Tebuthiuron	mg/kg	0.11	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	0
Gypsum Requirements										
Gypsum Requirements	tons/acre	NG	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0
Available Nutrients										
Ammonia - N	mg/kg		7	8	8	9	7	9	8.0	0.8
Nitrate-N	mg/kg	NG	< 1	1	< 1	1	< 1	1		
Phosphate-P	mg/kg	NG	39	40	38	40	38	40	39.3	1.0
Potassium -K	mg/kg	NG	369	377	399	356	356	399	375.3	18.0
Sulfur -S	mg/kg		10	13	11	13	10	13	11.8	1.5
Total Nutrients										
Total Nitrogen	mg/kg	NG	0.19	0.19	0.24	0.19	0.19	0.24	0.203	0.025
Total Phosphorus	mg/kg	NG	477.2	498.0	463.3	455.7	455.7	498.0	473.55	18.57
Total Carbon										
Total Organic Carbon	%	NG	1.56	1.68	2.22	1.62	1.56	2.22	1.770	0.304

Control–Subsoil

Parameters	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Minimum	Maximum	Mean	Standard Deviation
Physical Observations								
Material Type	Subsoil (Bnjtj)	Subsoil (Bnjtj)	Subsoil (Bnjtj)	Subsoil (Bnjtj)				
Field Texture	SCL	SCL	SCL	SCL				
Colour	Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.				
Measured Headspace Vapours	NM	NM	NM	NM				
Soil Staining	No	No	No	No				
Hydrocarbons								
Benzene								
Toluene								
Ethylbenzene								
Xylenes								
F1 (C_6 to C_{10})								
F2 (C_{10} to C_{16})								
F3 (C_{16} to C_{34})								
F4 (C_{34} to C_{50})								
Soil Moisture Content								
Routine								
pH	6.8	6.8	6.8	6.7	6.7	6.8	6.78	0.05
Electrical Conductivity (EC)	0.85	0.80	0.79	0.85	0.79	0.85	0.823	0.032
Sodium Adsorption Ratio (SAR)	1.8	1.8	1.9	1.8	1.8	1.9	1.83	0.05
Exchangeable Sodium Percent	1.4	1.3	1.5	1.4	1.3	1.5	1.40	0.08
Saturation	47	49	47	48	47	49	47.8	1.0
Soluble Salts								
Calcium (Ca) mg/kg	51	45	47	56	45	56	49.8	4.9
Magnesium (Mg) mg/kg	10	9	11	11	9	11	10.3	1.0
Sodium (Na) mg/kg	37	35	39	39	35	39	37.5	1.9
Potassium (K) mg/kg	8	7	7	8	7	8	7.5	0.6
Chloride (Cl) mg/kg	7.4	7.2	6.4	6.5	6.4	7.4	6.88	0.50
Sulphate (SO_4) mg/kg	49	46	45	49	45	49	47.3	2.1
Calcium (Ca) meq/L	5.50	4.67	5.08	5.83	4.67	5.83	5.270	0.504
Magnesium (Mg) meq/L	1.92	1.65	1.97	1.98	1.65	1.98	1.880	0.156
Sodium (Na) meq/L	3.48	3.19	3.64	3.61	3.19	3.64	3.480	0.205
Potassium (K) meq/L	0.46	0.42	0.42	0.48	0.42	0.48	0.445	0.030
Chloride (Cl) meq/L	0.45	0.41	0.39	0.38	0.38	0.45	0.408	0.031
Sulphate (SO_4) meq/L	2.18	1.96	2.02	2.13	1.96	2.18	2.073	0.100
Metals								
Arsenic (As)								
Barium (Ba)								
Beryllium (Be)								
Cadmium (Cd)								
Chromium (Cr), total								
Cobalt (Co)								
Copper (Cu)								
Lead (Pb)								
Mercury (Hg)								
Molybdenum (Mo)								
Nickel (Ni)								
Selenium (Se)								
Thallium (Tl)								
Vanadium (V)								
Zinc (Zn)								
Particle Size Analysis								
% Sand	48	48	45	50	45	50	47.8	2.1
% Silt	11	11	9	8	8	11	9.8	1.5
% Clay	41	41	45	41	41	45	42.0	2.0
CSSC Texture	SC	SC	C	SC			SC	
Sterilants								
Tebuthiuron	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	0
Gypsum Requirements								
Gypsum Requirements	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0
Available Nutrients								
Ammonia - N	6	6	5	6	5	6	5.8	0.5
Nitrate-N	1	< 1	< 1	< 1	< 1	1		
Phosphate-P	24	24	26	25	24	26	24.8	1.0
Potassium -K	416	331	338	351	331	416	359.0	38.9
Sulfur -S	35	31	32	34	31.0	35.0	33.0	1.8
Total Nutrients								
Total Nitrogen	0.11	0.07	0.08	0.09	0.07	0.11	0.088	0.017
Total Phosphorus	346.2	281.6	334.2	288.1	281.60	346.20	312.53	32.44
Total Carbon								
Total Organic Carbon	0.89	0.82	0.67	0.89	0.67	0.89	0.818	0.104

Site–Subsoil

Parameters	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Minimum	Maximum	Mean	Standard Deviation
Physical Observations								
Material Type	Fill	Fill	Fill	Fill				
Field Texture	CL	CL	CL	CL				
Colour	Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.				
Measured Headspace Vapours	NM	NM	NM	NM				
Soil Staining	No	No	No	No				
Hydrocarbons								
Benzene	< 0.02							
Toluene	< 0.02							
Ethylbenzene	< 0.02							
Xylenes	< 0.02							
F1 (C_6 to C_{10})	< 0.1							
F2 (C_{10} to C_{16})	< 10							
F3 (C_{16} to C_{34})	105							
F4 (C_{34} to C_{50})	184							
Soil Moisture Content	5.6							
Routine								
pH	7.7	7.7	7.8	7.8	7.7	7.8	7.75	0.06
Electrical Conductivity (EC)	1.21	1.24	0.92	1.14	0.92	1.24	1.128	0.145
Sodium Adsorption Ratio (SAR)	2.3	2.2	2.1	2.2	2.1	2.3	2.20	0.08
Exchangeable Sodium Percent	2.1	1.9	1.8	1.9	1.8	2.1	1.93	0.13
Saturation	43	49	40	50	40	50	45.5	4.8
Soluble Salts								
Calcium (Ca) mg/kg	44	52	29	44	29	52	42.3	9.6
Magnesium (Mg) mg/kg	17	20	11	17	11	20	16.3	3.8
Sodium (Na) mg/kg	47	52	33	47	33	52	44.8	8.2
Potassium (K) mg/kg	11	13	9	12	9	13	11.3	1.7
Chloride (Cl) mg/kg	7.1	6.8	4.2	7.0	4.2	7.1	6.28	1.39
Sulphate (SO_4) mg/kg	160	211	112	182	112	211	166.3	41.8
Calcium (Ca) meq/L	5.20	5.33	3.63	4.40	3.63	5.33	4.640	0.789
Magnesium (Mg) meq/L	3.26	3.44	2.35	2.86	2.35	3.44	2.978	0.483
Sodium (Na) meq/L	4.76	4.68	3.62	4.13	3.62	4.76	4.298	0.531
Potassium (K) meq/L	0.69	0.71	0.59	0.64	0.59	0.71	0.658	0.054
Chloride (Cl) meq/L	0.46	0.39	0.30	0.40	0.30	0.46	0.388	0.066
Sulphate (SO_4) meq/L	7.72	8.96	5.82	7.57	5.82	8.96	7.518	1.292
Metals								
Arsenic (As)	6.0							
Barium (Ba)	192.8							
Beryllium (Be)	< 0.6							
Cadmium (Cd)	< 0.1							
Chromium (Cr), total	10.7							
Cobalt (Co)	6.4							
Copper (Cu)	12.1							
Lead (Pb)	10.7							
Mercury (Hg)	< 0.5							
Molybdenum (Mo)	0.4							
Nickel (Ni)	14.8							
Selenium (Se)	< 0.5							
Thallium (Tl)	< 0.5							
Vanadium (V)	12.7							
Zinc (Zn)	48.7							
Particle Size Analysis								
% Sand	58	58	64	58	58	64	59.5	3.0
% Silt	16	17	12	18	12	18	15.8	2.6
% Clay	26	25	24	24	24	26	24.8	1.0
CSSC Texture	SCL	SCL	SCL	SCL			SCL	
Sterilants								
Tebuthiuron	0.0315	0.0336	0.0324	0.0328	0.0315	0.0336	0.03258	0.00087
Gypsum Requirements								
Gypsum Requirements	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0
Available Nutrients								
Ammonia - N	4	4	3	4	3	4	3.8	0.5
Nitrate-N	4	4	3	5	3	5	4.0	0.8
Phosphate-P	9	13	6	9	6	13	9.3	2.9
Potassium -K	380	371	310	357	310	380	354.5	31.1
Sulfur -S	123	143	93	121	93	143	120.0	20.6
Total Nutrients								
Total Nitrogen	0.04	0.05	0.07	0.03	0.03	0.07	0.048	0.017
Total Phosphorus	261.4	318.1	457.6	436.2	261.40	457.60	368.325	94.044
Total Carbon								
Total Organic Carbon	0.74	1.07	0.76	0.78	0.74	1.07	0.838	0.156

Notes:

1 Alberta Environment (AENV). June 2007. Alberta Tier I Soil and Groundwater Remediation Guidelines, Agricultural Land Use, Coarse Surface Soil.

AENV. 2001. Salt Contamination Assessment and Remediation Guidelines. Referenced guidelines are for Agricultural Land Use.

NG - No guidelines established.

Bold - Greater than the referenced guideline.

Soil Texture Abbreviations - S=Sand, Si=Silt, C=Clay, L=Loam, SCL=Sandy Clay Loam, SC=Sandy Clay.

Soil Colour Abbreviations - Bl.=Black, Dk.=Dark, Lt.=Light, Br.=Brown, Gr.=Grey or Greyish, Ol. = Olive, Ye.=Yellowish.

Parameter**	Material Type: Topsoil (A-horizon) Value	Topsoil (A-horizon) Rational	Subsoil (B-horizon) Value	Subsoil (B-horizon) Rational
EC (dS/m)	<2	Good	<3	Good
SAR	<4	Good	<4	Good

Appendix I

Chemical Contaminant Analyses
of Test Soils

TABLE 2: ANALYTICAL RESULTS FOR ECOTOXICITY SOIL SAMPLES

Parameters	Units	Guideline[1]	Control–Topsoil								Control–Subsoil								Site–Subsoil							
			Replicate #1	Replicate #2	Replicate #3	Replicate #4	Minimum	Maximum	Mean	Standard Deviation	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Minimum	Maximum	Mean	Standard Deviation	Replicate #1	Replicate #2	Replicate #3	Replicate #4	Minimum	Maximum	Mean	Standard Deviation
Physical Observations																										
Material Type			Topsoil (Ah)	Topsoil (Ah)	Topsoil (Ah)	Topsoil (Ah)					Subsoil (Bnjtj)	Subsoil (Bnjtj)	Subsoil (Bnjtj)	Subsoil (Bnjtj)					Fill	Fill	Fill	Fill				
Field Texture			FSL	FSL	FSL	FSL					SCL	SCL	SCL	SCL					CL	CL	CL	CL				
Colour			Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.					Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.					Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.	Dk. Gr. Br.				
Measured Headspace Vapours	% LEL/ppm		NM	NM	NM	NM					NM	NM	NM	NM					NM	NM	NM	NM				
Soil Staining			No	No	No	No					No	No	No	No					No	No	No	No				
Hydrocarbons																										
Benzene	mg/kg	0.073																	< 0.02							
Toluene	mg/kg	0.49																	< 0.02							
Ethylbenzene	mg/kg	0.21																	< 0.02							
Xylenes	mg/kg	12																	< 0.02							
F1 (C_6 to C_{10})	mg/kg	24																	< 0.1							
F2 (C_{10} to C_{16})	mg/kg	130																	< 10							
F3 (C_{16} to C_{34})	mg/kg	300																	105							
F4 (C_{34} to C_{50})	mg/kg	2,800																	184							
Soil Moisture Content	%	NG																	5.6							
Routine																										
pH	pH-unit	6 to 8.5	6.9	6.9	6.9	7.0	6.9	7.0	6.93	0.05	6.8	6.8	6.8	6.7	6.7	6.8	6.78	0.05	7.7	7.7	7.8	7.8	7.7	7.8	7.75	0.06
Electrical Conductivity (EC)	dS/m	**	0.68	0.70	0.64	0.68	0.64	0.70	0.675	0.025	0.85	0.80	0.79	0.85	0.79	0.85	0.823	0.032	1.21	1.24	0.92	1.14	0.92	1.24	1.128	0.145
Sodium Adsorption Ratio (SAR)	Ratio	**	0.2	0.3	0.2	0.5	0.2	0.5	0.30	0.14	1.8	1.8	1.9	1.8	1.8	1.9	1.83	0.05	2.3	2.2	2.1	2.2	2.1	2.3	2.20	0.08
Exchangeable Sodium Percent	%	NG	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1		1.4	1.3	1.5	1.4	1.3	1.5	1.40	0.08	2.1	1.9	1.8	1.9	1.8	2.1	1.93	0.13
Saturation	%	NG	67	67	71	78	67	78	70.8	5.2	47	49	47	48	47	49	47.8	1.0	43	49	40	50	40	50	45.5	4.8
Soluble Salts																										
Calcium (Ca)	mg/kg	NG	77	82	82	88	77	88	82.3	4.5	51	45	47	56	45	56	49.8	4.9	44	52	29	44	29	52	42.3	9.6
Magnesium (Mg)	mg/kg	NG	14	15	15	19	14	19	15.8	2.2	10	9	11	11	9	11	10.3	1.0	17	20	11	17	11	20	16.3	3.8
Sodium (Na)	mg/kg	NG	5	10	7	18	5	18	10.0	5.7	37	35	39	39	35	39	37.5	1.9	47	52	33	47	33	52	44.8	8.2
Potassium (K)	mg/kg	NG	16	17	17	20	16	20	17.5	1.7	8	7	7	8	7	8	7.5	0.6	11	13	9	12	9	13	11.3	1.7
Chloride (Cl)	mg/kg	NG	5.1	7.4	5.0	8.7	5.0	8.7	6.55	1.81	7.4	7.2	6.4	6.5	6.4	7.4	6.88	0.50	7.1	6.8	4.2	7.0	4.2	7.1	6.28	1.39
Sulphate (SO_4)	mg/kg	NG	20	25	23	31	20	31	24.8	4.6	49	46	45	49	45	49	47.3	2.1	160	211	112	182	112	211	166.3	41.8
Calcium (Ca)	meq/L	NG	5.81	6.12	5.78	5.64	5.64	6.12	5.838	0.202	5.50	4.67	5.08	5.83	4.67	5.83	5.270	0.504	5.20	5.33	3.63	4.40	3.63	5.33	4.640	0.789
Magnesium (Mg)	meq/L	NG	1.81	1.92	1.80	2.02	1.80	2.02	1.888	0.104	1.92	1.65	1.97	1.98	1.65	1.98	1.880	0.156	3.26	3.44	2.35	2.86	2.35	3.44	2.978	0.483
Sodium (Na)	meq/L	NG	0.38	0.66	0.43	1.01	0.38	1.01	0.620	0.287	3.48	3.19	3.64	3.61	3.19	3.64	3.480	0.205	4.76	4.68	3.62	4.13	3.62	4.76	4.298	0.531
Potassium (K)	meq/L	NG	0.62	0.69	0.64	0.68	0.62	0.69	0.658	0.033	0.46	0.42	0.42	0.48	0.42	0.48	0.445	0.030	0.69	0.71	0.59	0.64	0.59	0.71	0.658	0.054
Chloride (Cl)	meq/L	NG	0.21	0.31	0.20	0.32	0.20	0.32	0.260	0.064	0.45	0.41	0.39	0.38	0.38	0.45	0.408	0.031	0.46	0.39	0.30	0.40	0.30	0.46	0.388	0.066
Sulphate (SO_4)	meq/L	NG	0.65	0.78	0.69	0.83	0.65	0.83	0.738	0.082	2.18	1.96	2.02	2.13	1.96	2.18	2.073	0.100	7.72	8.96	5.82	7.57	5.82	8.96	7.518	1.292
Metals																										
Arsenic (As)	mg/kg	17																	6.0							
Barium (Ba)	mg/kg	750																	192.8							
Beryllium (Be)	mg/kg	5																	< 0.6							
Cadmium (Cd)	mg/kg	1.4																	< 0.1							
Chromium (Cr), total	mg/kg	64																	10.7							
Cobalt (Co)	mg/kg	20																	6.4							
Copper (Cu)	mg/kg	63																	12.1							
Lead (Pb)	mg/kg	70																	10.7							
Mercury (Hg)	mg/kg	6.6																	< 0.5							
Molybdenum (Mo)	mg/kg	4																	0.4							
Nickel (Ni)	mg/kg	50																	14.8							
Selenium (Se)	mg/kg	1																	< 0.5							
Thallium (Tl)	mg/kg	1																	< 0.5							
Vanadium (V)	mg/kg	130																	12.7							
Zinc (Zn)	mg/kg	200																	48.7							
Particle Size Analysis																										
% Sand	%	NG	62	66	66	68	62	68	65.5	2.5	48	48	45	50	45	50	47.8	2.1	58	58	64	58	58	64	59.5	3.0
% Silt	%	NG	5	4	3	1	1	5	3.3	1.7	11	11	9	8	8	11	9.8	1.5	16	17	12	18	12	18	15.8	2.6
% Clay	%	NG	32	29	30	30	29	32	30.3	1.3	41	41	45	41	41	45	42.0	2.0	26	25	24	24	24	26	24.8	1.0
CSSC Texture		NG	SCL	SCL	SCL	SCL			SCL		SC	SC	C	SC			SC		SCL	SCL	SCL	SCL			SCL	
Sterilants																										
Tebuthiuron	mg/kg	0.11	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	0	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	< 0.00016	0	0.0315	0.0336	0.0324	0.0328	0.0315	0.0336	0.03258	0.00087
Gypsum Requirements																										
Gypsum Requirements	tons/acre	NG	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0
Available Nutrients																										
Ammonia - N	mg/kg		7	8	8	9	7	9	8.0	0.8	6	6	5	6	5	6	5.8	0.5	4	4	3	4	3	4	3.8	0.5
Nitrate-N	mg/kg	NG	< 1	1	< 1	1	< 1	1			1	< 1	< 1	< 1	< 1	1			4	4	3	5	3	5	4.0	0.8
Phosphate-P	mg/kg	NG	39	40	38	40	38	40	39.3	1.0	24	24	26	25	24	26	24.8	1.0	9	13	6	9	6	13	9.3	2.9
Potassium -K	mg/kg	NG	369	377	399	356	356	399	375.3	18.0	416	331	338	351	331	416	359.0	38.9	380	371	310	357	310	380	354.5	31.1
Sulfur -S	mg/kg		10	13	11	13	10	13	11.8	1.5	35	31	32	34	31.0	35.0	33.0	1.8	123	143	93	121	93	143	120.0	20.6
Total Nutrients																										
Total Nitrogen	mg/kg	NG	0.19	0.19	0.24	0.19	0.19	0.24	0.203	0.025	0.11	0.07	0.08	0.09	0.07	0.11	0.088	0.017	0.04	0.05	0.07	0.03	0.03	0.07	0.048	0.017
Total Phosphorus	mg/kg	NG	477.2	498.0	463.3	455.7	455.7	498.0	473.55	18.57	346.2	281.6	334.2	288.1	281.60	346.20	312.53	32.44	261.4	318.1	457.6	436.2	261.40	457.60	368.325	94.044
Total Carbon																										
Total Organic Carbon	%	NG	1.56	1.68	2.22	1.62	1.56	2.22	1.770	0.304	0.89	0.82	0.67	0.89	0.67	0.89	0.818	0.104	0.74	1.07	0.76	0.78	0.74	1.07	0.838	0.156

Notes:

[1] Alberta Environment (AENV). June 2007. Alberta Tier I Soil and Groundwater Remediation Guidelines, Agricultural Land Use, Coarse Surface Soil.

AENV. 2001. Salt Contamination Assessment and Remediation Guidelines. Referenced guidelines are for Agricultural Land Use.

NG - No guidelines established.

Bold - Greater than the referenced guideline.

Soil Texture Abbreviations - S=Sand, Si=Silt, C=Clay, L=Loam, SCL=Sandy Clay Loam, SC=Sandy Clay.

Soil Colour Abbreviations - Bl.=Black, Dk.=Dark, Lt.=Light, Br.=Brown, Gr.=Grey or Greyish, Ol. = Olive, Ye.=Yellowish.

Parameter**	Material Type			
	Topsoil (A-horizon)		Subsoil (B-horizon)	
	Value	Rational	Value	Rational
EC (dS/m)	<2	Good	<3	Good
SAR	<4	Good	<4	Good