Equilibrium Environmental Inc.

Development of a Proposed Ecological Direct Soil Contact Selenium Guideline and Supporting Research

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EXECUTIVE SUMMARY

Selenium (Se) toxicity and interaction with sulphate (SO_4) was assessed for potentially updating the soil Se ecological direct contact guideline in Alberta and Canada. Se is an essential nutrient for plants but can cause toxicity at higher concentrations. The current soil Se guideline of 1 mg/kg is based on two studies (conducted in 1991 and 1974) of limited in scope that involved a small number of plant species. Research activities conducted under funding by the Petroleum Technology Alliance Canada (PTAC), combined with previously published work, demonstrates elevated soil SO_4 concentrations (a common occurrence in the Western Canadian Sedimentary Basin) can ameliorate Se toxicity. This deficiency can potentially occur at Se background concentration ranges in Canada (up to 4.7 mg/kg) when SO_4 concentrations are relatively high.

Six plant species (alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), northern wheatgrass (*Elymus lanceolatus*), red fescue (*Festuca rubra*)) and two invertebrate species (earthworms *(Eisenia andrei*) and springtails (*Folsomia candida*)) were tested in coarse and fine soils under control, low, medium, and high Se and SO_4 concentrations, to evaluate Se toxicity as a function of variable SO_4 concentrations. Se concentrations ranged from <0.3 to 31 mg/kg (administered as selenate) and SO_4 concentrations ranged from 28 to 1,482 mg/kg. Benchmark Dose Software (BMDS) was used to characterize and test dose-response curves and calculate 25% Effect Concentration (EC_{25}) values for use in guideline derivation. Plant toxicological enpoints included germination percent, root and shoot length, and dry weight. Intertebrate endpoints included number of live adults, juveniles, and dry weight of juveniles for invertebrates. EC_{25} values were plotted to derive a Species Sensitivity Distribution (SSD) as a function of soil texture and SO_4 concentration, following a detailed statistical analysis of model fit. The 25th percentile from each SSD was used for ecological guidelines development, as a function of SO_4 concentration.

Results for coarse textured soils suggest that under variable SO_4 concentrations, the existing Se guideline of 1 mg/kg may be appropriate and protective. However, of all the experiments conducted, those involving coarse soils were associated with the highest response variability, and lowest relative confidence, particularly with increasing SO_4 concentrations. It was postulated that this was due to difficulties in dosing plant roots growing in a readily drainable coarse soil with a water soluble substance (*i.e.*, Se as selenate), given the requirement of regular water addition for plant growth. Results for fine textured soils suggest a more appropriate minimum baseline Se soil quality guideline of 2 mg/kg is appropriate. At higher SO_4 concentrations (*i.e.*, > 1000 mg/kg), a guideline of 5 mg/kg would be more appropriate due to the amelioration of Se toxicity at higher SO_4 concentrations. The analysis by nature was complex given: Se induced toxicity at high Se concentrations combined with low SO_4 concentrations; SO_4 ameliorated Se toxicity at higher SO_4 concentrations; higher Se concentrations overcome SO_4 induced low Se dose nutritional deficiency; and, SO_4 may induce a distinct toxic effect at higher SO_4 concentrations. Further research work with coarse soils may improve the accuracy of the Se-SO_4 interaction and may allows for a more variable guideline to be developed as a function of SO_4 concentration.

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1 INTRODUCTION AND SCOPE

Selenium (Se) is an essential nutrient in plants, present in soils throughout Alberta and Canada. The Alberta Environment and Parks (AEP, 2019) Tier 1 guideline is 1 mg/kg, which is identical to the Canadian Council of Ministers of the Environment (CCME, 2009) soil quality guideline (SQG). It was based on protection of the ecological direct contact pathway and derived from two toxicological studies of limited scope (Carson, 1991; Singh and Singh, 1979).

It is not uncommon to find naturally occurring Se at concentrations above the 1 mg/kg guideline (Penny, 2004; CCME, 2009). It is furthermore not uncommon to find elevated Se in drilling waste disposal areas associated with oil and gas activities, as well as waste water discharges from coal mines. This leads to a challenge in determining whether an anthopogenic activity has lead to an increase in soil Se above the guideline, or whether it was simply a naturally elevated area of soil Se that was encountered.

A final question remains regarding the relevance of the guideline to natural systems. If 1 mg/kg Se is toxic towards soil dependent biota, it might be expected that areas with naturally elevated soil Se would be associated with reduced plant growth and health. No published information could be identified where naturally elevated selenium in Canadian soils was associated with such an effect. Clearly, there is a complexity of interaction at play, which may be in part related to Se being both an essential nutrient for, and a relatively low concentration toxicant towards, soil dependent biota. And the nutritional as well as adverse toxicological effects may be strongly dependent on factors such as soil texture, pH, redox conditions, etc. Another plausible explanation is that sulphate (SO_4) has the potential to alter the toxicity of Se via competition for uptake with soluble forms of Se (such as selenate or selenite), and a considerably large area of Canada is associated with elevated SO4 concentrations in soil that may overlap with areas of elevated Se.

This potential research topic of value was brought to the attention of the Petroleum Technology Alliance Canada (PTAC) by Equilibrium Environmental Inc. (EEI) based on an assessment of literature data conducted in 2011. PTAC endorsed the project, selected a Champion to guide activities and progress, and provided the necessary funding. Research was conducted during the years 2011 to 2019 to generate the required toxicological dataset and data analysis, required for an update to the Se SQG. The ultimate objective of the research was to determine whether the existing guideline of 1 mg/kg is adequately supported, and if not, conduct the studies needed to provide a more rigorous and defensible guideline for use in Alberta and potentially across Canada. Additional contractual obligations included designing and managing toxicology studies, analyzing results, and developing a preliminary SQG using protocols and methods developed by CCME (2006).

The first toxicological study was designed and initiated by Equilibrium in 2011, in collaboration with a Masters thesis completed at the Royal Roads University, British Columbia, by Prediger (2012), with linkage to Lakeland College, Alberta. Toxicology lab services were provided by

Alberta Innovates – Technology Futures (who later changed their name to Alberta Innotech). Research was conducted using a single plant species (*Medicago sativa* or alfalfa), artificial soils, with variable Se and SO_4 concentrations.

The second study, proposed and designed by EEI, funded and guided by PTAC, used the lab services of Alberta Innotech in 2014. Six common agricultural and garden species were studied, specifically, *Daucus carota* (carrot), *Elymus lanceolatus* (northern wheatgrass), *Festuca rubra* (red fescue), *M. sativa* (alfalfa), *Hordeum vulgare* (barley), and *Cucumis sativus* (cucumber). EEI provided standard coarse and fine textured soils collected near Vulcan, AB, which were low in SO_4 concentration and represented suitable controls. The primary objective of the 2014 study was to quantify Se toxicity to common plants in background low SO_4 concentration soils, to serve as a baseline for further study.

The third study took place in 2015, using the lab services of Alberta Innotech, involving the same six plant species and soil types as in 2014, but with the addition of low, mid, and high SO_4 concentration exposure groups. The primary objective was to quantify Se toxicity to common agricultural and garden plant species as a function of variable and increasing SO_4 soil concentrations.

In 2016 to 2017, the fourth toxicity study took place using soil invertebrates, for which a very limited Se toxicology dataset exists. Two species, *Folsomia candida* (springtails) and *Eisenia andrei* (earthworms), were selected as standard test organisms and exposed to varying concentrations of Se and SO4 in coarse and fine textured soils. One additional plant and invertebrate experiment was conducted involving a lower SO_4 concentration to investigate threshold ranges in SO_4-Se interactions.

Upon completion of these studies, EEI submitted a subset of soil and plant tissue samples to Exova laboratories (Exova) for further analytical chemistry work. The purpose was to provide some preliminary data on potential plant uptake and bioconcenetration as well as the applicability of developing a soil selenium guideline on a saturated paste basis.

The remainder of this report is organized as follows:

- Background (Section 2);
- Methodology (Section 3)
- 2011 to 2017 Experimental Results Summary (Section 4);
- Data Analysis and Interpretation (Section 5);
- Development of Preliminary Ecological Direct Soil Contact Guidelines (Section 6);
- Discussion (Section 7);
- Closure and References (Sections 8 and 9).

Supporting information is provided in a series of detailed appendices.

2 BACKGROUND INFORMATION

2.1 SELENIUM IN THE ENVIRONMENT

Selenium (Se) is a naturally occurring trace mineral element, commonly found in Cretaceous marine sedimentary or seleniferous rock (Zhao *et al.*, 2005). Se levels up to 11.7 mg/kg were found in Cretaceous sedimentary rock formations in the White Specks area of central Saskatchewan (Dunn, 1990). Other natural sources include coal and fossil fuel deposits (ASTDR, 2003, CCME, 2009; Rosenfeld and Beath, 2013). Natural surface soil Se concentrations across Canada may reach 4.7 mg/kg (CCME, 2009).

Anthropogenic sources of Se in soil include coal ash from coal plants, irrigation water sourced from seleniferous soils, and wastewaters from industrial processes (CCREM, 1987; US EPA, 2018). Se environmental impacts have been associated with drilling waste from oil and gas activities and copper refining. According to the CCME (2009), copper refining was a primary contributor to Se production and a source of environmental impact. Global markets for Se include the glass industry, chemical and pigments, metallurgy, and electronics (George, 2003).

2.2 SELENIUM FORMS IN ENVIRONMENT

Se properties, such as mobility, uptake, metabolism, bioavailability and toxicity depend on its chemical form in environment (Martens, 2003; Mayland *et al.*,1989). Se can exist in four oxidation states such as -2 (hydrogen selenide and metallic selenides), 0 (elemental selenium), +4 (selenite), and +6 (selenate). Hydrogen selenide (H_2Se) is thermodynamically unstable in aquatic solutions, whereas elemental Se is very stable and highly water insoluble (Martens, 2003). In soil and water, dissolved organic Se is mainly present as selenate (SeO_4) and selenite (SeO_3), and frequently both species are simultaneously encountered. The SeO_4/SeO_3 ratio in water correlates with pH and redox potential, with a greater ratio under oxidizing conditions and a lower ratio under reducing conditions (Adriano, 1986).

Between the two forms, a greater proportion of the SeO_4 mass in soil will be water soluble, representing a relatively greater potential for plant uptake and exposure. Metal-SeO_4 complexes or minerals have high solubilities in aerobic soils and are unlikely to form (Elrashidi *et al*., 1987), suggesting SeO_4 in oxygenated environments will be uncomplexed and more readily available for uptake into plants. SeO_4 is soluble under a relatively wide range of pH conditions. For example, in alluvia soils with a pH of 5.5 to 9, no adsorption was observed (Neal and Sposito, 1989).

Under near neutral and lower pH conditions ($\leq$ 7), SeO_3 has a greater tendency to adsorb onto aluminum and iron oxides, clay minerals, and calcite (Geering *et al.*, 1968; Carey and Allaway, 1969; Martens, 2003). Maximum SeO_3 adsorption onto various clay minerals (including montmorillonite) occurs in the pH range of 3 to 5 (Goldberg and Gaubig, 1988). This reduces the potential for exposure to plants (and animals consuming plants) as the Se mass is less accessible. Metal-SeO_3 complexes have high solubilities under alkaline conditions – as a result, metal-SeO_3 complexation is not a mechanism for reducing plant bioavailable concentrations at these pH levels. This is one rationale for why greater Se exposure may occur at alkali sloughs that frequently have pH values > 8, as relatively large pools of SeO_3 bound to soil are released and made available for uptake into plants and secondary consumers.

A third important form of Se in the environment is organic Se. In a study with various soils derived from different parent rocks (shale, granite, sandstone, serpentinite, limestone, volcanic ash, rhyolite, andesite, andesitic tuff, gabbro), approximately 42% on average of the total mass of Se was bound to several forms of organic material (fulvic acids, low molecular weight organic molecules, and humic acids; Yamada *et al.*, 1998). For the soluble mass of Se, approximately 51% was bound to organic material. Soluble organically bound Se is plant bioavailable, and uptake rates may exceed SeO_3 and SeO_4 uptake, based on a study with canola and wheat (Kikkert and Berkelaar, 2013).

2.3 BACKGROUND SELENIUM IN ALBERTA SOILS

Se was one of 30 trace elements surveyed in Alberta agricultural soils under the Soil Quality Monitoring Program (CCME, 2009). The survey was performed in 2002 to provide a benchmark database, and total selenium levels ranged from 0.1 to 2.3 mg/kg within the depth interval of 0 to 0.3 m (Penny, 2004).

2.4 SELENIUM TOXICITY AND DEFICIENCY

As an element, Se was first identified in 1817 by the Swedish chemist Jacob Berzeluis. The earliest record of Se poisoned livestock occurred in the 13th century, when Marco Polo described a necrotic equine hoof disease in China. Marco Polo stated that merchants "…*cannot venture among the mountains with any beasts of burden, on account on poisonous plant growing there, which, if eaten by them, has the effect of causing the hoofs of the animals to drop off*." (Reilly, 1996). Symptoms, resembling chronic selenosis, were further described in Colombia during 1560 and in Mexico over 200 years ago (Rosenfeld and Beath, 2013). In the United States, the acute and chronic poisoning of livestock exposed to naturally accumulated Se in plant tissue, described as ‘alkali disease’, was reported in 1860 by the US Army near South Dakota, and since 1919, it was a recognised poisoning in Wyoming (Rosenfeld and Beath, 2013). In the early 1930’s, it was first published that the toxic agent associated with ‘alkali disease’ may be Se in vegetation (Rosenfeld and Beath, 2013). Se was associated primarily with its poisonous qualities until 1957, when the German chemists Schwarz and Foltz presented the evidence that Se is essential for animal life, protecting against necrotic liver degradation (Schwarz and Foltz, 1957).

Se has been referred to as a ‘double-edged sword’ element (Brozmanová *et al.*, 2010) and an ‘essential toxin’ (Lenz and Lens, 2009), due to the narrow range between the dietary deficiency and toxicity. The daily requirements is 0.05 to 0.1 mg/kg to prevent Se deficiency, and at a 20-fold higher dose above deficiency levels, animals can suffer Se toxicity if dietary levels exceed 2 to 15 mg/kg (Mayland *et al.*, 1989; Ruyle, 1993).

Se deficiency in animals affects appetite, growth, fertility, and muscle strength (WHO, 2003; Fordyce, 2013). It may also lead to ‘white muscle disease’, a complex condition causing degeneration and apoptosis of muscles in animals (Fordyce, 2013). Penny (2004) stated that Se-deficient agricultural areas of Alberta are more common than Se toxic soils, and some areas of Se toxic soils have been associated with cases of ‘white muscle disease’ in livestock.

Under natural conditions, chronic Se toxicity is more common than acute toxicity since animals tend to avoid the consumption of generally unpalatable Se accumulator plants (Fordyce, 2013). Chronic toxicity in livestock may occur in the form of ‘alkali disease’ after long-term ingestion of typical forage plants containing 5 to 40 mg/kg, and it is characterized by emaciation, hoof loss, bone erosion, and other symptoms (Ruyle, 1993; Fordyce, 2013). In addition to alkali disease, high concentrations of Se have been shown cause congenital malformations and reproductive problems in rats, dogs, pigs and cattle (Fordyce, 2013, WHO, 2003). Se can also be directly toxic to plants, with toxic symptoms including chlorosis, black spots, and reduced yield (Efroymson *et al*., 1997; Hartikainen *et al*., 2000; Mikkelsen *et al*., 1987).

Whether Se is beneficial to higher plants is still under debate (CCME, 2009; Terry *et al*., 2000). Some researchers suggest Se can increase the tolerance of plants to UV-induced oxidative stress, delay senescence, promote the growth of ageing seedlings, and provide protection from pathogens and herbivores (Hartikainen *et al.*; 2000; Kuznetsov *et al*., 2003, Germ *et al*. 2007; Quinn *et al*., 2010; Wu *et al.,* 2016). Kuznetsov *et al.* (2003) demonstrated that Se regulates the water status of plants under drought conditions. Hartikainen *et al.* (2000) reported that at low concentrations, Se acted as an anti-oxidant, increasing the ryegrass yield. Wu *et al.* (2016) investigated the beneficial role of Se in protecting oilseed rape from cadmium (Cd) and lead (Pb) toxicity and noted that the medium application of Se (5,10, and 15 mg/kg) had a positive effect on growth while decreasing the oxidative damage caused by Cd and Pb.

2.5 SELENIUM UPTAKE BY PLANTS AND ANIMALS AND SULPHATE INTERACTIONS

The physiologic response of plants to Se varies greatly between the species (Rosenfeld and

Beath, 2013; Shrift, 1969; Brown and Shrift, 1982; Banuelos and Shrale, 1989; Wu *et al.,*1994, White *et al*.; 2007; Degenhardt *et al.,* 2016). According to their ability to accumulate Se, plants have been divided into several groups. Plants which cannot grow on seleniferous soils, are considered non-accumulators (White, 2015; USDA, 2016). Se-accumulator plants may contain up to 20 to 40 ppm Se in their dry tissue when grown under natural conditions (Rosenfeld and Beath, 2013; Shrift, 1969; Brown and Shrift, 1982). These plants typically like to grow in seleniferous soils, but they do not have specific requirement for it (USDA, 2016). Some plants can accumulate up to 1,000 to 15,000 mg/kg of Se in dry matter, and they are defined as hyperaccumulators (White, 2015; Feist and Parker, 2001). Some of these plants found in Se rich areas may require Se for growth (USDA, 2016).

Selenium can bioaccumulate in aquatic food webs and terrestrial organisms (Lemly, 1997; deBruyn and Chapman, 2007; CCME, 2009). Soil invertebrates can accumulate selenium to concentrations several times higher than concentrations in soil (Wu, 2004, CCME, 2009). Se level was reported increasing from soil to plants, from plants to grasshoppers, and from grasshoppers to mantis (Wu, 2004; CCME, 2009). Se concentrations increase within food webs and may pose risks to tertiary consumers (such as wildlife) (Woodbury *et al*., 1999).

Few recent studies have investigated the adverse effects and bioavailability of SeO_4 and SeO_3 for soil organisms, and the toxicological dataset is considered very limited (Stolfa *et al*., 2017). The toxicity of SeO_3 is higher in invertebrates living in aquatic environments, whereas SeO_4 is typically more toxic in terrestrial environments (Somogyi *et al*., 2007). Fisher and Koszorus (1992) studied the effects of SeO_3 on *E. fetida* and found that sodium SeO_3 concentrations up to 50 mg/kg in soil caused no mortality, but reduced the juvenile production and mass gain of juveniles. Somogyi *et al.* (2007; 2012) compared SeO_4 and SeO_3 toxicity to potworms *(E.albidus*) and found SeO_4 was approximately 4-fold more toxic than SeO_3.

Se uptake by plants differs between SeO_3 and SeO_4. SeO_4 is taken up by plant roots via sulphate transporters (Terry *et al*., 2000; White *et al*., 2004; Sors *et al*., 2005; Stolfa *et al*., 2017). For SeO_3, earlier studies suggested passive diffusion (Shrift and Ulrich, 1969; Brown and Shrift, 1982), however, more recent work has demonstrated uptake via phosphate transporters (Li *et al*., 2008; Zhang *et al*., 2013). SeO_4 is translocated and tends to accumulate in leaves (shoots) following root contact, whereas SeO_3 is retained in the roots and does not tend to translocate at the same level as SeO_4 (Cartes *et al*., 2011; Longchamp *et al*., 2015).

Gupta *et al.* (1993) compared the Se concentration in barley grain after sodium SeO_4, calcium SeO_3, and sodium SeO_3 soil treatment, and found that Se rates of 10 and 40 g/ha of sodium SeO_4 raised the grain Se level to 234 ug/kg and 959 ug/kg, respectively. In contrast, the addition of sodium SeO_3 or calcium SeO_3 at the same rate did not cause any significant difference in the grain Se level compared to the control group (29 ug/kg). Ali *et al*. (2018) noted that at the same rate of Se application, Se availability was higher in wheat grown in SeO_4 treated soils than that in SeO_3 treated soils. Kinetic studies conducted by de Souza *et al*. (1998) on Indian mustard revealed that SeO_4 plant uptake was 2-fold faster than that of SeO_3.

2.6 EFFECTS OF SULPHATE ON SELENIUM TOXICITY

Se uptake in plants as SeO_4, follows the same metabolic pathway as sulphate (Terry *et al*., 2000; White *et al*., 2004; Sors *et al*., 2005; Huang *et al*., 2007; Stolfa *et al*., 2017). Several studies have reported that sulphate reduces Se uptake by plants (Bell *et al*., 1992; Mikkelsen and Wan, 1990; White *et al*., 2004; Mackowiak and Amacher, 2008), although few studies have examined the effects of sulphate co-exposure on selenium toxicity in soil.

Gissel-Nielsen (1973) found that increasing sulphate concentrations in soil significantly decreased the uptake of SeO_4 by red clover and barley, whereas, SeO_3 uptake was affected to a lesser degree. Mikkelsen *et al*. (1988) found that Se accumulation by alfalfa grown in Se 1 mg/L solution was reduced from 948 mg/kg to 6 mg/kg in presence of sulphate. When sulphate-induced salinity increased from 0 to 6 dS/m, the authors noted that Se phytotoxicity was ameliorated.

The interaction between sulphate and Se toxicity in plants requires more research given the relatively large areas of seleniferous soils in Alberta and other provinces where concentrations of Se can be found at concentrations above regulatory guidelines. These areas are frequently co-located with areas of elevated sulphate, and the interplay and resulting potential for Se-induced toxicity to livestock and wildlife is likely complex. Elevated bioavailable Se at levels that may be toxic to livestock and wildlife may also be found in some alkali slough areas. Understanding this interaction is also of importance for anthopogenically derived exceedences of regulatory Se soil quality guidelines, where elevated sulphate concentrations are simultaneously observed.

2.7 SELENIUM GUIDELINE LEVELS

The current soil Se guideline in Canada and Alberta is 1 mg/kg. The guideline is variable amongst other provinces of Canada (Table 2.1). The limiting pathway is ecological direct soil contact (receptors of concern being microbes, invertebrates, and plants). The current guideline of 1.0 mg/kg has several limitations. First, the regional database of naturally occurring soil concentrations suggests Se may range up to 2.3 mg/kg in Alberta and 4.3 mg/kg for several provinces, which is greater than the Tier 1 guideline (CCME, 2009, Penny, 2004). This suggests widespread areas of soil dependent biota may be under naturally induce Se toxicity. Second, the guideline was developed based on two studies using the most toxic form of selenium in soil (SeO_4; Carlson *et al*. 1991; Singh and Singh, 1979), whereas typical environmental measurements of Se (via acid digestion as part of a metals analytical package) will simultaneously also measure SeO_3 and organic forms of Se that may be associated with lower toxic potency. Third, the SeO_4 based guideline of 1 mg/kg may be overprotective if there is amelioration due to elevated sulphate concentrations in soil. Areas with elevated soil sulphate concentrations are wide-spread across Alberta and other provinces in the Western Canadian Sedimentary Basin.

Table 2.1. Summary of Selenium Soil Quality Guidelines

Jurisdiction	Description	Soil Guidelines (mg/kg)		Reference
Canada	Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health	1.0 2.9	Agr/R/P C/I	CCME, 2009
British Columbia	Generic Numeric Soil Standards for Contaminated Sites	1.0	Agr/R/P/C/I	BCMOE, 2017
Ontario	Generic Site Condition Standards	1.2 1.5	Agr R/P/C/I	ONMOE, 2011
Alberta	Alberta Tier 1 Soil and Groundwater Remediation Guidelines	1.0 2.9	Agr/R/P C/I	AEP, 2016

Notes:
Agr: agricultural land use; R: residential land use; P: parkland land use; C: commercial land use; I: industrial land use

3 METHODOLOGY

The following sections present the approach and methods used to develop soil toxicity data for soil dependent biota under varying sulphate co-exposure levels.

3.1 TOXICITY TESTING

Toxicity testing work herein was based on SeO_4 as it has the greatest potential to induce toxicity to soil invertebrates and plants amongst the various Se species encountered in a soil matrix, due in part to its solubility under neutral pH conditions – this helps to ensure that potential toxicity was evaluated in a manner that excluded binding with soil during the experiment and a loss of bioavailable Se dose. Literature data indicates that SeO_4 uptake into, and toxicity towards, plants is higher than SeO_3. Use of SeO_4 toxicity data would ensure that any guidelines derived from this work would be adequately protective of SeO_3 exposures.

Experimental studies first commenced by EEI, and published by Prediger (2012), were for alfalfa (*M. sativa*) grown on artificial soils as part of a range finding study to determine levels of Se induced toxicity under various sulphate concentrations. Additional toxicity studies were carried out in the lab of Alberta Innovates (AI), under the guidance of EEI, and funding as well as oversight was provided by the Petroelum Technology Alliance Canada (PTAC) and Alberta Environment and Parks (AEP). Raw laboratory results are provided in Turner *et al.*, 2014, Degenhardt *et al*., 2016; and, Degenhardt *et al*., 2017) The toxicity testing work involved six plant and two invertebrate species. According to CCME (2006), coarse-grained and fine-grained soils should be considered separately, and guidelines developed for each soil type (CCME, 2006). EEI provided AI with natural coarse and fine textured soils, collected near Vulcan, AB. Dose-response data from artificial soil was not included in the final statistics and derivation of guidelines, to avoid potential interference with natural conditions of the representative soils from Alberta.

Six plant species were used for the trial as recommended by EC (2007) protocols. Plants were selected following discussions with AEP for relevance, and included alfalfa (*M. sativa*), barley (*H. vulgare*), carrot (*D. carota*), cucumber (*C. sativus*), northern wheatgrass (*E. lanceolatus*), and red fescue (*F. rubra*). The two invertebrate species were earthworms (*E. andrei*) and springtails (*F. candida*), which are he most common species used in soil ecotoxicology work in Canada.

3.2 QUALITY ASSURANCE

For quality assurance purposes, Se and sulphate (SO_4) soil concentrations were validated analytically by Exova laboratories (Exova) for each unique combination of treatment (Appendix I). In addition, the dose-response data from 2014 to 2017 studies were statistically verified for integrity by t-tests to eliminate potential statistical 'noise' or inconsistency from combination of several data sets. T-tests compared Se control data sets between different experiments and assisted in identifying experimental outlier exclusions (Appendix G).

3.3 ENDPOINT CALCULATION

The dose-response analysis was conducted using US EPA Benchmark Dose Software (BMDS), Version 2.7.0.4, which assesses goodness of fit for the dose-response curves by determining p-values for four different tests. Based on p-values and visual assessment (goodness of fit) for the control sulfate group, the Hill Model was selected. The Hill model, a preferred model in BMDS, is a sigmoidal dose-response curve consistent with receptor-mediated responses, and it is often used for the best representation of the data (US EPA, 2016).

The Hill Model was used to calculate the Se soil effective concentration (in mg/kg) causing a 25% adverse effect (EC_{25}) to the germination, root/shoot mass and length for the plants, and live adult and juvenile number/mass for invertebrates. An effective concentration of 25% is the preferred approach in Canada for determining unacceptable risk to the ecological soil contact pathway (CCME, 2006). The BMDS curves and calculated EC_{25} values are shown in Appendices B-F.

3.4 SPECIES SENSITIVITY DISTRIBUTION

Conversion of the dose-response data from toxicity tests in 2014-2017 to the ecological soil guidelines was done with the Species Sensitivity Distribution (SSD) curve, as recommended by CCME (2006). For agricultural and residential/parkland lands, CCME (2006) recommends the use of calculated EC_{25} values rather than no observed effect level (NOEC), lowest observed effect level (LOEC), and EC_{50} values to reduce uncertainty in guideline derivation and applying uncertainty factors. In some instances, it is necessary to use NOEC and LOEC values for guideline derivation depending on endpoint availability.

To avoid redundancy, the root and shoot length and dry biomass biological endpoints (EC_{25}, derived from BMDS) were combined as geometric mean, to yield one dry matter endpoint. In a similar way, the geometric mean for the earthworm biomass and juvenile numbers was utilized as a single endpoint characterizing reproduction. This calculation resulted in one dry matter endpoint and one germination endpoint for each plant species as well as one adult mortaility and one reproduction endpoint for each invertebrate species. Thus, the final SSD curve was built on sixteen endpoints for eight species, for every unique combination of the soil type and sulphate treatment. The soil type included coarse and fine loamy sand soils collected by EEI near Vulcan, AB, but not artificial soils, and the final guidelines were built on 2014 to 2017 toxicity tests. The EC_{25} from 2015 SO_4 control data set and 2014 experiment were combined as geometric mean to eliminate potential redundancy in control SO_4 data.

3.5 BEST MODEL FIT

The goodness of fit of the species sensitivity distribution curves and model results were evaluated with the MathWave Technologies EasyFit software, Version 5.6. The data points from statistical analysis of 2014 to 2017 toxicity experiments were processed by EasyFit, and probability distributions that best fit the data were was ranked according to the goodness of fit from 1 to 40 by Anderson-Darling tests. Anderson-Darling test ranking was preferred over Kolmogorov-Smirnov tests (Aldenberg *et al*., 2002) for the better sensitivity to the tails of distribution, and therefore, better working with potential outliers. The models with the highest rank (i.e., 1 or 2) were applied to derive the final guidelines.

3.6 PLANT UPTAKE AND TISSUE CONCENTRATIONS

Upon completion of the 2015 to 2017 experiments, plant tissues and soil samples collected by AI were submitted to EEI. EEI evaluated tissue weights and select samples with sufficient yield (dry weight > 0.5 g) for submission to Exova to determine Se and SO_4 (as sulfur) content in plants. Soil samples were submitted to Exova for Se concentration analysis by two methods: strong acid extractable Se and saturated paste Se. These analytical data were used to evaluate bioconcentration potential and assess relationships between toxicity and plant tissue concentrations for Se and SO_4.

4 EXPERIMENTAL STUDIES SUMMARY (2012 TO 2017)

This section describes methodology, materials, and observation results of toxicity testing designed by EEI (2012-2017) and conducted by Prediger (2012), Turner *et al.* (2014), and Degenhardt *et al.* (2016, 2017), resulting in the following experimental studies:

- Selenium (Selenate) Toxicity to *Medicago sativa* and the Hormetic Effect of Sulphate (Prediger, 2012);
- Phase 1 (2013-2014) Development of EcoContact Soil Selenium Guideline (Turner *et al*., 2014);
- Phase 2 (2015-2016) Development of EcoContact Soil Selenium Guideline (Degenhardt *et al.*, 2016),
- Phase 3 (2016-2017) Development of EcoContact Soil Selenium Guideline: Toxicity Testing Using Soil Invertebrates (Degenhardt *et al*., 2017)

4.1 SE TOXICITY TO ALFALFA (2012 TESTING)

4.1.1 Study Design

The research methodology generally followed Environment Canada's Biological Test Method: Test of Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil (EC, 2007). The test involved spiking artificial soil with eight different concentrations of sodium selenate, seeding *M. sativa* (alfalfa), conducting a 26 to 31 day growth test, and measuring root and shoot length and weight of the plants. Artificial soil and spiking was completed at Lakeland College in Vermilion, Alberta, and the growth tests were carried out in the growth chambers at AI in Vegreville, Alberta.

Soils were spiked with SO_4 in the form of magnesium sulphate ($MgSO_4$•$7H_2O$) at dry weight concentrations of 0 (no SO_4 added), 500, 1,500, and 3,000 mg/kg. Each of the four SO_4-spiked soils were spiked with Se in the form of sodium selenate (Na_2SeO_4) at dry weight concentrations of 0 (no Se added for a negative control), 0.5, 0.75, 1.0, 1.25, 2.0, 5.0, and 15.0 mg/kg, resulting in 32 different combinations of SO_4 and Se in the spiked artificial soils (four SO_4 concentrations by eight Se concentrations).

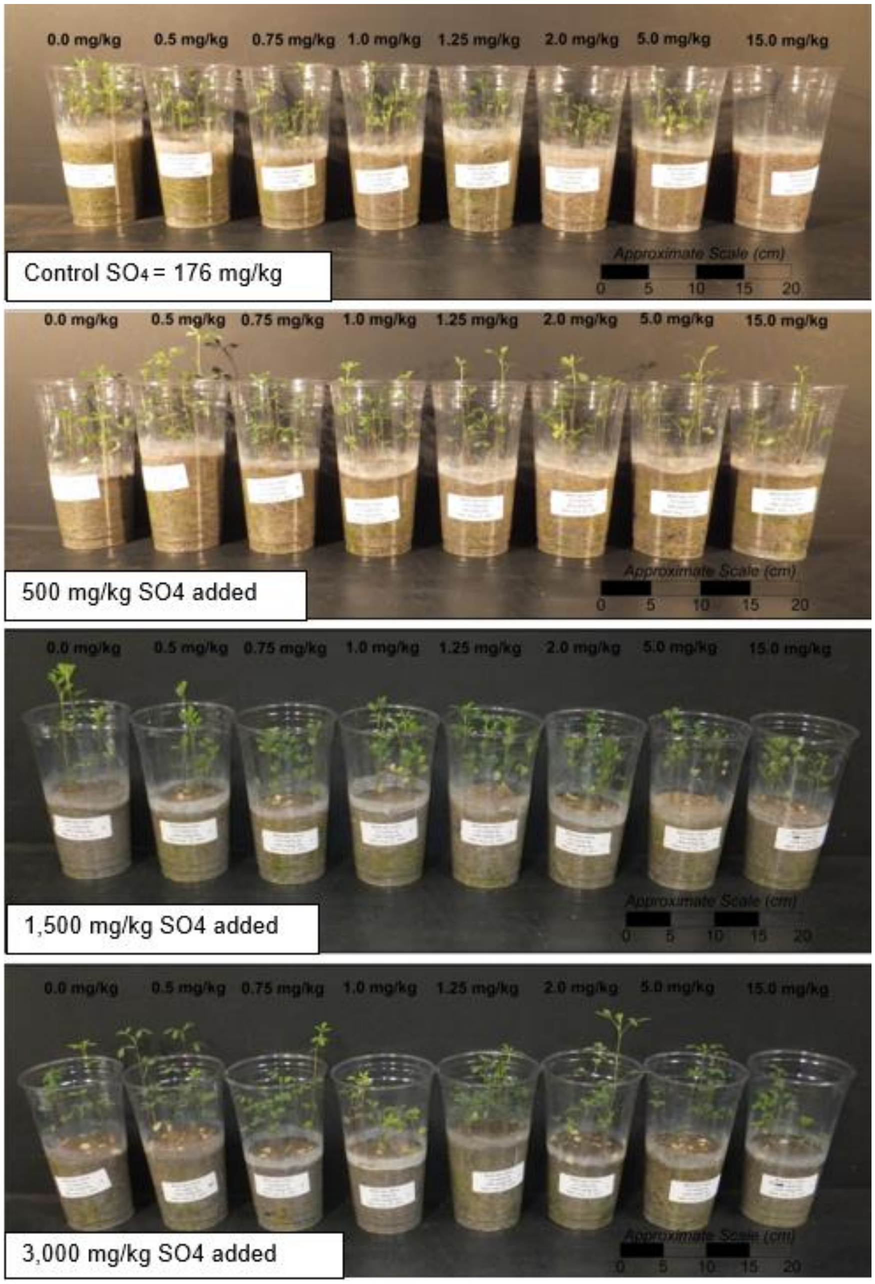
A composite sample of artificial soil was submitted to Exova in Edmonton, Alberta. Salinity analysis indicated the initial SO_4 concentration of 176 mg/kg. SO_4 concentrations as 500, 1 500, and 3, 000 mg/kg were identified based on the concentration of sulphate the artificial soil was spiked with. Assuming 176 mg/kg of SO_4 in background, all four SO_4 concentrations may be estimated as 176 mg/kg, 676 mg/kg, 1,676 mg/kg, and 3,176 mg/kg. The soil texture (hydrometer method) indicated the soil was a sandy loam, based on Agriculture Canada (1983), with a 73.6% sand content, a 10.4% silt content and a 16.0% clay content, which may be considered coarse soil (Prediger, 2012).

Approximately 500 g of hydrated soil was added to each growth vessel and the vessels were seeded with ten seeds of *M. sativa var. Algonquin*. *M. sativa* was selected for testing based on approved species in the Environment Canada methodology (EC, 2007). The seeds were certified and sourced from commercial suppliers across Canada (Prediger, 2012).

4.1.2 OBSERVATION

The biological endpoints included root and shoot mass and length. Results indicated that effective concentrations to cause a 25% adverse effect (EC_{25}) in control SO_4 ranged from 0.11 to 0.49 mg/kg. The addition of SO_4 reduced SeO_4 toxicity to alfalfa by more than 136-fold, depending on endpoint and SO_4 concentration (Prediger, 2012). In control SO_4 concentration of 176 mg/kg, the 15 mg/kg of Se caused 100% plant mortality (Figure 4.1). Comparatively, when soils were spiked with SO_4 at concentrations of 500, 1,500, or 3 000 mg/kg, the 15 mg/kg Se treatment appeared to cause little difference from the negative controls.

Figure 4.1. Photographs of Growth Vessels



Source: Prediger 2012

Se (as selenate) concentrations for each vessel are listed along top of photograph. Note the 0% survival at 15 mg/kg Se concentration in control SO_4 vessel (background SO_4 of 176 mg/kg).

4.2 SE TOXICITY TO PLANTS (2014 TESTING)

4.2.1 STUDY DESIGN

The study consisted of spiking field-collected soils with varying concentrations of Se (SeO_4 valence form Se^{6+}), counting the germinated plants, sowing test species, and measuring root/shoot lengths and dry biomass following Environment Canada (EC, 2007) methodology (Turner *et al*., 2014).

Coarse and fine textured loamy soils were collected by EEI from an agricultural field near Vulcan, AB. Soils were dried, homogenized and saturated at 20% moisture. Plant species selection and Se concentrations levels were finalized upon discussions between EEI and AEP. AEP suggested the use of common monocot and dicot agricultural species and some common garden species due to their sensitivity to Se (Banuelos and Schrale, 1989). A total of six plant species were selected for the trial (Table 4.1), all recommended by EC (2007).

Seeds were planted in coarse and fine substrates, and spiked with 10 mg/kg of SO_4 (coarse soils) and 12 mg/kg of SO_4 (fine soils). Se treatment consisted of 0.35 (control), 0.85, 1.35, 2.35, 5.35, and 15.35 mg/kg dry weight concentrations for coarse soils, and 0.65 (control), 1.15, 1.65, 2.65, 5.65, and 15.65 mg/kg dry weight concentrations for fine soils. Experiment lasted for 14-21 days in compliance with EC (2007). Five seeds were sown for northern wheatgrass, red fescue, barley, cucumber, and ten seeds were sown for alfalfa and carrot. Each treatment, including control, had six replicates. Testing vessels with seeds were set to grow in controlled environment chambers at 21/15 degree Celsius (°C), day/night temperature with 16 hrs photoperiod (Turner *et al*., 2014).

Table 4.1. Plant Species Selected for Toxicity Testing

Species Name		**Plant Se Accumulator Type**	**Type of species**
Scientific	**Common**		
Daucus carota	Garden carrot	Non-accumulator	Dicot
Elymus lanceolatus	Northern wheatgrass	Non-accumulator	Monocot
Medicago sativa	Alfalfa	Potential accumulator*	Dicot
Festuca rubra	Creeping red fescue	Potential accumulator*	Monocot
Hordeum vulgare	Barley	Non-accumulator	Monocot
Cucumis sativus	Cucumber	Non-accumulator	Dicot

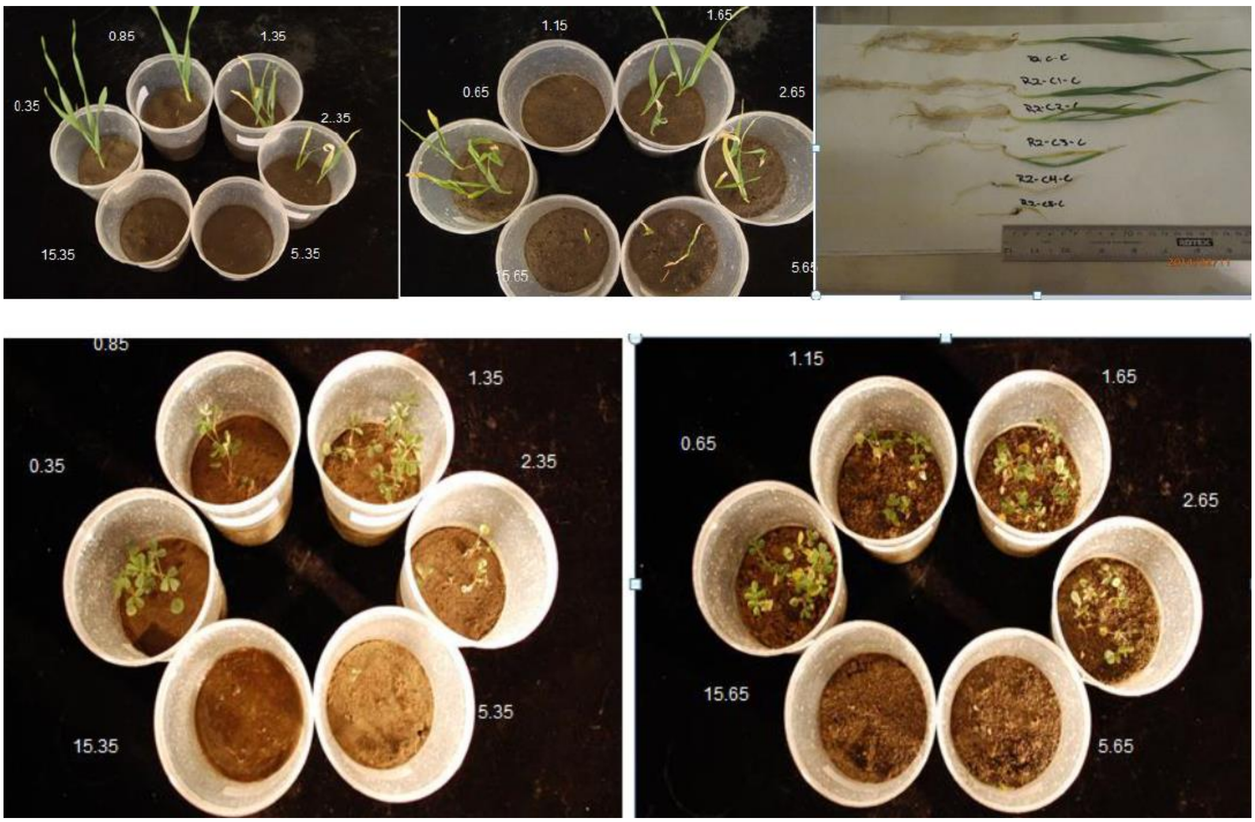
**M.sativa* and *R.fescue* are considered potentially Se accumulator species (Turner *et al*., 2014)

4.2.2 OBSERVATION

The biological endpoints included root and shoot mass and length, and germination percent. Seedlings responded differently to treatment levels between species and soil texture. An increased concentration of Se typically correlated with a decreased number of surviving individuals and decreased biomass for species by the end of the test. The effect of high Se levels

(15.35 mg/kg in coarse-textured soils, and 15.65 mg/kg in fine-textured soils) was universally detrimental to plant growth and survival during the study period (Turner *et al*., 2014; Figure 4.2 to 4.3).

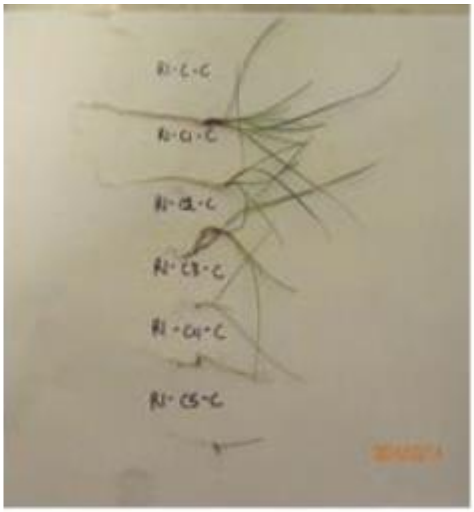
Figure 4.2. Negative Effects of Se on Barley and Alfalfa



Source: Turner et al., 2014

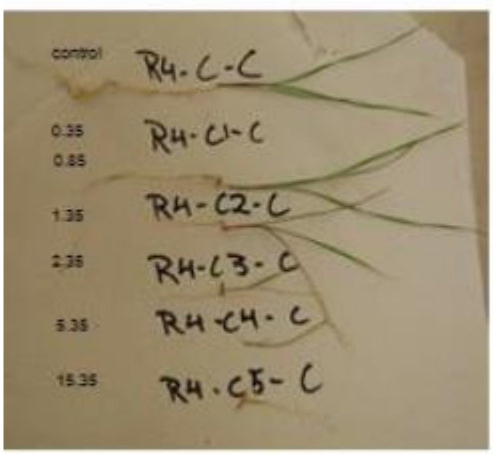
Barley pots are shown on an upper photo; bottom photo represents alfalfa.
The numbers in the diagram refer to the Se concentrations with 0.35 and 0.65 (mg/kg) in control coarse-textured and fine-textured soils, respectively.

Figure 4.3. Negative Se Effect on Red Fescue and Northern Wheatgrass



Root/shoot – Red fescue in coarse soil

Root/shoot – Red fescue in fine soil



Root/shoot – Northern wheatgrass in coarse soil

Root/shoot – Northern wheatgrass in fine soil

Source: Turner et al., 2014
Units: mg/kg; R – replicate, C- concentration

4.3 SE TOXICITY TO PLANTS (2015 TO 2016 TESTING)

4.3.1 STUDY DESIGN

Coarse and fine textured soils were collected by EEI near Vulcan, AB. Soils were dried, homogenized, and saturated at 20% moisture following Environment Canada protocol (EC, 2007). The mass of Na_2SO_4 required to reach the targeted Se levels was calculated and weighed using an analytical scale. It was then dissolved in 250 mL of distilled water and mixed in with the soil until it reached a homogeneous, crumbly consistency with clumps approximately 3-5 mm in diameter. To achieve the targeted SO_4 levels while maintaining Ca:Na ratios within 10–20 and Ca:Mg ratios within 2.5–5, a combination of $CaSO_4$, $MgSO_4$ and and Na_2SO_4 were used. The quantity of $CaSO_4$, $MgSO_4$, and Na_2SO_4 required to reach targeted SO_4 levels were calculated and weighed using an analytical scale. It was then dissolved in 500 mL of distilled water and mixed in with the soil until it reached a homogeneous, crumbly consistency with clumps approximately 3-5 mm in diameter (Degenhardt *et al*., 2016).

Plant selection and Se concentrations levels were finalized after discussions between EEI and Alberta Environment and Parks (AEP). The same six species, as in 2014 (Section 3.2, Table 3.1) were chosen: alfalfa, barley, carrot, cucumber, northern wheatgrass, and red fescue. Prior to the start of the study, seeds from a recognized supplier were obtained and tested for seedling emergence as per EC (2007) to ensure seeds of each species had 80% or higher emergence.

Five seeds (barley, cucumber) or ten seeds (alfalfa, northern wheatgrass, red fescue and carrot) were planted into coarse, fine or artificial soil; each soil had 16 treatments (four levels of Se and four levels of SO_4), and each treatment was replicated four times (Degenhardt *et al*., 2016). The test was terminated after 14 days of exposure for barley and cucumber, and after 21 days of exposure for alfalfa, carrot, northern wheatgrass and red fescue, following EC (2007) protocol.

Testing vessels with seeds were set to grow in a greenhouse at 24/15°C, day/night temperature and 18 hrs photoperiod. Approximately 500 mL weight equivalent of soil was used in each pot. Pots were 1 L polypropylene food containers with lids. All pots were labelled, seeds chosen and sown individually and then watered in accordance with Environment Canada’s contaminated soils test. Lids were placed on pots for the first seven days or until plant leaves reached the lids. Watering was done when necessary, about every three days. Visual assessments were recorded on the condition of the emerged plants on a weekly basis, with attention to delayed emergence, impaired development, necrosis, defoliation, desiccation, malformation, mottling, staining, wilting, discoloration, or chlorosis (Degenhardt *et al*., 2016).

The targeted concentrations for Se and SO_4 were determined based on the results from Phase 1 (2013-2014) PTAC funded Development of EcoContact Soil Se Guideline project (Turner *et al*., 2015). Composite soil samples from each of 16 treatments were submitted to Exova (Edmonton, AB) for analysis. Targeted and actual concentrations (coarse and fine soils) are summarized in Table 4.2.

Table 4.2. 2015 Targeted and Actual Concentrations for Se and SO_4

Sample ID*	Targeted Se, mg/kg	Actual Se, mg/kg	Targeted SO_4, mg/kg	Actual SO_4, mg/kg
Coarse soils				
Control SO_4 treatment**				
Se0-S0	No spiking	0.3	No spiking	21.9
Se1-S0	1.15	0.9		36.6
Se2-S0	2.65	2.4		40.5
Se3-S0	5.65	6.2		63.6
Low-level SO_4 treatment				
Se0-S1	No spiking	0.3	500	894
Se1-S1	1.15	0.9	500	858
Se2-S1	2.65	2.5	500	927
Se3-S1	5.65	5.4	500	840
Mid-level SO_4 treatment				
Se0-S2	No spiking	0.4	1,000	1,095
Se1-S2	1.15	0.9	1,000	1,101
Se2-S2	2.65	2.8	1,000	981
Se3-S2	5.65	5.4	1,000	1,140
High-level SO_4 treatment				
Se0-S3	No spiking	0.3	1,500	1,242
Se1-S3	1.15	1.1	1,500	1,314
Se2-S3	2.65	2.8	1,500	1,371
Se3-S3	5.65	5.9	1,500	1,356
Fine soils				
Control SO_4 treatment				
Se0-S0	No spiking	0.6	No spiking	19.8
Se1-S0	1.40	1.3		67.8
Se2-S0	2.95	3.2		96
Se3-S0	5.95	6.5		33.3
Low-level SO_4 treatment				
Se0-S1	No spiking	0.6	500	981
Se1-S1	1.40	1.4	500	897
Se2-S1	2.95	3.5	500	933
Se3-S1	5.95	8.4	500	1,038
Mid-level SO_4 treatment				
Se0-S2	No spiking	0.6	1,000	1,260
Se1-S2	1.40	1.5	1,000	1,137
Se2-S2	2.95	3.1	1,000	1,227
Se3-S2	5.95	9.5	1,000	1,236
High-level SO_4 treatment				
Se0-S3	No spiking	0.7	1,500	1,407
Se1-S3	1.40	1.4	1,500	1,515
Se2-S3	2.95	3.2	1,500	1,452
Se3-S3	5.95	7.8	1,500	1,551

**In sample ID, Se indicates selenium, where Se0 = control, Se1 is low, Se2 = mid, and Se3 = high Se concentration, and S indicates SO_4, where S0 = control, S1 = low, S2 = mid, and S3 = high SO_4 concentration*

***Concentrations are grouped by SO_4 treatment for benchmark dose-response analysis, discussed in Section 4.*

In January 2017, upon discussion with EEI, additional treatments with a lower SO_4 concentration (targeted SO_4 = 500 mg/kg) at four different Se levels were added. This additional experiment chemistry results are summarized in Table 4.3.

Table 4.3. 2017 Targeted and actual concentrations for Se and SO_4

Sample ID*	Targeted Se, mg/kg	Actual Se, mg/kg	Targeted SO_4, mg/kg	Actual SO_4, mg/kg
Coarse soils				
Se0	No spiking	0.4	500	681
Se1	1.0	0.9	500	672
Se2	2.5	2.9	500	732
Se3	5.0	5.8	500	651
Fine soils				
Se0	No spiking	0.8	500	714
Se1	1.0	1.1	500	624
Se2	2.5	2.9	500	702
Se3	5.0	5.8	500	666

**In sample ID, Se indicates selenium, where Se0 = control, Se1 is low, Se2 = mid, and Se3 = high Se concentration. Note that targeted SO4 level is the same for all group in 2017 additional experiment.*

4.3.2 Observation

The toxicity of Se was most evident in the coarse textured soil. The root biomass for all species in coarse soil (except cucumber at low selenium concentration) substantially decreased with increasing selenium without SO_4. Barley and northern wheatgrass had the greatest increase in root weight when SO_4 was added. SO_4 effect on root biomass was most evident in coarse soils at low SO_4 level (average 880 mg/kg) for most species.

For all species, significant reduction in shoot length was observed in Se3 (high dose) treatment without SO_4, and for alfalfa and northern wheatgrass the same effect was observed under Se2 (mid-dose) treatment. Cucumber and fescue responded positively (through increase in shoot weight) to low levels of Se. Overall, SO_4 addition had a positive or neutral effect on the shoot length in all Se spiked treatments. The greatest increase in shoot length with SO_4 was observed in grass species (barley, northern wheatgrass, and red fescue).

Similar to shoot length effects, shoot weight decrease was observed in higher Se concentration without any SO_4 addition. However, the high SO_4 treatments (Se control), also significantly reduced shoot weight in fescue, alfalfa and northern wheatgrass. The higher EC associated with higher SO_4 may have also adversely affected the growth of those species. The antagonistic effect of SO_4 and Se was most evident at SO_4-low (880 mg/kg) treatments for most species (Degenhardt *et al*., 2016).

In fine soil, the root biomass response to Se was inconsistent amongst the species. Higher root length was observed in four species (barley, carrot, cucumber and red fescue), at low and medium

Se concentration. The lowest root length within SO_4-control group was observed at the highest Se concentration, for all species, except cucumber. With added SO_4, an increased root length was most evident in alfalfa and barley. Overall, the toxic effect of Se on root length in fine soils was most evident at the highest Se concentration (Se3) for all species except cucumber, and the antagonistic effect of Se and SO_4 was also most evident at that concentration.

Reduction in shoot biomass was observed in all species with increased Se level without SO_4. Shoot length and weight were significantly reduced under Se3 and SO_4-control treatments. The addition of SO_4 (at all three levels) substantially increased shoot length and weight for all species at the highest Se concentration (Se3). The antagonistic effect of Se and SO_4 was evident in all species with similar response across the species tested (Degenhardt *et al*., 2016).

4.4 SE TOXICITY TO INVERTEBRATES (2016 TO 2017 TESTING)

This study extended toxicity testing to assess the reproduction and survival of two standard soil invertebrate species under various Se and SO_4 concentrations in fine and coarse textured soils. A number of standardized biological test methods have been established for measuring toxicity of samples of contaminated or potentially contaminated soil using specified terrestrial test organisms (EC, 2004 and 2014). *Folsomia candida* (springtails) and *Eisenia andrei* (earthworms) were selected as the standard invertebrate test organisms for this project (Degenhardt *et al*., 2017).

Biological endpoints for springtails test included the mean number of surviving adults (first generation) and live progeny after 28 days in each treatment. Endpoints for earthworms test included mean number of surviving adults (first generation) after 28 days and mean number of live juveniles, and their mean dry weight after 56 days in each treatment.

4.4.1 STUDY DESIGN

Breeding stocks of *E. andrei* and *F. candida* were obtained from the Method Development and Applications Unit, Science and Technology Branch, of Environment Canada (Ottawa, Ontario) on August 17, 2016. The organisms used in this toxicity test were derived from the same population (Degenhardt *et al.*, 2017).

Preparation and maintenance of synchronized cultures of *F.candida* were completed following Environment Canada's Biological Test Method Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil. Age synchronization was performed following the standard operating procedure (SOP) for Springtail Age-Synchronization (SOP No. 15/20/1.4/S), developed by the Soil Toxicology Laboratory at Environment Canada. Culturing, acclimation and maintenance of *E. andrei* was completed by following the Earthworm Culture Maintenance protocol (SOP No. 15.12/2.4/S 15.12/2.4/S) (Degenhardt *et al.*, 2017).

Coarse and fine soils were provided by EEI and prepared for test using the same method as discussed in Section 4.3. Baseline physicochemical properties were determined prior to spiking the soils to the targeted Se (in the form of sodium selenate Na_2SeO_4) and SO_4 levels. The mass of Na_2SeO_4 required to reach the targeted Se levels was calculated from baseline soil properties, and the required amount was then weighed using an analytical scale. To achieve the targeted SO_4 concentrations, a combination of $CaSO_4$, $MgSO_4$ and Na_2SO_4 were used. The quantities of $CaSO_4$, $MgSO_4$ and Na_2SO_4 required to reach the targeted SO_4 levels were calculated based on the baseline soil properties; the required amount was then weighed using an analytical scale, and mixed in their dry form prior to being gradually rehydrated with de-ionized water. Soil was mixed until it reached a homogeneous, crumbly consistency with clumps approximately 3-5 mm in diameter (Degenhardt *et al*., 2017).

Test soils were spiked with five different treatment levels of Se (Se0 (<1ppm), Se1 (2 ppm), Se2 (5 ppm), Se3 (10 ppm) and Se4 (20 ppm). For each Se treatment level, the soil was also spiked with four different SO_4 concentrations. Targeted concentrations of Se and SO_4 were determined based on results from Phase 1 and 2 (2013-2015) PTAC funded Development of EcoContact Soil Se Guideline project (Degenhardt *et al.,* 2016; Turner *et al*., 2014; Section 4.2 and 4.3). A negative control test with artificial soil was included in this study to ensure toxicity tests were done in a consistent, standardized approach.

Composite soil samples from each of 20 treatments were submitted to Exova (Edmonton, AB) for analysis. Targeted and actual concentrations (coarse and fine soils), grouped by SO_4 treatment for dose-response analysis, are summarized in Table 4.4.

In January 2017, upon discussion with EEI, additional treatments with a lower SO_4 concentration (targeted SO_4 = 500 mg/kg) at four different Se levels (Se0 (<1ppm), Se1 (1 ppm), Se2 (2.5 ppm), Se3 (5 ppm)) were added in conjunction with plant toxicity work. This additional experiment soil chemistry results were shown in Table 4.3, Section 4.3.

Table 4.4. Targeted and actual concentrations for Se and SO_4 for coarse and fine soils

Sample ID*	Targeted Se, mg/kg	Actual Se, mg/kg	Targeted SO_4, mg/kg	Actual SO_4, mg/kg
Coarse soils				
Control SO_4 treatment**				
Se0-S0	No spiking	<0.3	No spiking	45.3
Se1-S0	2	2.3		27.6
Se2-S0	5	1.3		25.2
Se3-S0	10	7.1		36.6
Se4-S0	20	12.3		47.1
Low-level SO_4 treatment				
Se0-S1	No spiking	0.3	500	879
Se1-S1	2	2.2	500	789
Se2-S1	5	4.6	500	840
Se3-S1	10	8.1	500	879
Se4-S1	20	20.5	500	741
Mid-level SO_4 treatment				
Se0-S2	No spiking	0.3	1,000	837
Se1-S2	2	2.1	1,000	1,008
Se2-S2	5	3.7	1,000	1,227
Se3-S2	10	8.9	1,000	915
Se4-S2	20	15.5	1,000	843
High-level SO_4 treatment				
Se0-S3	No spiking	<0.3	1,500	1,077
Se1-S3	2	2.1	1,500	1,188
Se2-S3	5	5.6	1,500	1,161
Se3-S3	10	8.6	1,500	1,230
Se4-S3	20	18.8	1,500	1,071
Fine soils				
Control SO_4 treatment				
Se0-S0	No spiking	0.6	No spiking	50.7
Se1-S0	2	2.7		93.6
Se2-S0	5	5.5		110.7
Se3-S0	10	10.9		100.2
Se4-S0	20	30.6		99.3
Low-level SO4 treatment				
Se0-S1	No spiking	0.7	500	984
Se1-S1	2	4.5	500	921
Se2-S1	5	8.0	500	870
Se3-S1	10	17.7	500	882
Se4-S1	20	26.8	500	939
Mid-level SO_4 treatment				
Se0-S2	No spiking	0.6	1,000	1,080
Se1-S2	2	3.3	1,000	1,206
Se2-S2	5	5.4	1,000	1,089
Se3-S2	10	14.8	1,000	1,119
Se4-S2	20	24.9	1,000	1,215
High-level SO_4 treatment				
Se0-S3	No spiking	0.7	1,500	1,482
Se1-S3	2	3.0	1,500	1,377
Se2-S3	5	7.7	1,500	1,470
Se3-S3	10	13.6	1,500	1,404
Se4-S3	20	31.2	1,500	1,410

4.4.2 Observation: Springtails

Results for *F. candida* were considered valid based on passing the following criterion:

- The percent survival of adults in the negative control soil (Se0-S0) and the artificial soil were greater than 80% (or >8 live adults per test vessel).
- The mean reproduction rate for the adults in the negative control soil was greater than 100 progeny per test vessel.

Mean number of live adults and live progeny produced after 28 days differed significantly ($p<0.05$) among treatments in the coarse textured soil. Treatments Se0-S0, Se0-S1, Se1-S2 and Se3-S1 had significantly more live adults surviving after 28 days, while Se4-S0 had the fewest. While not statistically significant, more live progeny were observed in treatments with elevated Se and SO_4 concentrations compared to treatments with elevated Se concentration and no SO_4 (Degenhardt *et al*., 2017).

Similar to coarse textured soil results, there were substantial differences ($p<0.1$) among treatments in mean number of live adults and mean number of live progenies produced after 28 days in fine textured soils. Treatment Se0-S0 and Se4-S3 had substantially more live adults surviving after 28 days, while Se3-S0 and Se4-S0 had the lowest adult survival rates. While not statistically significant for all treatments, survival rates for adults and live progeny production are greater in treatments with elevated Se and SO_4 concentrations compared to those treatments with elevated Se concentrations alone. The average surviving adults and progeny across all treatments in the fine textured soil were less than those in the coarse textured soil (Degenhardt *et al*., 2017).

4.4.3 Observation: Earthworms

Results for *E. andrei* were considered valid based on passing the following criterion:

- Survival of adults in the negative control soil (Se0-S0) was greater than 90%;
- Mean reproduction rate for adults in the negative control soil was > 3 live juveniles per test vessel; and,
- Average dry weight of individual live juveniles in the negative control soil was > 2 mg.

There were significant differences ($p<0.05$) among treatments in the average live adults after 28 days and average live juveniles after 56 days in the coarse textured soils. Nine treatments had 100% adult survival rate after 28 days: Se0-S1, Se0-S3, Se1-S3, Se2-S1, Se2-S3, Se3-S2, Se4-S1, Se4-S2 and Se4-S3, while Se4-S0 had no adults survived after 28 days. On average, Se0-S0 had the highest number of juveniles produced per treatment (8.6 live juveniles per vessel) followed by Se0-S1 (6.7 live juveniles per vessel) and Se0-S2 (4.7 live juveniles per vessel). Despite high adult survival rates, 13 out of 20 treatments had very low reproduction rates (≤1 live juvenile per vessel). Although not statistically significant, results from Se1 treatments showed that the addition of SO_4 had effect on reproduction rates, suggesting a reduction in Se toxicity to *E. andrei* (Degenhardt *et al*., 2017).

Similar to the results in the coarse textured soils, there were significant differences ($p<0.05$) among treatments in the average live adults after 28 days and average live juveniles after 56 days. Eighteen out of twenty treatments had greater than 80% adult survival after 28 days. Only Se4-S0 and Se4-S2 treatments had adult survival rates less than 80% after 28 days. Similar to the coarse textured soils, the highest number of live juveniles was in Se0-S0 treatment (6.1 live juveniles per vessel), followed by Se0-S1 (2.2 live juveniles per vessel). Most treatments (16 out of 20) had very low reproduction rates (≤1 live juvenile per vessel). While not statistically significant, results from Se1, Se2 and Se3 treatments had higher reproduction rates with the addition of SO_4, suggesting a reduced toxicity of Se for *E. andrei* with a higher SO_4 concentration (Degenhardt *et al*., 2017).

The average surviving adults across all treatments were similar between the fine and coarse textured soil, while more progeny were found in the coarse textured soil treatments compared to the fine textured soil treatments. Similar to *F. candida*, *E. andrei* may favour the coarse-textured soil over the fine-textured soil due to the ease of burrowing (Degenhardt *et al*., 2017).

5 DATA ANALYSIS AND INTERPRETATION

5.1 DOSE-RESPONSE CURVES

5.1.1 EC_{25} CALCULATION

To estimate EC_{25} endpoints, raw data from 2014 to 2017 toxicity testing were initially grouped by species and SO_4 level, and by Se treatment within each SO_4 group. For each combination of treatment, statistical parameters were determined such as average dose-response and standard deviation between replicates. Results were subsequently used as BMDS input. Within the BMDS, the Hill model was chosen among continuous models, with an automatic adverse direction, benchmark response (BMR) type set to relative deviation, and the benchmark response factor (BMRF) set to 25, to represent a 25% adverse effect.

The continuous models deal with responses measured on numerical scales, increasing or decreasing with dose, and having a measure of variability (i.e., standard deviation). The Hill model is the preferable model in BMDS for receptor-mediated responses, as it fits sigmoidal, S-shape dose-response curves with plateau. The relationship shows a steep curve in the beginning and a saturation plateau in the end (Hill 1910), which is a classical toxicological dose response.

The equation of the Hill continuous model is:

$$\mu\,(X) = \gamma + \frac{\nu \times X^{\mathrm{n}}}{k^{\mathrm{n}} + X^{\mathrm{n}}}$$

Where γ = *intercept (control)*

k = *dose with half-maximal change (must be positive number)*

n = power (must be a positive number ≤ 18; if n is restricted, the number must be > 1)

v = maximum change

(US EPA, 2016)

An adverse direction choice refers to whether adversity increases as the dose-response curve rises ‘up’ or falls ‘down’. ‘Up’ or ‘down’ directions are suggested if the direction of adversity for the endpoint being studied is known. An ‘automatic’ choice means that the software chooses the adverse direction based on the shape of the dose-response curve (US EPA, 2016). To avoid the potential biases towards SO_4 treated groups, an automatic direction was selected.

The BMR type refers to the method of choice to derive the response level and included ‘Rel. Dev.’ (relative deviation), ‘Abs. Dev.’ (absolute deviation), ‘St. Dev.’ (standard deviation), ‘Point’ and ‘Extra’ (US EPA, 2016). The relative deviation means that response associated with BMR is calculated as background (control) estimate plus or minus the product of background estimate by BMRF, and therefore, it is based on the user-defined percentage (i.e., 25%) of the model-estimated control mean. The absolute deviation calculates the BMR as background estimate plus or minus the BMRF (US EPA, 2016), and does not reflect the percentage decline associated with a control group. The standard deviation method produces the BMR as background estimate plus or minus the product of the BMRF by the standard deviation for the control group data (US EPA, 2016), and may depend on heterogeneity within the control group. ‘Point’ method gives the BMRF value as a response associated with the BMR (US EPA, 2016), and would not reflect the control group performance in any case. ‘Extra’ means the response associated with the BMR will be the background estimate plus or minus the product of BMRF by difference between the background estimate and the model estimate of the maximum/ minimum response (US EPA, 2016), and may depend on the difference between treatments, which may substantially vary between the groups. Thus, considering the EC_{25} BMR type, the most desirable is the relative deviation:

$$\textit{Rel. Dev. Response} = m(0) - \left(BMRF \times m(0)\right)$$

where m(0) is the mean response from Se control group, and

BMRF is the benchmark response factor (0.25)

Since a 25% reduction is calculated from corresponding background values, the relative deviation approach provides more conservative values compared to the absolute approach. The relative deviation considers possible control group weak and low performance, and therefore, is equally protective for the stressed and healthy plants under various field conditions.

In the current BMDS version, the distribution of continuous measures is assumed normal, with a constant (homogenous) variance or one that changes as a power function of the mean value:

Var(i) = α[mean(i)]ρ • ρ(rho) = 0, constant variance • ρ(rho) $\neq$ 0, modeled variance

Constant variance should be assumed by default unless data output clearly indicates otherwise

(US EPA, 2016). A typical example of preliminary data processing from the raw data statistics to BMDS input is shown in Tables 5.1 to 5.3, followed by Figure 5.1 with the BMDS output curve and calculated EC_{25}. Table 5.2 shows an example of the raw data statistics for each unique treatment, from 2014 toxicity testing experiments, fine soils, alfalfa, and negative control (Se = 0.65 mg/kg, SO_4 = 12 mg/kg). The model input required the dose, the number of replicates, the mean response (per dose group), and the standard deviation for the modeling purposes. Alfalfa experiments were based on ten seeds per pot/replicate, and six replicates per treatment. The root and shoot weights were measured as total dry root and shoot mass per one pot. The root and shoot length (Table 5.1) was pre-averaged from the total number of plants per pot/replicate (maximum 10 plants). Germination percent was calculated from the total number of seedlings per pot. For the species with five seeds per pot (barley, cucumber, northern wheatgrass, and red fescue) the number of germinated seedlings was multiplied by two.

Table 5.1. Se Control Endpoints by Replicate: Fine Soils, Alfalfa, SO_4 = 12 mg/kg

ID	Se	Root Weight	Shoot Weight	Root Length	Shoot Length	End of Test Germination
Rep #	*mg/kg*	*g*	*g*	*mm*	*mm*	*%*
R1	0.65	0.05	0.13	134.7	43.29	70
R2	0.65	0.02	0.05	239.7	42.00	40
R3	0.65	0.06	0.13	187.8	37.75	90
R4	0.65	0.02	0.06	143.7	23.71	60
R5	0.65	0.07	0.14	154.2	43.67	90
R6	0.65	0.05	0.12	167.8	38.63	80
Average	***na***	**0.046**	**0.105**	**171.3**	**38.17**	**71.67**
St. Dev.	***na***	**0.019**	**0.038**	**38.3**	**7.49**	**19.41**

All replicates were averaged as shown in Table 5.1 and placed in order by increasing Se level within the same soil, species, and SO_4 treatment group. Table 5.2 is an example of an average statistics per Se treatment within fine soils, alfalfa, SO_4 control (12 mg/kg). Bold font indicates the values corresponding to Table 5.1.

Table 5.2. Se Average Endpoints by Treatment: Fine Soils, Alfalfa SO_4 = 12 mg/kg

ID	Se	Root Weight	Shoot Weight	Root Length	Shoot Length	Germination
Trt #	*mg/kg*	*g*	*g*	*mm*	*mm*	*%*
Se0	**0.65**	**0.046**	**0.105**	**171.30**	**38.17**	**71.67**
Se1	1.15	0.049	0.101	111.15	50.83	70.00
Se2	1.65	0.044	0.102	123.55	37.04	80.00
Se3	2.65	0.021	0.072	86.77	28.29	78.33
Se4	5.65	0.004	0.030	24.60	11.11	75.00
Se5	15.65	0.004	0.010	26.14	6.86	41.67

The next step includes preparation of the general input table for BMDS entry (Table 5.3). The input consists of Se doses, number of replicates averaged, mean response, and standard deviation between replicates. Table 5.3 shows an example of BMDS input for fine soils, alfalfa, SO_4 control (12 mg/kg), for shoot weight. Bold font indicates the values corresponding to Tables 5.1 to 5.2.

Table 5.3. BMDS Input Table for Se Dose-Response Curve: Fine Soils, Alfalfa, SO4 = 12 mg/kg, Shoot Weight

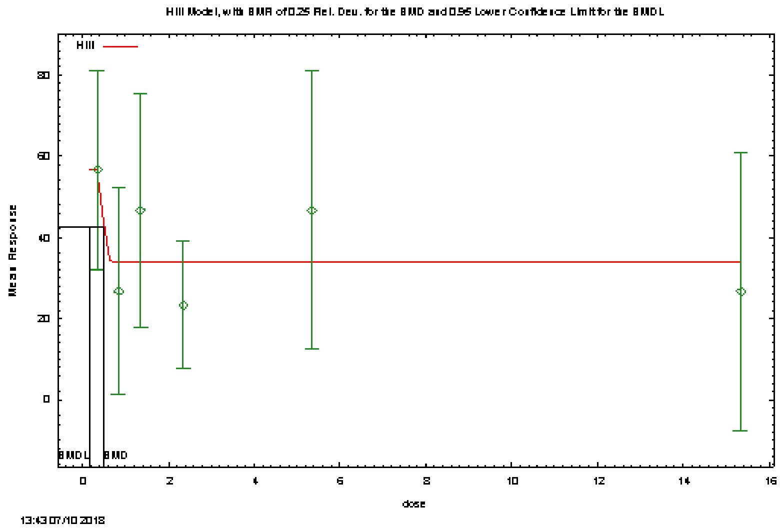
ID	Se Dose	Replicates	Mean Response	St. Dev
	mg/kg	*n*	*g*	*g*
Se0	**0.65**	6	**0.105**	**0.038**
Se1	**1.15**	6	**0.101**	0.032
Se2	**1.65**	6	**0.102**	0.024
Se3	**2.65**	6	**0.072**	0.017
Se4	**5.65**	6	**0.030**	0.031
Se5	**15.65**	6	**0.010**	0.015

Figure 5.1 shows the BMDS curve built with the Hill model using the values presented in Table 5.3, for alfalfa shoot weight (fine soils, SO_4 = 12 mg/kg), with Se doses placed on x-axis and corresponding shoot weight values (mm) on y-axis. The specified effect (0.25 or 25%) indicates the endpoint of interest (EC_{25}), and the benchmark dose (BMD) is calculated from an intersection point on BMDS curve, with a 25% reduction in shoot weigh (g), measured on y-axis. According to the BMDS computation, the BMD, or EC_{25} is equal to 2.5 mg/kg. BMDL value (Figure 5.1) indicates the confidence limit for BMD.

The P-values for four different tests (Figure 5.1) indicate the goodness of fit. Test 1 assesses the likelihood that responses and variances do not differ among treatments. If this test is accepted there may not be a dose-response relationship. Test 2 determines if the variances are homogeneous, and if not, the BMDS suggests to switch to the non-constant variance model. Test 3 assesses if the model fits the data, and Test 4 assesses if the variances are adequately modeled. BMDS uses a P-value of 0.1 to accept or reject models. Test 1 accepts a P-value of <0.1. If Test 2 has a P-value <0.1, a non-constant variance model should be run in the BMDS. For Test 3 and Test 4 a P-value >0.1 is desired to accept the model.

The typical dose-response curve normally takes the inverse-sigmoidal form (Rand, 1995), with BMDS-computable BMDL and BMD, and acceptance by all 4 tests. However, it was not always achievable, because of heterogeneity of the data, and the final decision of acceptance or rejection the EC_{25} values was based on the BMDS output, when computable. This approach was utilized for conservative purpose, rather than relying on an overall curve appearance or rejection/acceptance by Test 1, Test 3, and Test 4 statistical outputs (Test 2 gives an option to switch from a homogeneous model to a non-homogeneous one, and thus, an appropriate model by Test 2 is found easily).

Figure 5.1. BMDS Output and Se EC_{25} Computation: Fine Soils, Alfalfa, SO_4 = 12 mg/kg, Shoot Weight

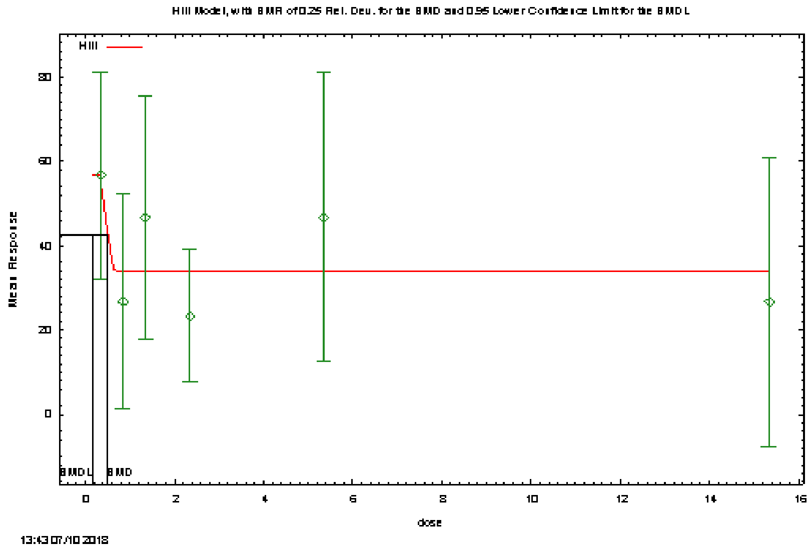


C:\Users\v.winter\Documents\BMDS\BMDS2704\Data\hil_Dax_Setting.out

```
The p-value for Test 1 is greater than .05.  There may not be a
diffence between responses and/or variances among the dose levels
Modelling the data with a dose/response curve may not be appropriate

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =          0.25

Risk Type        =     Relative deviation

Confidence level =           0.95

             BMD =       0.494132

            BMDL =      0.178782

            BMDU =       1.55485
```

Figure 5.2 shows an example of BMDS curve for coarse soil, barley, germination, control SO_4, with a steep slope and computable BMD/BMDL, but non-acceptable results for Test 1. Despite P-value > 0.5 indicates that there may not be a difference between responses among the treatments (likely due to greater standard deviations; Figure 5.2), the substantially low BMDS-calculated EC_{25} of 0.49 is accepted for conservative purpose.

Figure 5.2. BMDS Curve with no Difference between Responses: Coarse Soils, Barley, SO_4 = 10 mg/kg, Germination



C:\Users\v.winter\Documents\BMDS\BMDS2704\Data\hil_Dax_Setting.out

```
The p-value for Test 1 is greater than .05.  There may not be a
diffence between responses and/or variances among the dose levels
Modelling the data with a dose/response curve may not be appropriate

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =          0.25

Risk Type        =     Relative deviation

Confidence level =           0.95

             BMD =       0.494132

            BMDL =      0.178782

            BMDU =       1.55485
```

When EC_{25} computations failed because of the lower effect level, a lower toxicological endpoint was selected. For example, if an EC_{25} could not be determined, lower endpoints such as the EC_{24} or EC_{10} were selected. CCME (2006) accepts the range of EC_{20} to EC_{30} in the case of an EC_{25} not being available. For conservative purpose, EEI did not use endpoints greater than EC_{25}, and accepted endpoints from EC_{10} to EC_{24}, when EC_{25} was non-computable (Table 5.5).

Table 5.5. Endpoints Less than EC_{25} Included in Statistics

Toxicological Endpont	Se, mg/kg	Species	Biological Endpoint	Soil Texture	SO_4 Level, mg/kg
EC_{10}	1.75	Alfalfa	Root weight	Coarse	685
EC_{10}	1.73	Alfalfa	Shoot length	Fine	1,481
EC_{10}	2.91	Barley	Root length	Fine	677
EC_{10}	4.25	Barley	Shoot length	Fine	962
EC_{10}	6.82	Barley	Germination	Fine	1,215
EC_{10}	3.92	Barley	Root weight	Fine	1,481
EC_{10}	3.33	N. Wheatgrass	Germination	Fine	1,481
EC_{10}	0.98	Red Fescue	Root length	Fine	962
EC_{11}	10.1	Cucumber	Shoot length	Fine	962
EC_{11}	0.95	Earthworms	Live adults	Coarse	826
EC_{12}	2.5	Barley	Shoot weight	Fine	1,215
EC_{12}	1.02	Cucumber	Shoot length	Fine	1,215
EC_{12}	1.09	N. Wheatgrass	Shoot weight	Fine	1,215
EC_{13}	1.04	R. Fescue	Root length	Fine	962
EC_{14}	0.64	N. Wheatgrass	Germination	Coarse	12
EC_{14}	1.12	N. Wheatgrass	Germination	Fine	54
EC_{14}	2.03	N. Wheatgrass	Shoot length	Coarse	685
EC_{15}	3.44	Barley	Shoot length	Coarse	1,321
EC_{15}	4.06	Carrot	Shoot weight	Fine	1,481
EC_{15}	1.34	Cucumber	Germination	Coarse	1,321
EC_{15}	1.48	Cucumber	Germination	Fine	1,481
EC_{15}	10.2	N. Wheatgrass	Germination	Fine	962
EC_{15}	0.5	R. Fescue	Shoot length	Coarse	1,079
EC_{16}	2.18	N. Wheatgrass	Shoot length	Fine	677
EC_{16}	1.15	N. Wheatgrass	Shoot weight	Fine	962
EC_{17}	6.4	Barley	Shoot length	Coarse	1,079
EC_{17}	1.6	N. Wheatgrass	Root length	Coarse	880
EC_{17}	2.96	N. Wheatgrass	Root length	Fine	1,215
EC_{18}	1.0	Alfalfa	Shoot weight	Fine	1,215
EC_{18}	13.3	Carrot	Shoot length	Coarse	1,481
EC_{18}	0.65	N. Wheatgrass	Shoot length	Coarse	1,079
EC_{19}	1.8	Alfalfa	Root length	Fine	1,215
EC_{19}	2.4	Cucumber	Shoot length	Coarse	1,321
EC_{19}	0.54	N. Wheatgrass	Germination	Coarse	800
EC_{19}	1.1	R. Fescue	Germination	Fine	1,481
EC_{20}	1.28	Cucumber	Germination	Fine	677
EC_{20}	2.91	Cucumber	Root length	Coarse	880
EC_{20}	1.91	Cucumber	Shoot length	Coarse	880
EC_{20}	30.64	Earthworms	Live adults	Fine	91

Table 5.5. Endpoints Less than EC_{25} Included in Statistics

Toxicological Endpont	Se, mg/kg	Species	Biological Endpoint	Soil Texture	SO_4 Level, mg/kg
EC_{20}	0.66	R. Fescue	Shoot weight	Coarse	1,079
EC_{21}	1.28	Cucumber	Germination	Fine	677
EC_{22}	4.04	Barley	Shoot weight	Fine	962
EC_{22}	1.06	Carrot	Root length	Fine	677
EC_{22}	2.4	Cucumber	Shoot weight	Fine	1,481
EC_{22}	13.52	N. Wheatgrass	Shoot length	Fine	1,215
EC_{23}	1.77	Cucumber	Germination	Coarse	880
EC_{23}	1.42	N. Wheatgrass	Root length	Fine	1,481
EC_{24}	1.8	Alfalfa	Root length	Fine	962
EC_{24}	1.24	Carrot	Root length	Fine	1,481
EC_{24}	2.04	N. Wheatgrass	Shoot length	Coarse	685
EC_{24}	10.7	N. Wheatgrass	Shoot length	Fine	962

3 out of 51 lower effect levels were observed in SO_4 control groups, whereas 48 out of 51 were observed within SO_4-treated groups; EC_{25} values become non-computable more often in SO_4 control groups; under equal probabilities, control groups have approximately 10 non-computable EC_{25} values (five SO_4 treatment groups and 51 lower EC values), 18 lower EC values within coarse soils, and 33 lower EC values within fine soils (correlates with a generally lower toxicity in fine soils)

The lowest acceptable toxicological endpoint was an EC_{10} value When EC_{10} calculations failed due to lower than a 10% effect level (very little toxicity observed), a conservative assumption was made that the EC_{25} value was equal to the highest Se dose. This is conservative in that the highest dose in these instances is associated with an $EC_{<10}$ values instead of a EC_{25} value. Based on the BMDS curve (Figure 4.2), corresponding T-tests (Table 4.4), and professional judgement, EC_{25} assumed to be equal to 15.35 mg/kg of Se, the highest concentration in coarse soils in 2014 toxicity studies.

5.1.2 SO_4 – Se Interaction

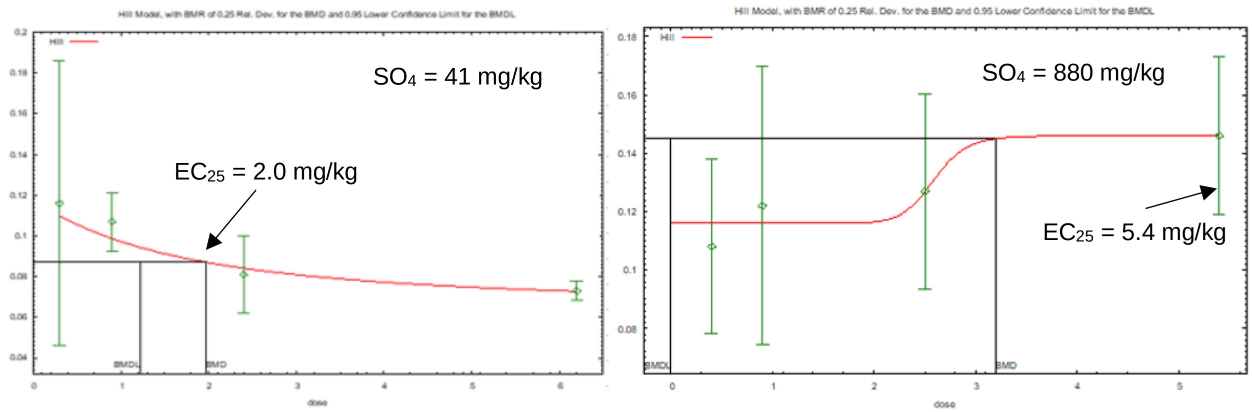
As previously mentioned, a typical dose-response curve is of sigmoidal form (sometimes called a 'S'-shaped curve). The most common way to express this curve is by plotting the percent of population effect versus dose (Rand, 1995). The y-axis shows percentage of subjects with a toxicological response and the x-axis represent the corresponding dose, often presented on a logarithmic scale if the dose range is relatively large. A second method to represent dose-response curves includes plotting the main biological response (the group performance, such as shoot weight in plants, number of juveniles in invertebrates, *etc.*) on the y-axis versus the corresponding dose on the x-axis. In this case, the typical dose-response curve resembles an inverted 'S', reflecting lower performance (such as survivability, mean plant height, *etc*.) with increased dose.

A lower performance with increased Se dose was observed from the 2014 toxicity experiment, with SO_4 concentration ranging from 10 to 12 mg/kg – a similar result was observed for the SO_4 control groups in 2015 to 2017 experiments. When higher SO_4 treatments were applied during 2015 to 2017 studies, the general dose-response curve shape shifted towards a so-called 'U-shaped effect'. This term was adopted from Calabrese and Baldwin (1999) where it has been used to describe a hermetic effect. Other terms describing this type of toxicological response included biphasic, non-monotonic, bell-shaped, J-shaped, overshoot, rebound effect, bitonic, functional antagonism, preconditioning, and adaptive response (Calabrese 2008), which have a common feature: the substantial improve of the low-dosed group compared to control – in other words, under a low exposure scenario, less toxicity is observed compared to the control group. This could be the result of a nutrient effect, where the control is deficient in the nutrient, before doses increase to toxic levels.

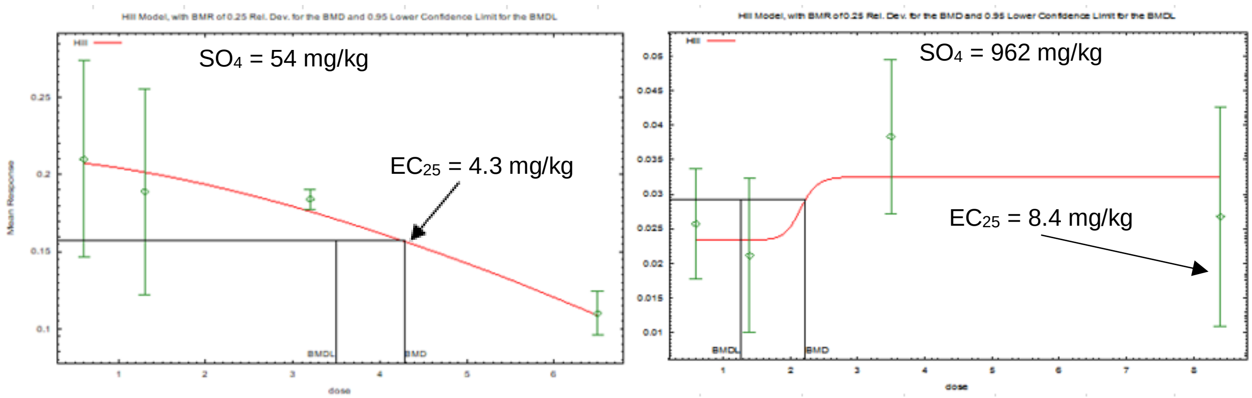
Figure 5.3 (and Table 5.6) illustrates the 'U-shaped effect', observed in 2015 to 2017 studies. Select Se dose-response curves from control SO_4 groups (left) are compared to SO_4–treated groups (right) with the same soil type and biological endpoint. With increasing SO_4 concentrations, it is readily discernable that Se toxicity is reduced, and in some cases, higher Se levels are associated with improved plant performance. This may be due to overcoming a SO_4 induced nutritional Se deficiency at low Se doses via competitive uptake. For conservative purpose, whenever 'U-shaped effect' was observed, EC_{25} was assumed to be equal to the highest Se dose.

Figure 5.3. Dose-response Curves and SO_4 Effect

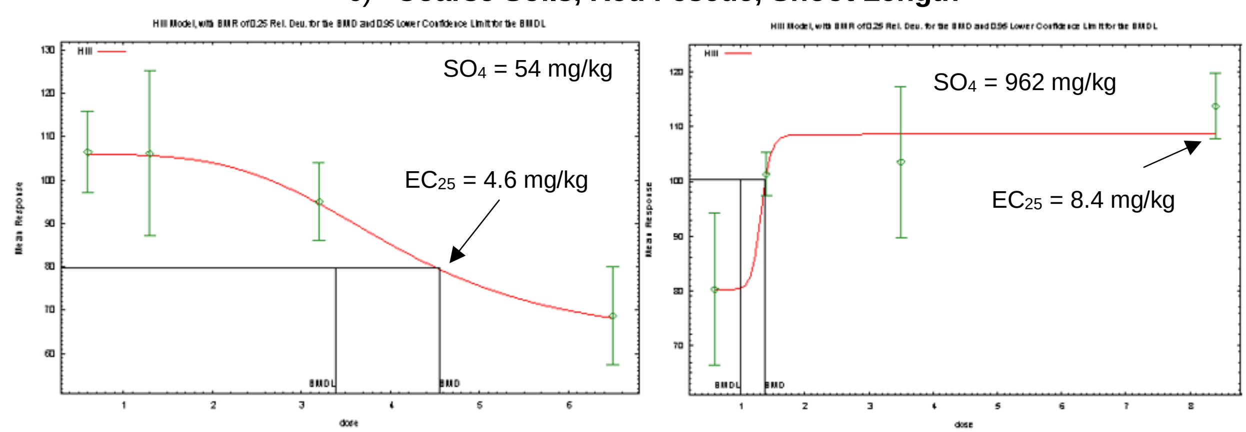
a) Coarse Soils, Barley, Root Weight



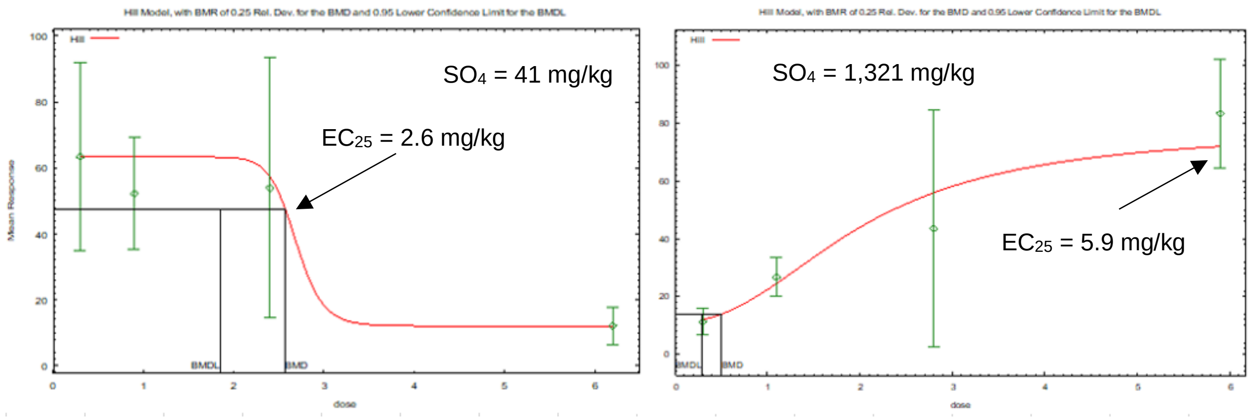
b) **Fine Soils, Cucumber, Shoot Weight**



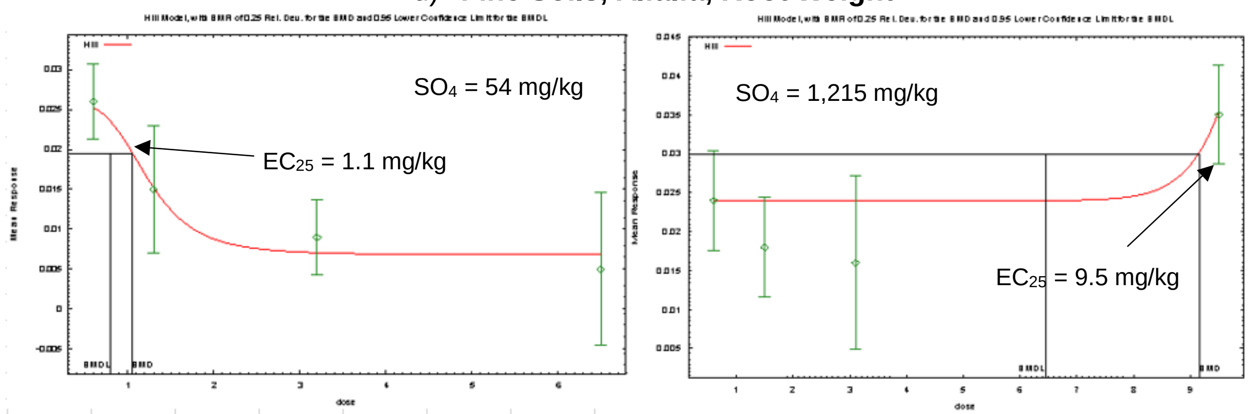
c) **Coarse Soils, Red Fescue, Shoot Length**



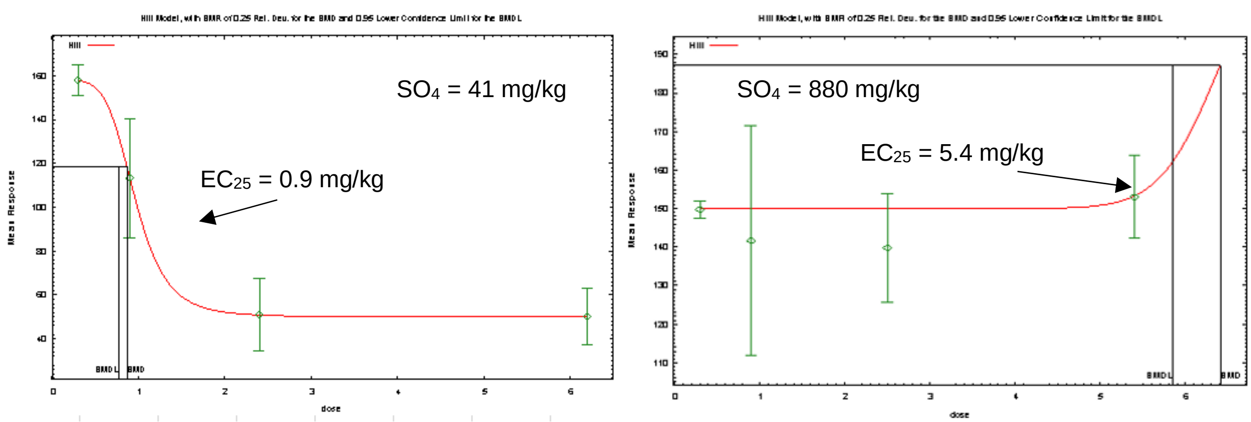
Coarse Soils, Carrot, Root Length



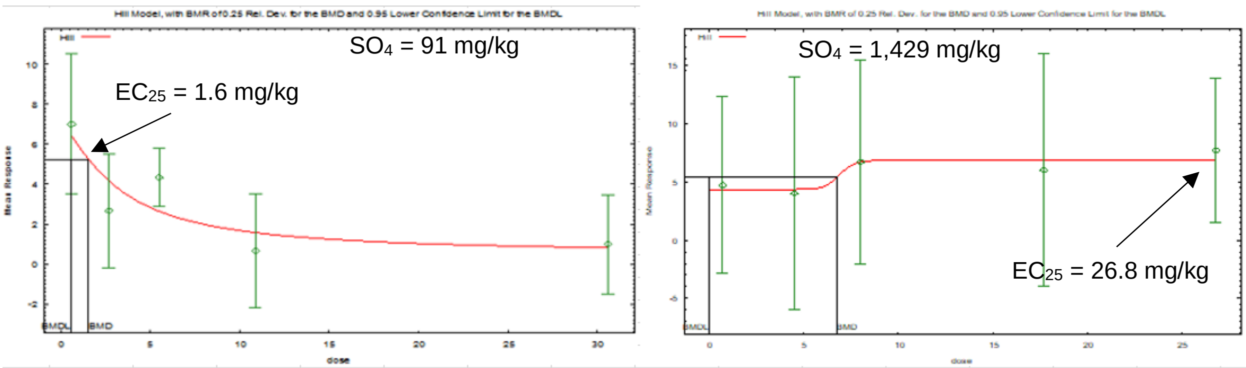
d) Fine Soils, Alfalfa, Root Weight



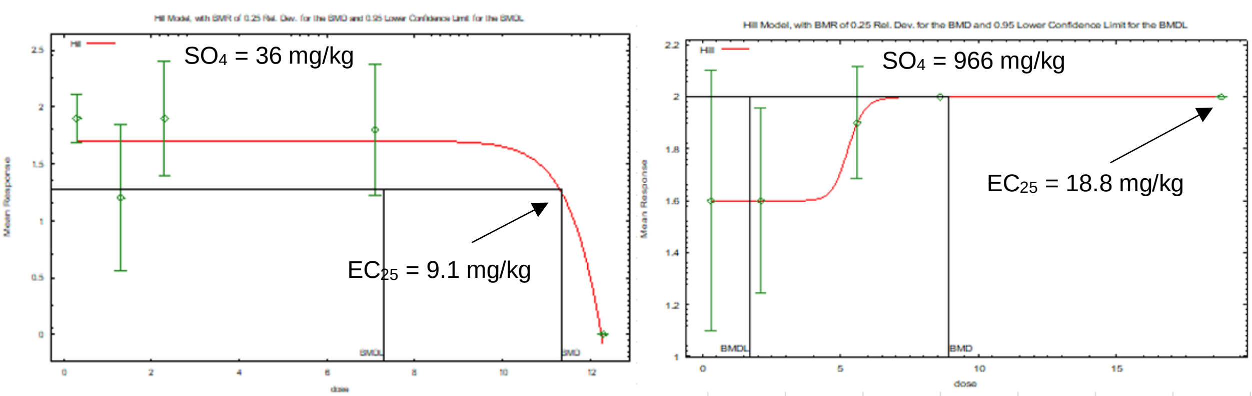
e) Coarse Soils, N. Wheatgrass, Shoot Length



f) **Fine Soils, Springtails, Live Adults #**



g) **Coarse Soils, Earthworms, Live Adults**



5.1.3 SO_4 TOXICITY

To evaluate possible SO_4 toxicity interferences, SO_4 endpoints (EC_{25}) were calculated by BMDS using the same methodology as Se (Section. 4.1.1). Se control dose-response data for coarse and fine soils were arranged by SO_4 treatment and analysed using the Hill model. Red font in Table 4.6 indicates the EC_{25} values are lower than the highest SO_4 treatment. The corresponding BMDS output data are present in Appendix F.

Red fescue shoot mass (fine soils) and juvenile earthworms (fine and coarse soils) demonstrated a toxic response at relatively lower SO_4 concentrations. This complicates the toxicological analysis within the range of Se and SO_4 doses tested, given that Se induced toxicity at high Se concentrations combined with low SO_4 concentrations, SO_4 ameliorated Se toxicity at higher SO_4 concentrations, higher Se concentrations overcome SO_4 induced low Se dose nutritional deficiency, and SO_4 may induce a distinct toxic effect at higher SO_4 concentrations.

Table 5.6. Summary of EC_{25} (SO_4, mg/kg) by Species; Se Control Treatment

a) 2015-2017 (Plants)

	Biological Endpoint				
Species	**Root Weight**	**Shoot Weight**	**Root Length**	**Shoot Length**	**Germination**
Coarse Soils					
Alfalfa	1,321	**1,051**	**1,250**	**1,134**	**1,225**
Barley	1,321	1,321	1,321	1,321	1,321
Carrot	**1,236**	1,321	**1,234**	**1,217**	1,321
Cucumber	1,321	1,321	1,321	1,321	1,321
N. Wheatgrass	1,321	1,321	**1,305**	**1,301**	1,321
R. Fescue	1,321	1,321	**1,016**	**1,252**	**1,260**
Fine Soils					
Alfalfa	1,481	1,481	1,481	1,481	1,481
Barley	1,481	1,481	1,481	1,481	1,481
Carrot	**1,245**	1,481	1,481	**2185**	1,588
Cucumber	1,481	1,481	1,481	1,481	1,481
N. Wheatgrass	1,481	1,481	1,481	1,481	1,481
R. Fescue	1,481	**661**	1,481	**140**	1,481

b) 2016-2017 (Invertebrates)

	Biological Endpoint		
Species	**Live Adults**	**Juveniles**	**Juvenile Weight**
Coarse Soils			
Springtails	1,145	1,145	*na*
Earthworms	1,145	**883**	**789**
Fine Soils			
Springtails	1,429	1,429	*na*
Earthworms	1,429	**697**	**309**

5.1.4 QUALITY ASSURANCE AND T-TESTS

For quality assurance purposes, the dose-response data set from Se control groups tested in 2015 and 2017, were evaluated with T-tests. T-tests compared the 2015 to 2016 negative control SO_4 treatment (36-91 mg/kg SO_4) to the 2017 low SO_4 control treatment (667-685 mg/kg) data pool. Additional T-tests compared the 2015 to 2016 negative control SO_4 group (36-91 mg/kg SO_4) to the 2015 to 2016 low SO_4 control group (826-962 mg/kg SO_4) and 2017 low SO_4 control group (667-685 mg/kg), to evaluate potential SO_4 toxicity interference.

The T-test comparison between data sets was introduced to ensure consistency of conditions between two seasons of experimental study. The 2015 experiment took place in late summer - early fall, whereas 2017 experiment was conducted during winter (Degenhardt, pers. communication), and therefore, 2017 and 2015 plants might receive different level of artificial versus natural light. The larger amount of artificial light could potentially increase the plant growth, and therefore, introduce the bias towards the low level of SO_4 treatment.

Another potential interference is that caused by SO_4 toxicity itself, and T-tests were conducted to ensure all Se control groups were consistent in their response to increased SO_4 level. In addition to T-tests, SO_4 toxicity was evaluated using the BMDS software (Section 4.1.3). Following a detailed analysis, approximately 7% and 5% of the 2015 to 2017 plant biological endpoints were excluded from the coarse and fine soil data pools, respectively. For the invertebrate dataset, 4% and 8% (coarse and fine, respectively) were excluded. The remaining dataset was considered of sufficient quality to support detailed quantitative analysis for potential guideline development support.

5.1.5 EC_{25} SUMMARY

Table 5.7 summarizes all toxicological endpoints (EC_{25}), calculated with BMDS from 2014-2017 studies, described in Section 4.2-4.4. Light orange highlights indicate the endpoints obtained from the curves with distinct sigmoidal-shaped effect. Light grey highlights indicate the endpoints excluded from the data pool based on the quality assurance analysis (discussed in Section 5.1.4). Corresponding BMDS curves are shown in Appendices B to F.

Table 5.7. Summary of EC_{25} (Se, mg/kg) by Species and SO_4 Treatment

a) 2014 (Plants)

	Biological Endpoint				
Species	**Root Weight**	**Shoot Weight**	**Root Length**	**Shoot Length**	**Germination**
Coarse Soils, SO_4 = 10 mg/kg					
Alfalfa	0.6	0.7	0.7	1.5	14.3
Barley	0.3	0.5	0.7	1.1	0.5
Carrot	1.2	1.2	1.2	1.8	1.6
Cucumber	1.2	2.3	1.2	1.9	1.3
N. Wheatgrass	0.8	0.9	1.5	1.8	0.6
R. Fescue	0.4	0.4	0.6	0.7	13.3
Fine Soils, SO_4 = 12 mg/kg					
Alfalfa	2.2	2.5	1.3	2.4	15.1
Barley	2.1	2.6	2.6	4.2	15.7
Carrot	2.1	2.3	2.8	2.5	5.6
Cucumber	2.6	2.8	2.1	1.8	15.7
N. Wheatgrass	1.9	1.9	2.6	2.4	15.7
R. Fescue	2.1	2.3	2.7	2.9	16.8

Table 5.7. Summary of EC_{25} (Se, mg/kg) by Species and SO_4 Treatment cont…

b) 2015 to 2017 (Plants)

	Biological Endpoint				
Species	**Root Weight**	**Shoot Weight**	**Root Length**	**Shoot Length**	**Germination**
Coarse Soils					
SO_4 = 41 mg/kg					
Alfalfa	0.9	1.0	0.8	1.2	1.5
Barley	2.0	2.4	2.4	2.1	6.2
Carrot	1.0	2.1	2.6	1.8	1.7
Cucumber	3.9	4.2	2.6	3.6	6.2
N. Wheatgrass	0.8	0.8	0.9	0.9	0.5
R. Fescue	6.2	6.0	0.8	2.2	6.2
SO_4 = 684 mg/kg					
Alfalfa	1.8	5.8	5.8	5.8	5.8
Barley	5.8	0.8	5.8	1.0	5.8
Carrot	5.8	5.8	5.7	5.8	5.8
Cucumber	0.5	0.7	0.5	5.8	0.6
N. Wheatgrass	1.5	0.9	1.7	2.0	1.8
R. Fescue	5.8	5.8	5.8	5.8	5.8
SO_4 = 880 mg/kg					
Alfalfa	0.9	0.9	0.9	1.0	1.0
Barley	5.4	5.4	5.4	5.4	5.4
Carrot	5.4	1.6	5.4	6.0	2.3
Cucumber	2.9	1.6	0.9	1.9	1.8
N. Wheatgrass	5.4	5.4	1.6	5.4	0.5
R. Fescue	5.4	5.4	1.9	5.4	5.4
SO_4 = 1,079 mg/kg					
Alfalfa	0.5	1.1	0.8	2.5	1.7
Barley	5.4	5.4	5.4	6.4	5.4
Carrot	0.5	1.4	0.8	5.5	0.5
Cucumber	2.9	2.6	5.4	1.5	1.9
N. Wheatgrass	0.5	0.5	0.6	5.4	0.6
R. Fescue	5.4	0.7	5.1	5.4	0.5
SO_4 = 1,321 mg/kg					
Alfalfa	0.5	0.6	5.9	5.9	0.5
Barley	5.9	5.7	2.9	3.4	5.8
Carrot	5.9	5.9	5.9	5.9	5.9
Cucumber	2.8	2.1	5.9	2.4	1.3
N. Wheatgrass	5.9	5.9	5.9	5.9	0.5
R. Fescue	5.9	5.9	5.9	5.9	5.9

Table 5.7. Summary of EC_{25} (Se, mg/kg) by Species and SO_4 Treatment cont…

Fine Soils					
SO_4 = 54 mg/kg					
Alfalfa	1.1	5.7	1.4	3.5	6.5
Barley	6.3	2.3	3.2	3.2	6.5
Carrot	0.8	1.1	6.2	4.3	6.5
Cucumber	2.2	4.3	7.0	3.5	6.5
N. Wheatgrass	1.8	1.2	2.1	2.6	1.1
R. Fescue	1.7	3.5	4.0	4.6	8.3
SO_4 = 677 mg/kg					
Alfalfa	0.9	0.8	5.4	1.8	5.8
Barley	5.8	5.8	2.9	5.8	5.8
Carrot	5.8	5.8	1.1	5.8	5.8
Cucumber	1.0	5.8	5.8	5.8	1.3
N. Wheatgrass	1.0	1.2	1.5	2.2	5.8
R. Fescue	4.3	5.8	5.8	6.2	5.9
SO_4 = 962 mg/kg					
Alfalfa	1.4	8.9	1.8	8.4	8.4
Barley	18.2	4.0	9.0	4.3	11.6
Carrot	8.4	8.4	8.4	8.4	8.4
Cucumber	8.4	8.4	8.4	10.1	9.5
N. Wheatgrass	8.4	1.2	8.4	10.7	10.2
R. Fescue	8.4	8.4	1.0	8.4	9.0
SO_4 = 1,215 mg/kg					
Alfalfa	9.5	1.0	1.8	9.5	9.5
Barley	5.3	2.5	9.5	9.5	6.8
Carrot	9.5	9.5	9.5	9.5	9.5
Cucumber	9.5	9.5	9.6	1.0	9.5
N. Wheatgrass	1.2	1.1	3.0	13.5	9.5
R. Fescue	9.5	9.5	1.0	9.5	9.5
SO_4 = 1,481 mg/kg					
Alfalfa	1.0	1.0	0.9	1.7	7.8
Barley	3.9	7.8	7.8	7.8	7.8
Carrot	7.8	4.1	7.8	13.3	8.6
Cucumber	7.8	2.4	7.8	14.6	1.5
N. Wheatgrass	0.9	1.2	1.4	7.8	3.3
R. Fescue	7.8	7.8	7.8	7.8	1.1

Table 5.7. Summary of EC_{25} (Se, mg/kg) by Species and SO_4 Treatment cont…

c) 2016-2017 (Invertebrates)

	Biological Endpoints		
Species	**Live Adults**	**Juveniles**	**Juvenile Weight**
Coarse Soils			
SO_4 = 36 mg/kg			
Springtails	1.2	1.8	*na*
Earthworms	9.1	0.2	3.1
SO_4 = 684 mg/kg			
Springtails	5.8	5.8	*na*
Earthworms	5.8	5.8	5.38
SO_4 = 826 mg/kg			
Springtails	15.2	2.8	*na*
Earthworms	0.1	1.3	3.0
SO_4 = 966 mg/kg			
Springtails	15.6	18.8	*na*
Earthworms	18.8	1.9	18.8
SO_4 = 1,145 mg/kg			
Springtails	2.9	3.3	*na*
Earthworms	20.5	7.1	9.6
Fine Soils			
SO_4 = 91 mg/kg			
Springtails	1.6	7.0	*na*
Earthworms	30.6	1.0	1.1
SO_4 = 677 mg/kg			
Springtails	5.8	5.8	*na*
Earthworms	5.8	5.29	4.5
SO_4 = 919 mg/kg			
Springtails	16.0	13.5	*na*
Earthworms	26.6	1.1	3.1
SO_4 = 1,142 mg/kg			
Springtails	31.2	31.2	*na*
Earthworms	16.2	4.0	2.8
SO_4 = 1,429 mg/kg			
Springtails	26.8	26.8	*na*
Earthworms	27.2	24.8	1.5

5.2 PLANT BIOCONCENTRATION

Plant tissue samples with sufficient yield were analyzed for Se and sulphate (as sulphur (S)) concentrations. Alfalfa, carrot, northern wheatgrass, and red fescue did not produce enough dry mass and the main was focused on the shoot yield from barley and cucumber.

Bioconcentration factors (BCFs) were calculated for Se and S as plant-to-soil ratios, calculated from the concentration in plant tissue (ug/g) divided the corresponding concentration in soil (mg/kg). Table 5.8 provides a summary of Se and S analytical data with calculated BCF, grouped by soil type, species endpoint, and Se treatment, in ascending SO_4 treatment order within each group.

The highest Se bioconcentration was observed within SO_4 control groups, with low to mid Se treatment (Table 5.8, red font), whereas the highest S bioconcentration (Table 5.8, blue font) was observed within negative control groups (no Se, no SO_4 added). Se concentration in plant tissues collected from Se control soils (0.3-0.8 mg/kg in soil) did not exceed 3 ug/g, while within Se-treated soils (0.9-9.5 mg/kg) it falls in the range from 19.7 to 1,115 mg/kg. The median BCF for Se from the samples with combined Se- and SO4-treatment is equal to 29 mg/kg for coarse soils, and 21 mg/kg for fine soils. Figure 5.4 represent Se BCF versus corresponding SO_4 treatment in bar charts. The Se BCF values are greater than those reported in the literature, likely due to the tissue samples being taken from very young plants where accumulation is the greatest on a mg/kg dry tissue basis.

Table 5.8. BCF for Se and S with Corresponding Soil and Plant Tissue Concentrations

Sample ID*	Se in soil, mg/kg	Se in plant tissue, ug/g	BCF for Se	S in soil, mg/kg	S in plant tissue, ug/g	BCF for S
Coarse Soils						
Alfalfa, shoots						
Se3	5.8	200	34	217	8,700	40
Barley, roots						
Se0	0.4	2	5	227	6,700	30
Barley, shoots						
Se0	0.4	1	3	227	4,100	18
Se0-S3	0.3	0.8	3	414	5,900	14
Se1	0.9	18	20	224	6,300	28
Se1-S1	0.3	14	47	298	6,400	21
Se1-S2	0.9	9.7	11	367	7,000	19
Se1-S3	1.1	9	8	438	6,300	14
Se2	2.9	110	38	244	13,000	53
Se2-S1	2.5	55	22	309	7,900	26
Se2-S2	2.8	37	13	327	6,900	21
Se2-S3	2.8	46	16	457	7,900	17
Se3	5.8	410	71	217	16,000	74
Se3-S1	5.4	170	31	280	10,000	36
Se3-S2	5.4	57	11	380	12,000	32
Cucumber, shoots						
Se0	0.4	2	5	227	17,000	75
Se1	0.9	25	28	224	15,000	67
Se1-S1	0.9	26.5	29	286	17,700	62
Se2	2.9	110	38	244	16,000	66
Se3	5.8	260	45	217	19,000	88
Se3-S1	5.4	316	59	280	16,600	59
Fine Soils						
Barley, shoots						
Se0-S0	0.6	0.9	1.5	7	9,400	**1,424**
Se0	0.8	3.0	3.8	238	8,400	35
Se0-S1	0.6	0.6	1.0	327	11,100	34

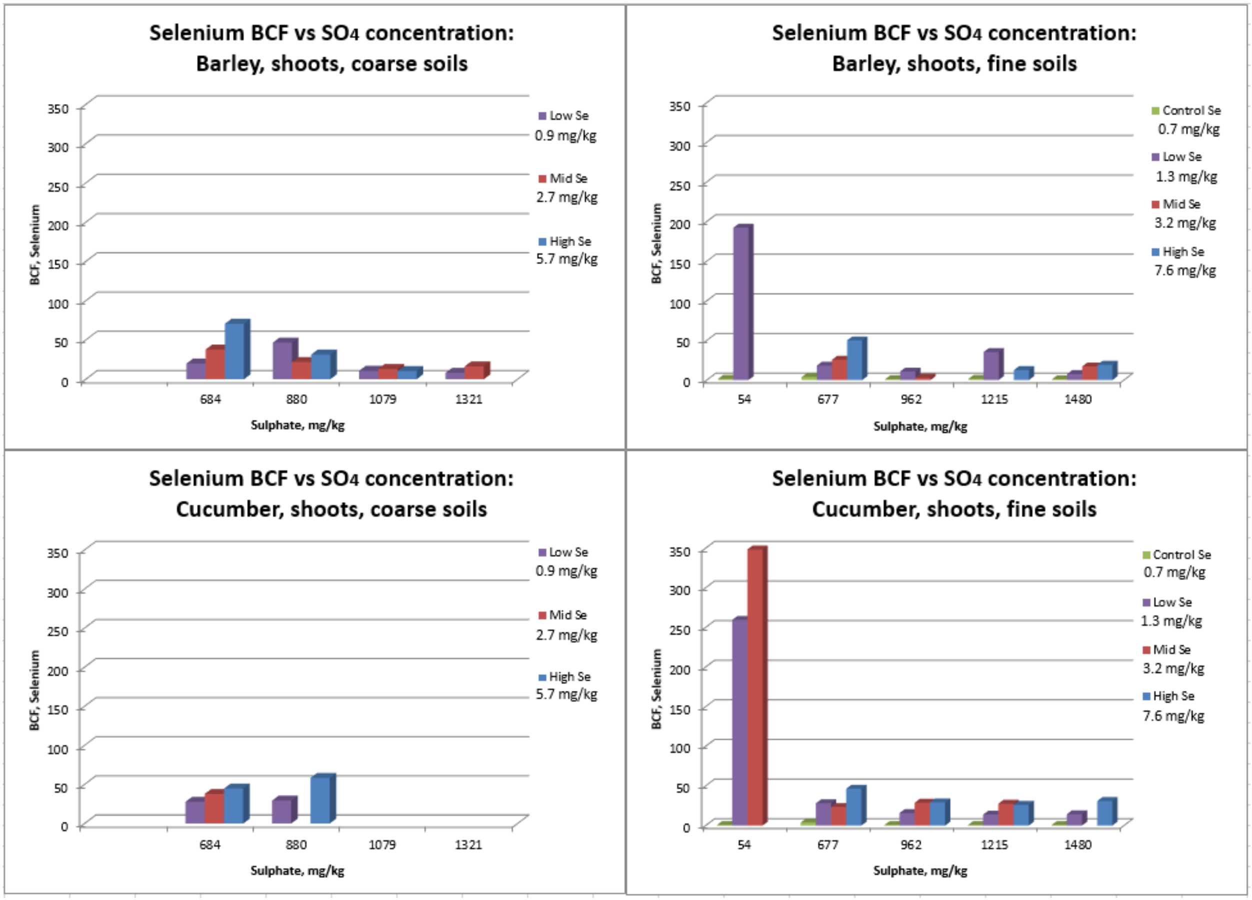
Table 5.8. BCF for Se and S with Corresponding Soil and Plant Tissue Concentrations

Sample ID*	Se in soil, mg/kg	Se in plant tissue, ug/g	BCF for Se	S in soil, mg/kg	S in plant tissue, ug/g	BCF for S
Se0-S2	0.6	0.9	1.5	420	11,000	26
Se0-S3	0.7	0.6	0.9	469	9,700	21
Se1-S0	1.3	250	**192**	23	11,000	487
Se1	1.1	20	18.2	208	10,000	48
Se1-S1	1.4	15	10.7	299	9,700	32
Se1-S2	1.5	53	35.3	379	10,000	26
Se1-S3	1.4	11	7.5	503	12,100	24
Se2	2.9	74	25.5	234	8,600	37
Se2-S1	3.5	10	2.9	311	10,000	32
Se2-S3	3.2	55	17.2	484	12,000	25
Se3	5.8	290	50.0	222	13,000	59
Se3-S2	9.5	120	12.6	412	12,000	29
Se3-S3	7.8	150	19.2	517	14,000	27
Cucumber, shoots						
Se0-S0	0.6	0.4	0.7	7	8,750	**1,326**
Se0	0.8	3.0	3.8	238	15,000	63
Se0-S1	0.6	0.4	0.7	327	18,800	57
Se0-S2	0.6	0.4	0.7	420	20,300	48
Se0-S3**	1.7	1.1	0.7	496	18,100	36
Se1-S0**	1.3	338	**260**	23	5,080	225
Se1	1.1	31	28.2	208	17,000	82
Se1-S1	1.4	22	15.8	299	19,100	64
Se1-S2**	1.5	21	13.9	379	17,700	47
Se1-S3	1.4	20	14.3	503	21,800	43
Se2-S0**	3.2	1,115	**348**	32	9,800	306
Se2	2.9	68	23.4	234	17,000	73
Se2-S1	3.5	101	28.9	311	23,300	75
Se2-S2	3.1	86	27.6	409	20,000	49
Se3	5.8	270	46.6	222	18,000	81
Se3-S1	8.4	246	29.3	346	19,300	56
Se3-S2	9.5	249	26.2	412	19,400	47
Se3-S3	7.8	242	31.0	517	22,600	44

**Sample IDs are listed according to AI identification system. Se and S are for selenium and sulphate treatment, numbered from 0 to 3 by increasing concentration. Sample IDs Se0, Se1, Se2, and Se3 identifiy 2017 experiment, treated with different Se but single SO_4 dose (684 mg/kg for coarse, and 677 mg/kg for fine soils); this low SO_4 treatment falls between -S0 and -S1 (2015 experiment).*

***Calculated as an average from two sample sets, submitted in 2015 (one replicate) and 2017(pooled replicates.*

Figure 5.4. Selenium BCF versus SO_4 Concentration



Note that number of bars per treatment reflect the number of samples analyzed (i.e., negative control data for barley and cucumber was not included for insufficient yield; cucumber tissues from the highest SO_4 groups, coarse soils, were less than 0.7 g, etc.)

5.3 SATURATED PASTE VS ACID EXTRACTABLE SE

To assess Se bioavailabilty, select soils samples were analyzed for the saturated paste. The saturated paste method was chosen for the more representative measure of the amount of Se in soils available for plants under typical field conditions (Gartley, 2011). Analytical chemistry data is shown in Table 5.9. Values bolded and in red for Se on a saturated paste basis were considered anomalous and not readily explainable given total Se concentrations.

Table 5.9. Summary of Se Saturated Paste and Acid Extractable Data

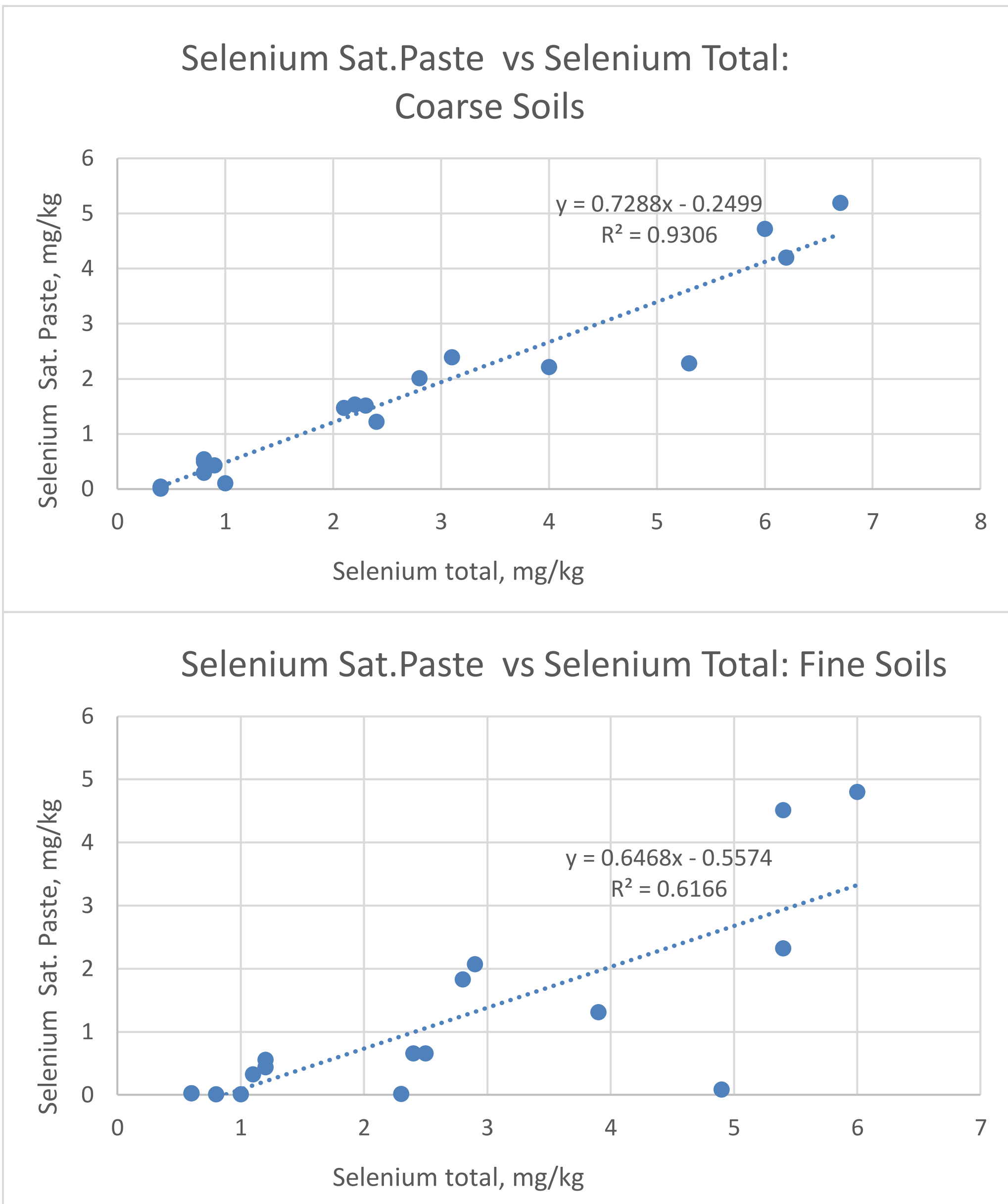
Sample ID*	Se Total	Soil Saturation Percentage	Se, Saturated Paste	Se, Saturated Paste	Se, Total to Se, Saturated Paste Ratio
	mg/kg	%	mg/kg	mg/L	
Coarse Soils, 2017 (Various species)**					
Se1 (Alfalfa)	0.8	42	0.50	1.18	1.6
Se2 (Alfalfa)	2.8	47	2.01	4.27	1.4
Se3 (Alfalfa)	6.0	44	4.72	10.80	1.3
Se 0 (Carrot)	0.4	46	0.01	0.02	56
Se1 (Carrot)	0.9	46	0.43	0.92	2.1
Se2 (Carrot)	2.4	46	1.22	2.64	2.0
Se3 (Carrot)	4	46	2.21	4.84	1.8
Se0 (Cucumber)	0.4	46	0.03	0.07	13
Se2 (Cucumber)	2.2	46	1.53	3.32	1.4
Se1 (Red fescue)	0.8	44	0.54	1.22	1.5
Se2 (Red fescue)	3.1	45	2.39	5.33	1.3
Se3 (Red fescue)	5.3	42	2.28	5.49	2.3
Se0 (Plants)	0.4	45	0.04	0.10	9.2
Coarse Soils, 2015 (Composite)**					
Se1-S0	1	43	0.11	0.25	9.5
Se1-S2	0.8	51	0.30	0.58	2.7
Se2-S2	2.1	45	1.47	3.31	1.4
Se2-S1	2.3	44	1.51	3.44	1.5
Se3-S1	6.7	52	5.19	9.89	1.3
Se3-S2	6.2	43	4.20	9.69	1.5
Fine Soils, 2017 (Various species)					
Se0 (Alfalfa)	0.6	56	0.025	0.05	24
Se2 (Alfalfa)	2.4	53	0.655	1.23	3.7
Se3 (Alfalfa)	3.9	57	1.310	2.29	3.0
Se0 (Carrot)	0.8	54	0.010	0.02	80.0
Se1 (Carrot)	1	55	**0.007**	**0.01**	149
Se2 (Carrot)	2.3	52	**0.014**	**0.03**	164
Se3 (Carrot)	4.9	52	**0.083**	**0.16**	59
Se0 (Red fescue)	0.6	53	0.02	0.05	24
Se1 (Red fescue)	1.1	63	0.33	0.52	3.4
Se2 (Red fescue)	2.5	60	0.66	1.09	3.8
Se3 (Red fescue)	5.4	61	2.320	3.83	2.3
Fine Soils, 2015 (Composite)					
Se1-S0	1.2	57	0.44	0.76	2.8
Se1-S2	1.2	56	0.55	0.98	2.2
Se2-S1	2.9	54	2.07	3.83	1.4
Se2-S2	2.8	52	1.83	3.55	1.5
Se3-S1	6.0	55	4.80	8.78	1.3
Se3-S2	5.4	48	4.51	9.46	1.2

**Sample IDs are listed according to AI identification system. Se and S are for selenium and sulphate treatment, numbered from 0 to 3 by increasing concentration. Sample IDs Se0, Se1, Se2, and Se3 from 2017 experiment were treated with different Se but single SO_4 dose (684 mg/kg for coarse soils, and 677 mg/kg for fine soils); this low SO_4 treatment falls between -S0 and -S1 from 2015 experiment.*

***Available soil samples received from AI were labeled originally as 'composite' for 2015 experiment, and by species in 2017, except one, which was labeled as 'plants' (presumable composite sample).*

Figure 5.5 presents a regression analysis performed to assess the validity of the data and evaluate the ratio. Both regressions have in general a predictable slope. The correlation coefficient for coarse soils is greater than for the fine soils (0.7288 versus 0.6468). This difference is primarily due to several 2017 data points for carrots in fine soils that had unusually low saturated paste values given the corresonding total Se values (red highlighted values in Table 5.9). It is not clear as to why these saturate paste results different from the rest of the experiments, and it may be an effect unique to carrots. Excluding these values produced a correlation coefficient similar to that of the coarse soil analysis.

Figure 5.5. Linear Regression of Se Sat. Paste vs Se Total



6 DERIVATION OF PRELIMINARY ECOLOGICAL DIRECT SOIL CONTACT GUIDELINES

6.1 SPECIES SENSITIVITY DISTRIBUTIONS

The species sensitivity distribution (SSD) concept was proposed in the late 1970s and mid-1980s in the United States and Europe for deriving environmental soil criteria (Aldenberg *et al*., 2002). It is currently used as a part of the weight of evidence method for ecological soil contact guidelines (CCME 2006). SSDs represent the statistical distributions of measures of species sensitivity to a tested compound or mixture, to assist in estimating concentrations expected to be safe for the majority of species of interest. The SSD curve is built on a sample of toxicity data with effect concentrations derived from acute or chronic toxicity tests and visualized as a cumulative distribution function. As a measure of toxicity, CCME (2006) recommends use of EC_{25} effect-endpoint, if available, or the closest value (generally between 20% and 30%).

6.1.1 SE SPECIES SENSITIVITY DISTRIBUTION CURVES

The final SSD curves were based on sixteen endpoints from eight species (six plants and two invertebrates), for two soil types (coarse and fine loam soils) and varying SO_4 co-exposure levels. Geometrical means from shoot and root EC_{25} were combined into single dry mass endpoint, and for earthworms, the final juvenile EC_{25} was calculated as a geometric mean from the juvenile number EC_{25} and the juvenile dry weight EC_{25}. A summary of the final toxicological endpoints for coarse and fine soils grouped by SO_4 treatment is provided in Table 6.1. Grey highlights indicate endpoints excluded from the data set for quality assurance purpose (see Section 5.1.4). Identical values in Table 6.1 are frequently due to the highest Se dose per treatment rather than BMDS-calculated value, since the EC_{25} computations fell outside of the applied Se treatment range. In other words, under the maximum Se dose tested, no significant toxicity was observed.

The EC_{25} presented in Table 6.1 by each SO_4 treatment were ranked, and rank percentiles were determined according to the CCME (2006) Protocol. Rank percentiles were plotted against corresponding EC_{25} values and a typical sigmoid curve was produced. This approach is called Hazen plotting positions (HPP) where the rank percentile represents an estimation of the population affected by a particular dose. SSD curves are shown in Figure 6.1, with coarse soil (left) and fine soils (right), grouped by SO_4 treatment. HPP points are shown by blue dots, with the red dashed line indicating the 25% effect level for all of the biological endpoints. The intersection between an SSD curve and 25% line, furher evaluated by EasyFit 5.6 Professional software, provides the value that can be used to assist in deriving ecological Se guidelines.

$$j = \frac{i}{n+1}$$

where,
j = *rank percentile*
i = *rank of the data point in the data set*
n = *total number of data points in the data set*

Table 6.1. Se Toxicological Endpoints (EC_{25}) Used for SSD Curves

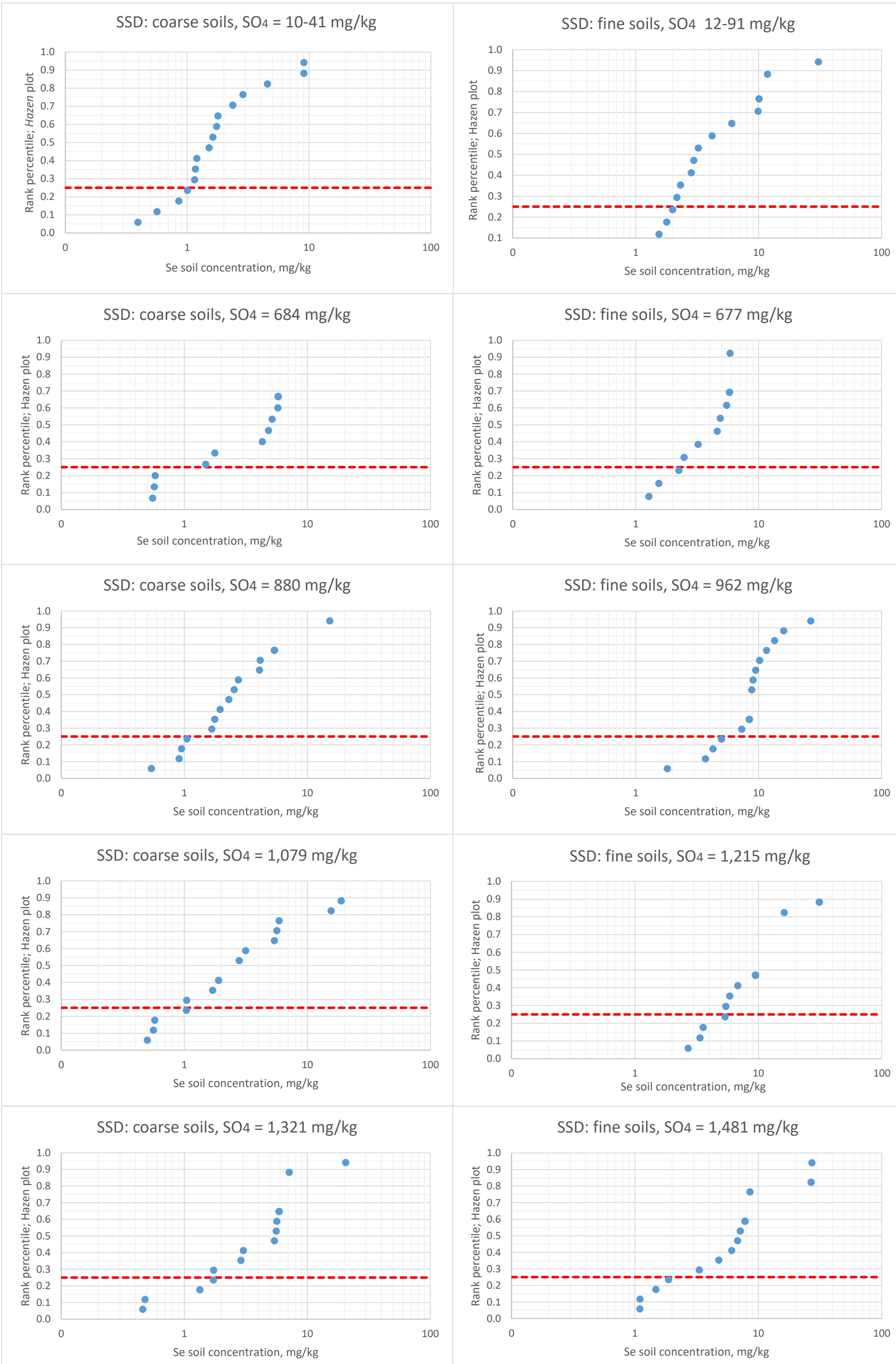
a) Coarse Soils

Species	Biological Endpoint	SO_4 Treatment: Control	Low	Mid-1	Mid-2	High
Alfalfa	Dry Mass	0.9	4.3	0.9	1.0	1.7
	Germination	4.6	5.8	1.1	1.7	0.5
Barley	Dry Mass	1.2		5.4	5.6	5.6
	Germination	1.7	5.8	5.4	5.4	5.4
Carrot	Dry Mass	1.5	5.75	4.1	1.0	5.9
	Germination	1.6		2.3	1.9	5.9
Cucumber	Dry Mass	2.4	0.6	1.7	2.8	3.0
	Germination	2.9	0.6	1.8	1.9	1.3
N. Wheatgrass	Dry Mass	1.0	1.5	2.5	0.6	1.7
	Germination	0.6	1.8	0.5	0.6	0.5
R. Fescue	Dry Mass	1.2	5.8	4.1	3.2	5.9
	Germination	9.1	5.8	5.4	0.5	5.9
Springtails	Adults	1.2	0.6	15.2	15.6	2.9
	Juveniles	1.8	5.2	2.8	18.8	7.1
Earthworms	Adults	9.1	5.8	1.0	18.8	20.5
	Juveniles	0.4	4.8	2.0	5.9	5.6

b) Fine Soils

Species	Biological Endpoint	SO_4 Treatment: Control	Low	Mid-1	Mid-2	High
Alfalfa	Dry Mass	2.2	2.2	3.7	3.6	1.1
	Germination	9.9		8.4	9.5	7.8
Barley	Dry Mass	3.2	4.6	7.3	5.9	7.2
	Germination	10.1	5.8	11.6	6.8	7.8
Carrot	Dry Mass	2.3	2.5	8.4	9.5	4.8
	Germination	6.1	5.8	8.4	9.5	8.6
Cucumber	Dry Mass	3.0	3.2	8.8	5.5	6.8
	Germination	10.1	1.3	9.5	9.5	1.5
N. Wheatgrass	Dry Mass	2.0	1.5	4.3	2.7	1.9
	Germination	4.2		10.2	9.5	3.3
R. Fescue	Dry Mass	2.8	5.5	5.0	5.4	7.8
	Germination	11.8	5.9	9.0	9.5	1.1
Springtails	Adults	1.6		16.0	31.2	26.8
	Juveniles	1.8		13.5	31.2	26.8
Earthworms	Adults	30.6	5.8	26.6	16.2	27.2
	Juveniles	1.0	4.9	1.8	3.4	6.1

Figure 6.1. Se SSD Curves by Hazen Plotting



Red dashed line represent the 25% rank of all of the biological data for a particular dosing level

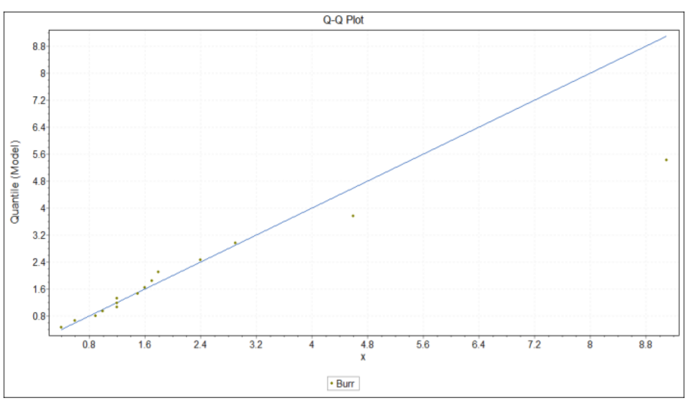
The calculated toxicological endpoints (EC_{10}-EC_{25} summarized in Section 5.1.5) and corresponding HPP ranks were analyzed by EasyFit 5.6 Professional software (Matwave Technologies, 2017) for the best distribution fit using probability density function, where 61 various models were compared, evaluated, and ranked by Anderson-Darling, Kolmogorov-Smirnov, or Chi-Squared statistical procedures. The Anderson-Darling rank was preferred because of the highest sensitivity towards the tails (lowest end of the tail is used to derive guideline values), and therefore, higher accuracy in prediction for the most sensitive species.

Ten best-ranked models for each SO_4 treatment group were further evaluated based on cumulative distribution functions with mathematical equations for each model. Figure 6.2 shows an example of the 'Goodness of Fit' summary for coarse soils, control SO_4 group, with the ten highest-ranked models (Anderson-Darling tests) and goodness of fit details for the Burr model (rank 1), followed by a quantile plot that can be used to evaluate the proximity of existing HPP points to the selected model (Burr in this instance).

Figure 6.2. Select Easy Fit Outputs for Coarse Soils, Control SO_4

Goodness of Fit - Summary

#	Distribution	Kolmogorov Smirnov		Anderson Darling		Chi-Squared	
		Statistic	Rank	Statistic	Rank	Statistic	Rank
2	Burr	0.10812	1	0.21491	1	0.12734	18
7	Dagum	0.12087	4	0.25	2	0.12359	13
16	Frechet (3P)	0.12232	5	0.25611	3	0.12575	17
42	Pearson 5	0.12055	3	0.2678	4	0.14057	20
43	Pearson 5 (3P)	0.12609	7	0.26918	5	0.12542	16
44	Pearson 6	0.12631	8	0.26953	6	0.12504	15
34	Log-Logistic (3P)	0.12277	6	0.27679	7	0.15283	21
35	Log-Pearson 3	0.13335	9	0.28963	8	0.11287	10
19	Gen. Extreme Value	0.12026	2	0.29542	9	0.05207	6
38	Lognormal (3P)	0.13934	11	0.33194	10	0.12471	14



Goodness of Fit - Details [hide]

Burr [#2]

Kolmogorov-Smirnov

Sample Size	16
Statistic	0.10812
P-Value	0.98175
Rank	1

α	0.2	0.1	0.05	0.02	0.01
Critical Value	0.25778	0.29472	0.32733	0.36571	0.39201
Reject?	No	No	No	No	No

Anderson-Darling

Sample Size	16
Statistic	0.21491
Rank	1

α	0.2	0.1	0.05	0.02	0.01
Critical Value	1.3749	1.9286	2.5018	3.2892	3.9074
Reject?	No	No	No	No	No

Chi-Squared

Deg. of freedom	1
Statistic	0.12734
P-Value	0.72121
Rank	18

α	0.2	0.1	0.05	0.02	0.01
Critical Value	1.6424	2.7055	3.8415	5.4119	6.6349
Reject?	No	No	No	No	No

To build a theoretical SSD curve (*e.g.*, based on the Burr model with the highest Anderson-Darling rank for the coarse soils, control SO_4; Figure 6.2), the following equation was used that represents the cumulative distribution function for the Burr model:

Cumulative Distribution Function

$$F(x) = 1 - \left(1 + \left(\frac{x}{\beta}\right)^{\alpha}\right)^{-k}$$

Where,

F(x) = *Calculated HPP rank on the cumulative distribution function*

β = *Burr coefficient determined by EasyFit for a particular dataset (e.g., 1.0233)*

α = *Burr coefficient determined by EasyFit for a particular dataset (e.g., 3.2559)*

k = *Burr coefficient determined by EasyFit for a particular dataset (e.g., 0.436)*

x = *EC_{25} value*

SSD curves (up to seven models per SO_4 group) were compared to the original HPP plot (Figure 6.3). The final SSD curve model (per group) was selected based on the best fit as summarized in Figure 6.4, with corresponding cumulative distribution functions and calculated 25% values.

6.1.2 SO_4 SPECIES SENSITIVITY DISTRIBUTION CURVES

To understand the influence of SO_4 toxicity on the analysis, BMDS and EasyFit analyses were completed using Se control data for coarse and fine soils. Table 6.2 summarises EC_{25} values calculated using the BMDS. SO_4 toxicity was observed more frequently in coarse soils compared to fine soils and the most sensitive endpoints were earthworm juveniles in fine soils and alfalfa and red fescue in coarse soils.

Table 6.2. SO_4 Toxicological Endpoints (EC_{25}) Used for SSD Curves

Species	Biological Endpoint	Coarse Soils	Fine Soils
Alfalfa	Dry Mass	1,184	1,481
	Germination	1,225	1,481
Barley	Dry Mass	1,321	1,481
	Germination	1,321	1,481
Carrot	Dry Mass	1,251	1,563
	Germination	1,321	1,588
Cucumber	Dry Mass	1,321	1,481
	Germination	1,321	1,481
N. Wheatgrass	Dry Mass	1,312	1,481
	Germination	1,321	1,481
R. Fescue	Dry Mass	1,221	671
	Germination	1,260	1,481
Springtails	Adults	1,145	1,429
	Juveniles	1,145	1,429
Earthworms	Adults	1,145	1,429
	Juveniles	835	464

Figure 6.5 represents SSD model curves for SO_4, over HPP points, with approximated 25% threshold falling below the highest SO_4 treatment level in coarse soils, and close to the second mid treatment in fine soils, with higher degree of uncertainty for the fine soils. The SO_4 toxicity threshold in fine soils appears to differ substantially for plant and invertebrate species, where plants appear to be more tolerant up to the highest SO_4 soil concentrations.

Figure 6.3. Select Se SSD Theoretical Models over Hazen Plotting Points

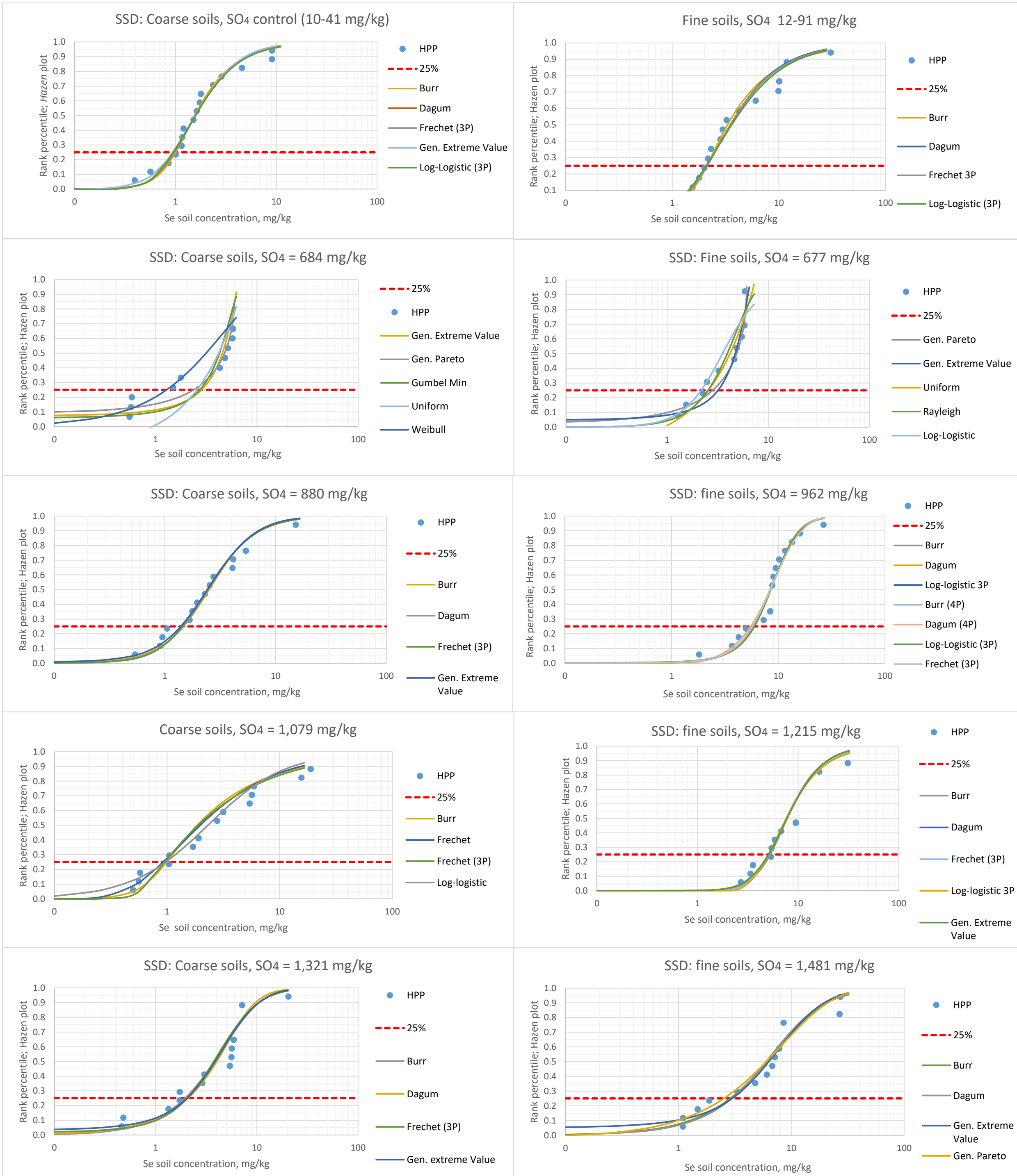


Figure 6.4. Final SSD Curve Models over Hazen Plotting Points

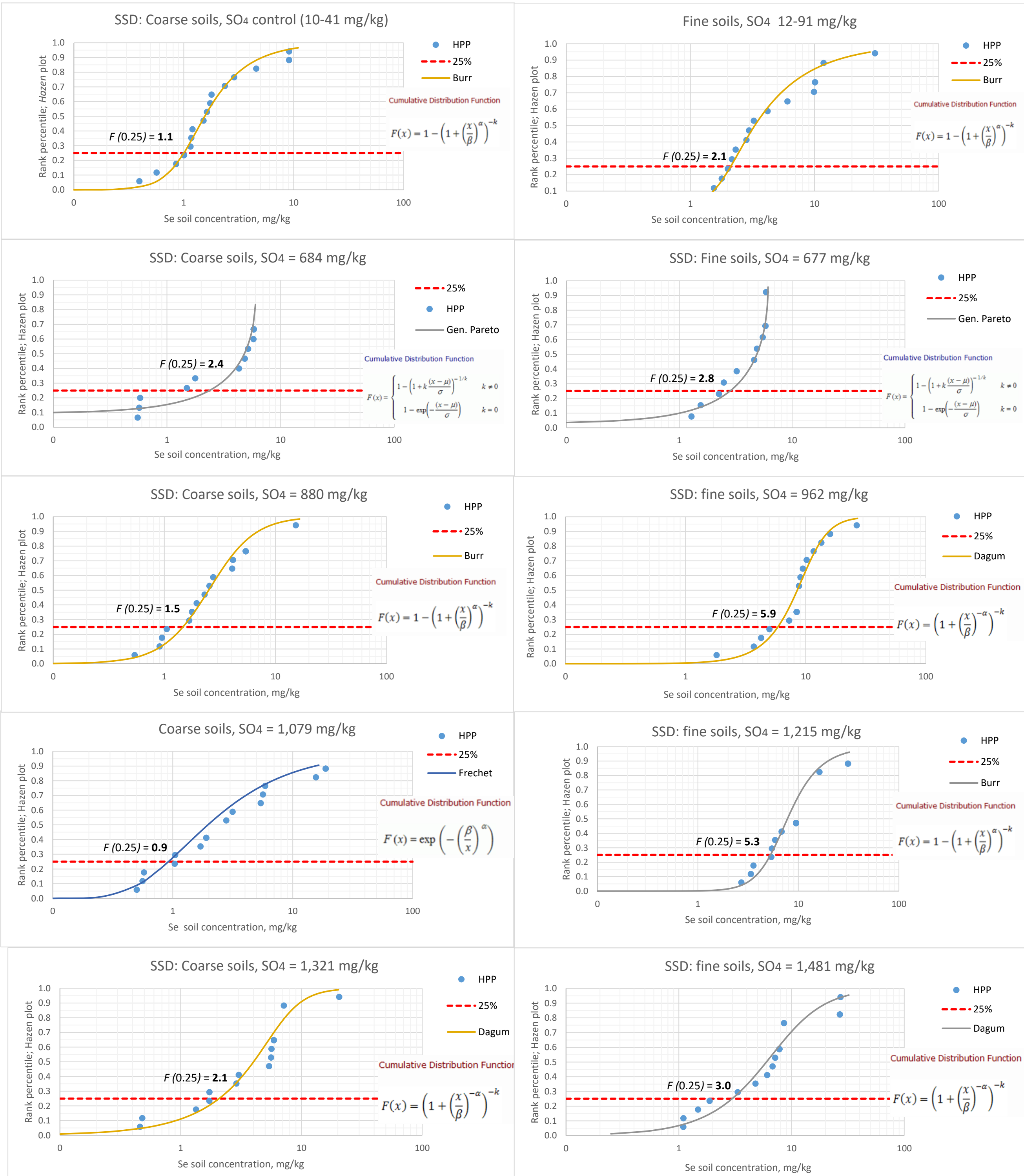
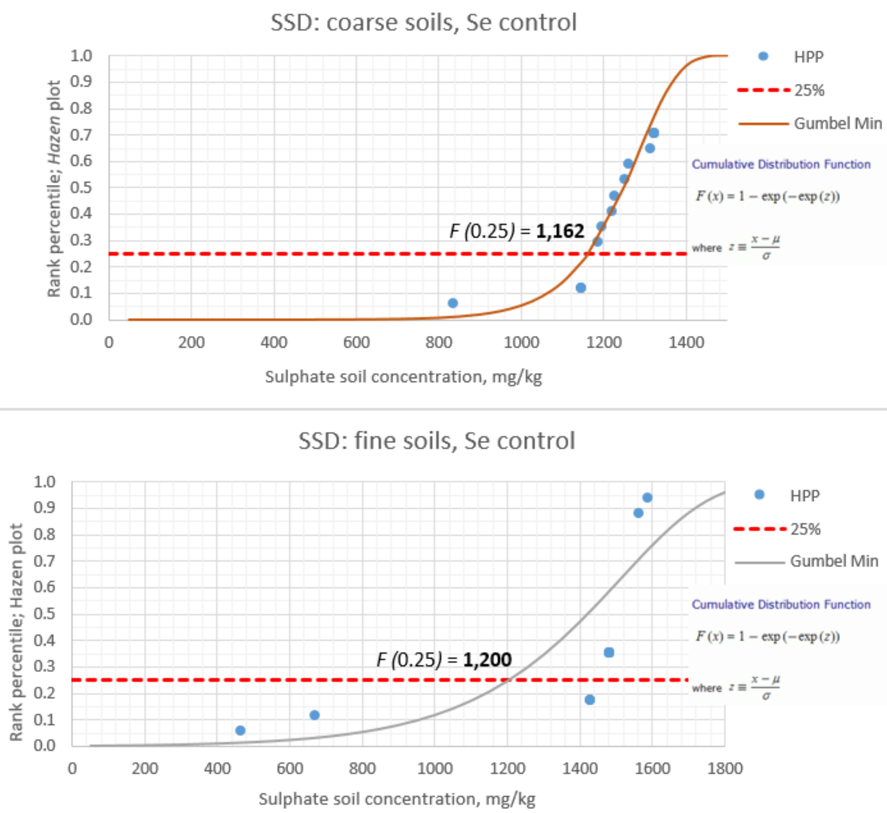


Figure 6.5. SO_4 SSD Curves (Se Control)



Data are shown for SO_4 toxicity towards plant and invertebrate species, plotted by the HPP position

6.2 POTENTIAL SRGS

Based on SSD model curves and 25% threshold calculations discussed in Section 6.1.1, and possible SO_4 toxicity limitations (Section 6.1.2), the proposed Se guidelines would be considered applicable to the 50 to 1,500 mg/kg of SO_4 soil concentration range, and values for 1,500 mg/kg SO_4 are expected to be conservative for higher sulphate concentrations outside of the study range. Table 6.3 summarizes the 25% thresholds calculated for each SO_4 group and Table 6.4 presents potential soil quality guidelines, taking into consideration restrictions imposed by SO_4 toxicity interference at the higher SO_4 concentrations.

Results for coarse textured soils suggest that under variable SO_4 concentrations, the existing Se guideline of 1 mg/kg may be appropriate and protective. However, of all the experiments conducted, those involving coarse soils were associated with the highest response variability, and lowest relative confidence, particularly with increasing SO_4 concentrations. It was postulated that this was due to difficulties in dosing plant roots growing in a readily drainable coarse soil with a water soluble substance (i.e., Se as selenate), given the requirement of regular water addition for plant growth. Results for fine textured soils suggest a more appropriate minimum baseline Se soil quality guideline of 2 mg/kg is appropriate. At higher SO_4 concentrations (i.e., > 1000 mg/kg), a guideline of 5 mg/kg would be more appropriate due to the amelioration of Se toxicity at higher

SO_4 concentrations. The analysis by nature was complex, given: Se induced toxicity at high Se concentrations combined with low SO_4 concentrations; SO_4 ameliorated Se toxicity at higher SO_4 concentrations; higher Se concentrations overcome SO_4 induced low Se dose nutritional deficiency; and, SO_4 may induce a distinct toxic effect at higher SO_4 concentrations.

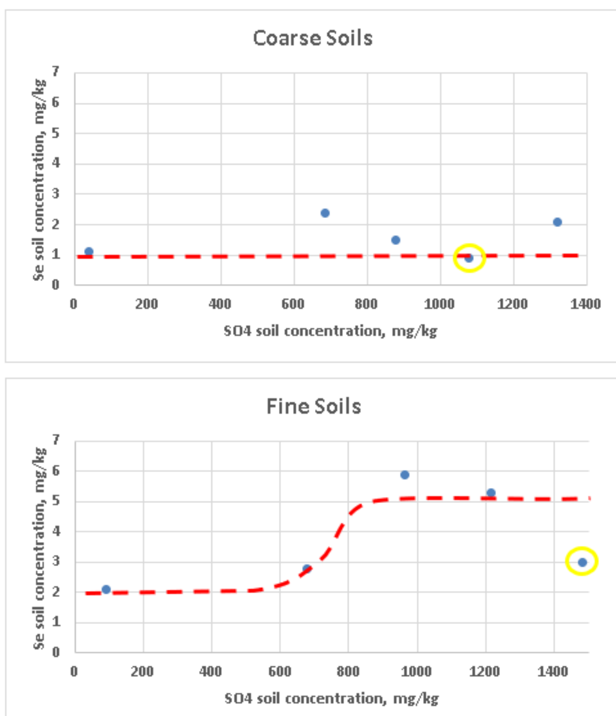
Table 6.3. Se Guidelines Ground Summary from SSD Modeling

Coarse soils					
SO_4, mg/kg	10-41	684	880	1,079	1,321
Se, mg/kg	1.1	2.4	1.5	0.9	2.1
Fine soils					
SO_4, mg/kg	12-91	677	962	1,215	1.481
Se, mg/kg	2.1	2.8	5.9	5.3	3.0

Table 6.4. Se Guidelines Proposed for Various SO_4 Concentrations

Coarse soils				
SO_4, mg/kg	50	500	1,000	1,500
Se, mg/kg	1.0	1.0	1.0	1.0
Fine soils				
SO_4, mg/kg	50	500	1,000	1,500
Se, mg/kg	2.0	3.0	5.0	5.0

Figure 6.6. Possible Se Soil Quality Guidelines as a Function of SO_4 Concentration



yellow circle indicates guideline data point that was limited by the maximum concentration of selenium used in the study – it is considered more of an artifact of dataset limitations rather than an indication that toxicity is occurring from Se that is not ameliorated by SO_4

7 DISCUSSION

7.1 GENERAL CONDITIONS

Proposed guidelines herein are based on a generally restrictive and conservative approach – the guidelines are also associated with several considerations. The most prominent considerations including Se treatment range boundaries, heterogeneity of Se mixtures within various SO_4 treatment groups, and SO_4 toxicity. The guidelines herein are not complete. Given the potential for selenium to bioconcentrate, risk to secondary receptors such as livestock and wildlife must be incorporated into the guideline derivation process, which was not part of the current scope of work.

7.2 CONSIDERATIONS

The first consideration is the range of Se dosing in the 2015 to 2017 plants experiments, which were not exceeding 5.9 mg/kg in coarse soils, and 9.5 mg/kg for fine soils. In the 2017 experiments with low SO_4 treatment groups, the highest Se concentration was equal to 5.8 mg/kg for coarse soils, which led towards using BMDS-calculated values as low as EC_{10}, when they were computable, and the assumption of a NOAEL if no computable effect was observed within the Se dose range. The approach is however conservative. This consideration had an impact on the number of functional endpoints available for SSD curve modeling. The more endpoints that are involved, the more adequately the SSD curve is built, and the better its accuracy in predicting an appropriate guideline. Using the highest Se dose by default as a NOAEL in multiple instances (due to BMDS computation failures and a conservative NOAEL being defined and used instead of an EC_{25}) resulted in several EC_{25} endpoints having the same HPP rank, resulting in one Hazen plotting position and limiting the total data pool at the higher tail of the SSD, which can have an influence on the slope of the SSD curve within the effects range.

The second consideration is due to the variability in final Se dose levels that were created in the lab by mixing SeO_4 with soil for the different SO_4 treatment groups (lab results shown in Table 4.2, Section 4.3.1). For example, for fine soils, the maximum Se concentration was equal to 9.5 mg/kg in the second mid SO_4 group (1,215 mg/kg), whereas in the high SO_4 group (1,481 mg/kg) it was equal to 7.8 mg/kg (Table 4.2; Section 4.3.1). Such difference may give an implication that the strength of the SO_4 amelioration of Se toxicity may be decreasing at higher Se doses, when the opposite may be true and the strength of SO_4 amelioration may be maintained at higher Se concentrations.

Toxicity due to increasing SO_4 concentrations was observed. In some respects, this precludes the study of ameliorating effects at SO4 levels higher than those studied herein, as the influence of this factor on the results would be more difficult to separate and control. SO_4 toxicity was observed for three SSD points, so the net total influence on the study results is considered relatively minor.

One final consideration is that a conservative approach was taken for BMDS output results acceptance. By default, any computable values equal or less than EC_{25} and equal or greater than EC_{10} were accepted and incorporated into the final statistical pool, regardless the steepness of BMDS curve shape and the standard deviation range. This approach was chosen to avoid any bias towards higher SO_4 concentration group and to avoid subjective interpretations. In addition, it helped utililize all available toxicity data, expect those failed the quality assurance test (Section 5.1.5). The net effect was a more conservative guideline – this approach was considered appropriate given the existing data pool.

7.3 BIOCONCENTRATION FACTORS

In coarse soils, BCF for Se ranged from 8 to 71 in SO_4-treated group. No SO_4 control samples were available for analysis due to insufficient yield. The greatest plant tissue Se BCF values ranged from 192 to 348 in barley and cucumber shoots (dry mass, fine soils; Table 5.8, Section 5.2) associated with the lowest SO_4 control groups. Increasing SO_4 co-exposure doses reduced the corresponding maximum BCF values to 2.9 – 50. This supports the hypothesis that observed Se toxicity was related to plant tissue concentration, and furthermore that increasing SO_4 concentrations ameliorate Se toxicity via competitive uptake leading to reduced plant tissue concentrations. This observation is consistent with the results from Mikkelsen *et al*. (1988), where Se accumulation by alfalfa was reduced by 158-fold in the presence of SO_4.

The highest BCF for sulphate (as S) was observed within SO_4 control group in fine soils (225 – 1,424), gradually lowering with increased SO_4 concentration (21 – 82). The same pattern, decreasing SO_4 BCF with increasing SO_4 concentration, was observed within coarse soils (14 – 88). This is a common observation for various ions and metals where greater relative uptake on a tissue weight basis is associated with younger, compared to more mature, plants. For comparison, literature derived BCF values provided by US EPA (1996) for mature grains, fruit and vegetables are provided in Table 7.1.

Table 7.1. Literature Bioconcentration Factors for Se

Selenium	Study		Bioconcentration factors (Br)		Geometric
grains and cereals	4	5.5 - 7.0	0.002	0.11	0.002
potatoes	2	5.5 - 6.8	0.018	0.096	0.042
leafy vegetables	7	5.5 - 7.8	0.002	0.076	0.016
legumes	4	5.5 - 6.8	0.024	0.11	0.024
root vegetables	8	5.5 - 7.6	0.004	0.096	0.022
garden fruits	8	5.5 - 6.8	0.008	0.078	0.02
sweet corn	default	ND	0.002	0.002	0.002

Table adapted from US EPA, 1996
Min and max values are given in mg Se /kg plant dry weight/ mg Se/kg soil

8 CLOSURE

Equilibrium Environmental Inc. has prepared this document for the exclusive use of Petroleum Technology Alliance Canada solely for the purpose of assisting in the decision-making process for development and/or revision of ecological contact soil selenium guidelines. Any uses which a third party makes of this document, or any reliance on decisions made based on it, are the responsibility of such third parties. Equilibrium Environmental Inc. accepts no duty or care to any other person or any liability or responsibility whatsoever, for any losses, expenses, damages, fines, penalties, or other harm that may be suffered or incurred by any other person as a result of the use of, reliance on, any decision made, or any action taken based on this document. Nothing in this document is intended to constitute or provide a legal opinion.

The data review, analysis and recommendations were limited to the experimental data that has been collected to date, and the accuracy of laboratory work and chemistry data by other parties cannot be verified and is not implied. Equilibrium Environmental Inc. believes information presented in this report is accurate but cannot guarantee or warrant its accuracy. If the study conditions or applicable standards change, or if any additional information becomes available at a future time, modifications to the findings, conclusions, and recommendations in this document may be necessary. Any questions regarding this document should be directed to Anthony Knafla at (403) 286-7706.

Sincerely,

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