

Update on Boron Toxicity Testing and Tier 1 Guideline Development



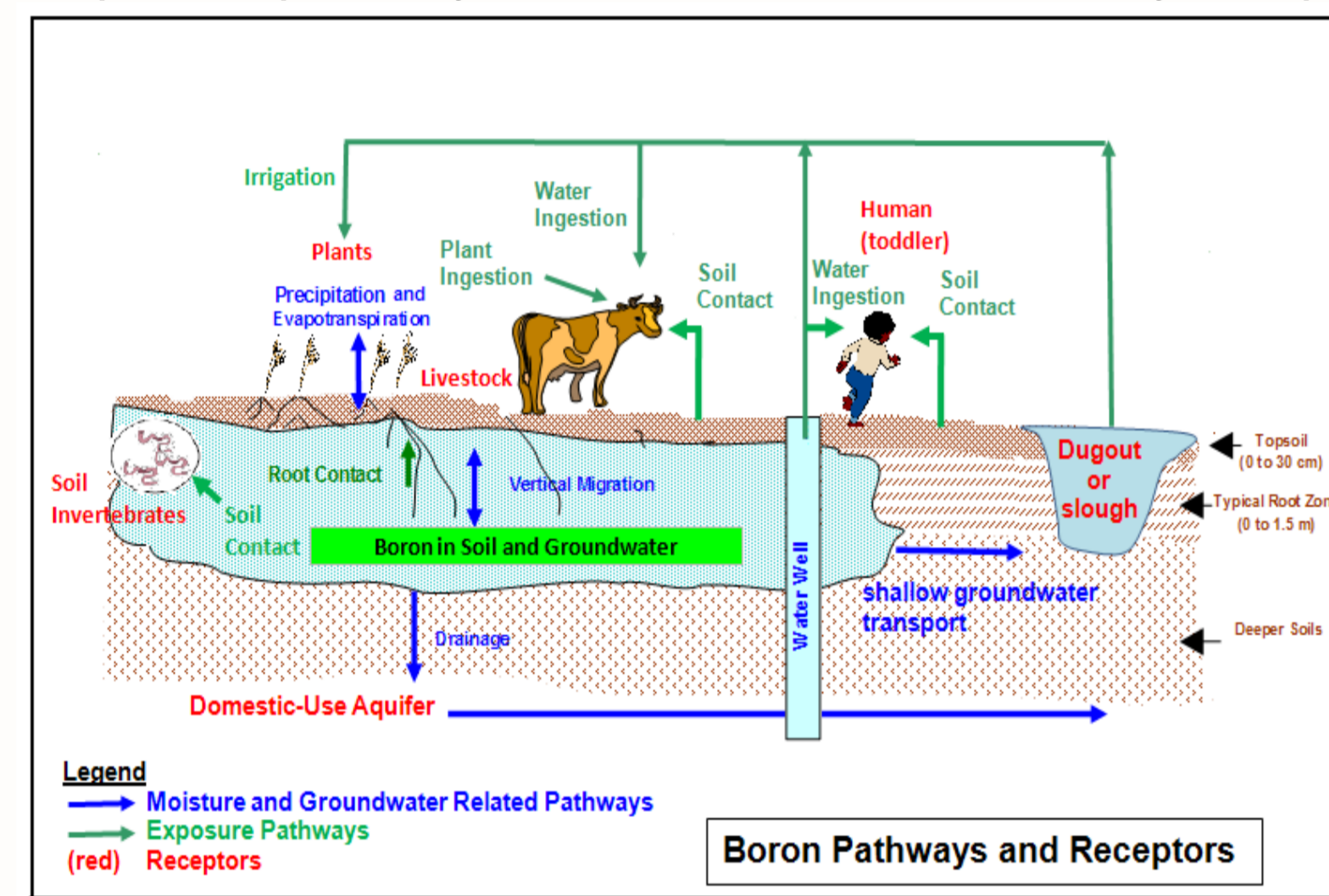
Equilibrium Environmental Inc.

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1. Introduction

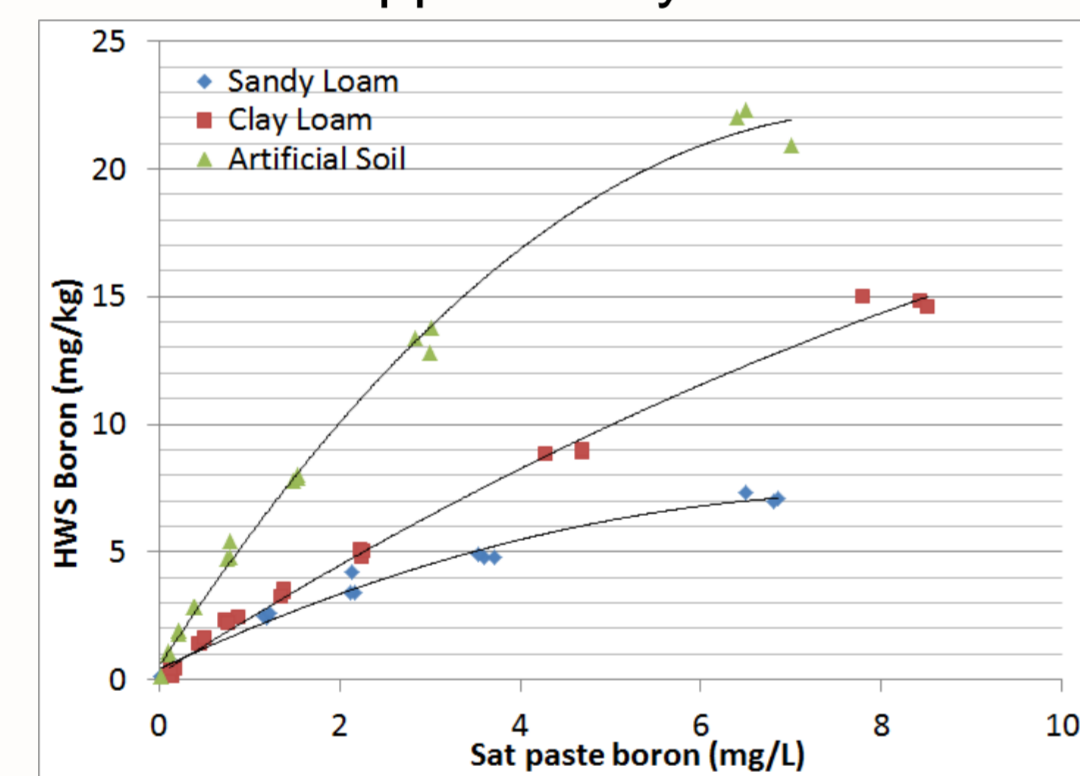
Boron is a non-metallic element that occurs naturally in soil and groundwater systems, sourced from the weathering of boron-containing minerals (Butterwick et al., 1989). It is an essential micronutrient, and plants accumulate boron from the soil to perform a variety of cellular functions. It is known that different plant species have different boron requirements and tolerances, and variations can be quite significant even between different varieties of the same species (Nable, 1997). Boron also undergoes significant binding to the soil matrix, and is highly dependent on specific soil conditions such as texture, clay content, and organic matter (Gupta, 1985). Increased boron uptake beyond a certain threshold can also lead to symptoms of toxicity in plants, as well as in other receptors such as soil invertebrates, humans, livestock/wildlife, and aquatic species. The figure below shows a conceptual diagram of boron exposure pathways in the environment with key receptors noted.



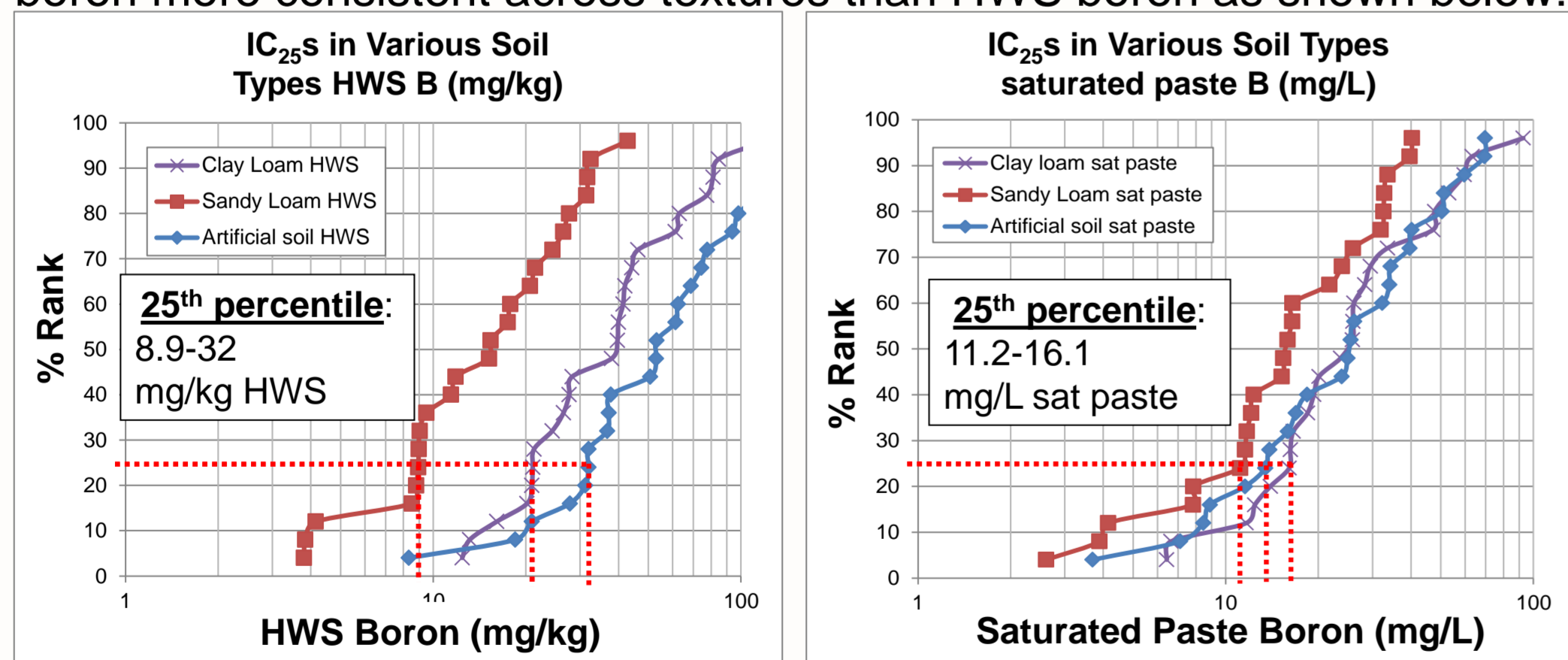
Saturated paste B (mg/L) is more closely related to toxicity and less influenced by soil texture, thus a more practical choice to develop guidelines than the traditional HWS boron test (EEI, 2013). Updated results from boron toxicity tests performed by Exova (supported by PTAC) are described here, primarily for agricultural plants, boreal plants, and soil invertebrates. These results can be combined with literature to generate preliminary species sensitivity distribution (SSD) curves based on IC₂₅ values.

2. Agricultural Plants

- Exova performed agricultural plant toxicity testing in 2011-2013 supported by PTAC for six agricultural plant species and three soil types
- Species: cucumber, carrot, durum wheat, Northern wheat-grass, alfalfa, and barley
- Soil types: clay loam, sandy loam, artificial soil
- sorption varies across the agricultural soil types, the least occurs in sandy loam and the most in artificial soil
- Benchmark Dose Software (BMDS) used to generate IC₂₅'s for four endpoints: (shoot length, shoot biomass, root length, root biomass)



- Sat paste boron more consistent across textures than HWS boron as shown below:



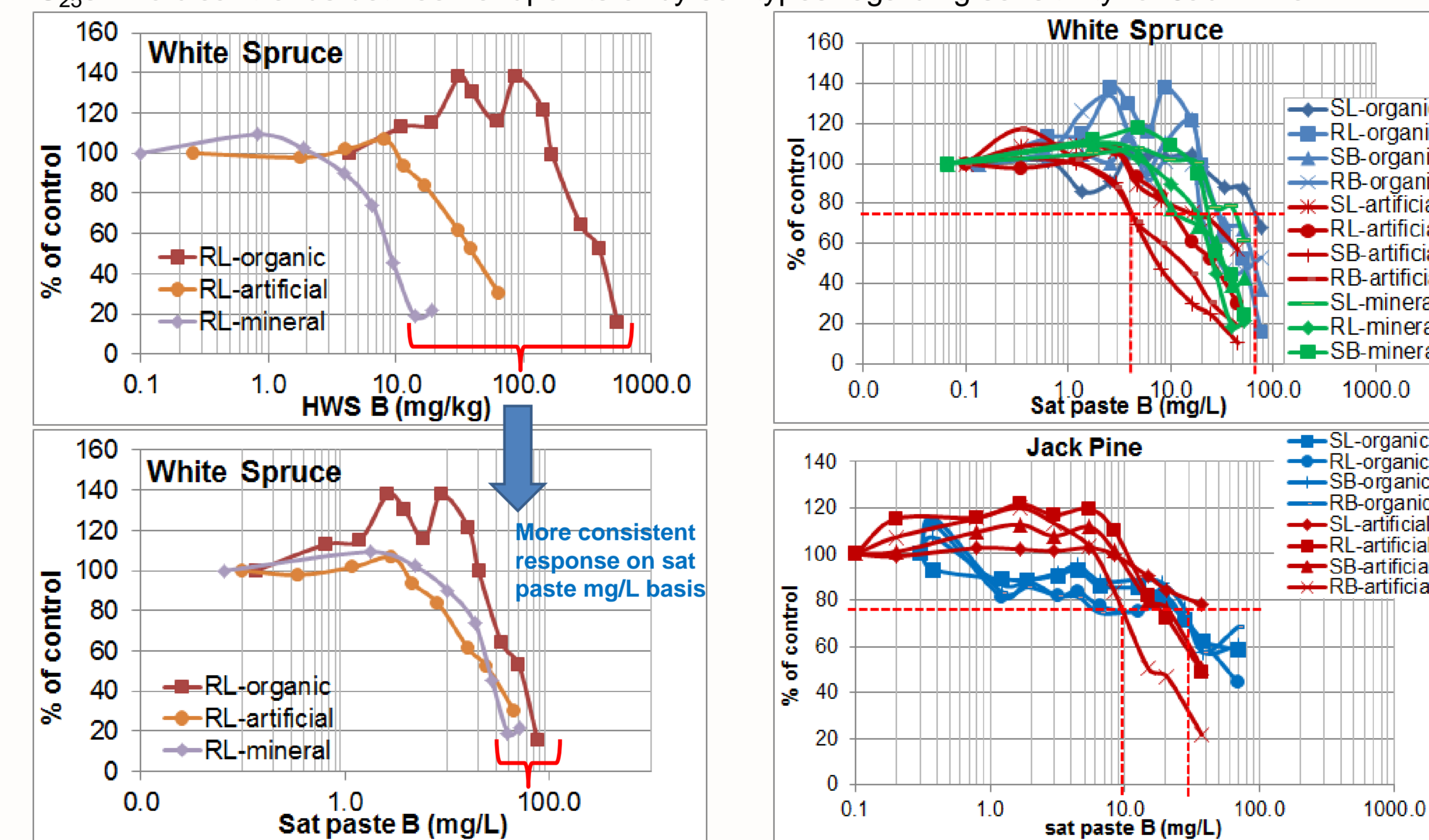
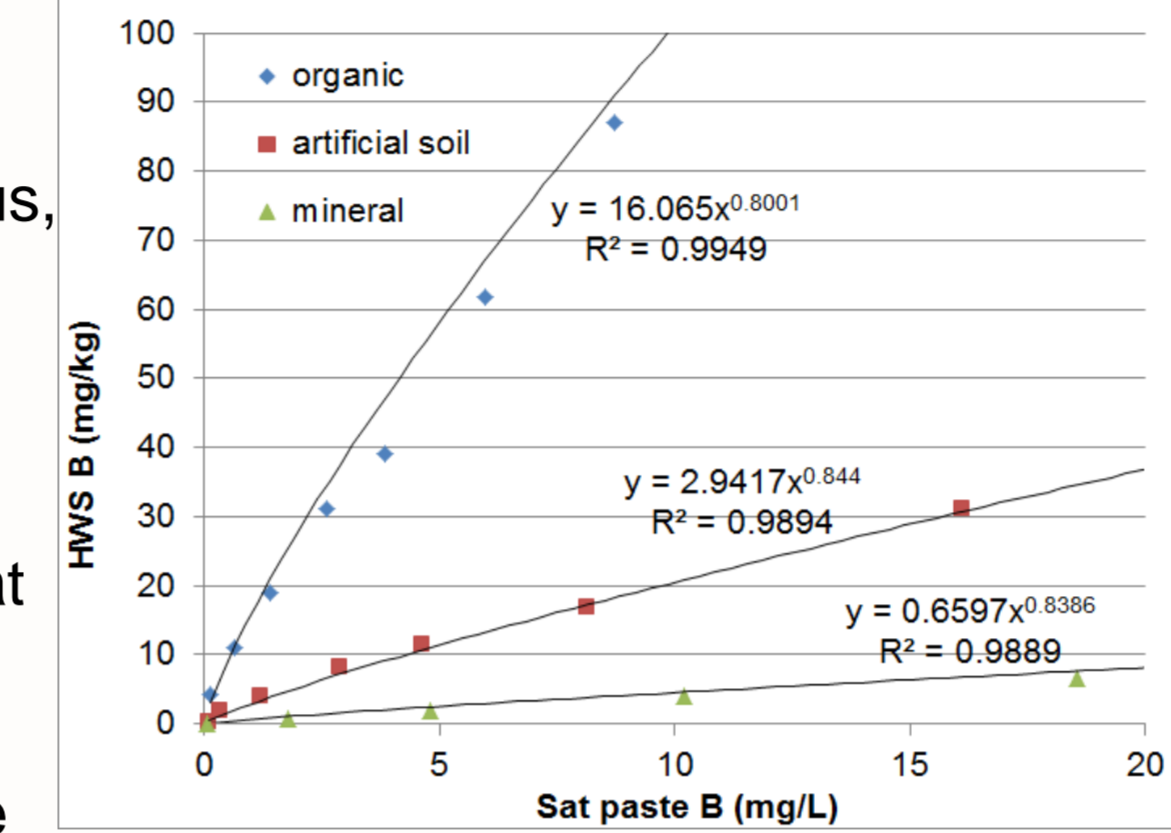
- The newer toxicity data can be supplemented with literature data for other relevant plant species, much of which comes from older sand culture experiments
- Seven of the most sensitive species from literature are shown in the table below and consists of fruit and vegetables. Blackberry is the most sensitive species with an estimated IC₂₅ of ~1.9 mg/L soil solution boron (interpolated from Eaton 1944 sand culture experiments)

Common Name	Botanical Name	Tolerance Based on	Calculated IC ₂₅ (mg/L)	Test Duration
Blackberry ¹	<i>Rubus Mammoth Thornless</i>	total dry weight	1.9	8 months
Strawberry ¹	<i>Fragaria Klondike</i>	total dry weight	2.9	5 months
Cherry ¹	<i>Prunus avium (L.) Mazzard</i>	total dry weight	2.5	8 months
Snap bean ²	<i>Phaseolus vulgaris</i>	Pod yield	3.1	2.5 months
Peach ¹	<i>Prunus persica (L.) Batsch</i>	total dry weight	3.2	8 months
Grape ¹	<i>Vitis vinifera L. (geomean of Malaga and Sultanina)</i>	total dry weight	4.2	5-8 months
Cowpea ²	<i>Vigna unguiculata Walp.</i>	Seed yield	4.6	2-3 months

1: analysis performed on raw data from Eaton 1944
2: Data sourced from Francois (1989), obtained from Maas and Grattan (1999)

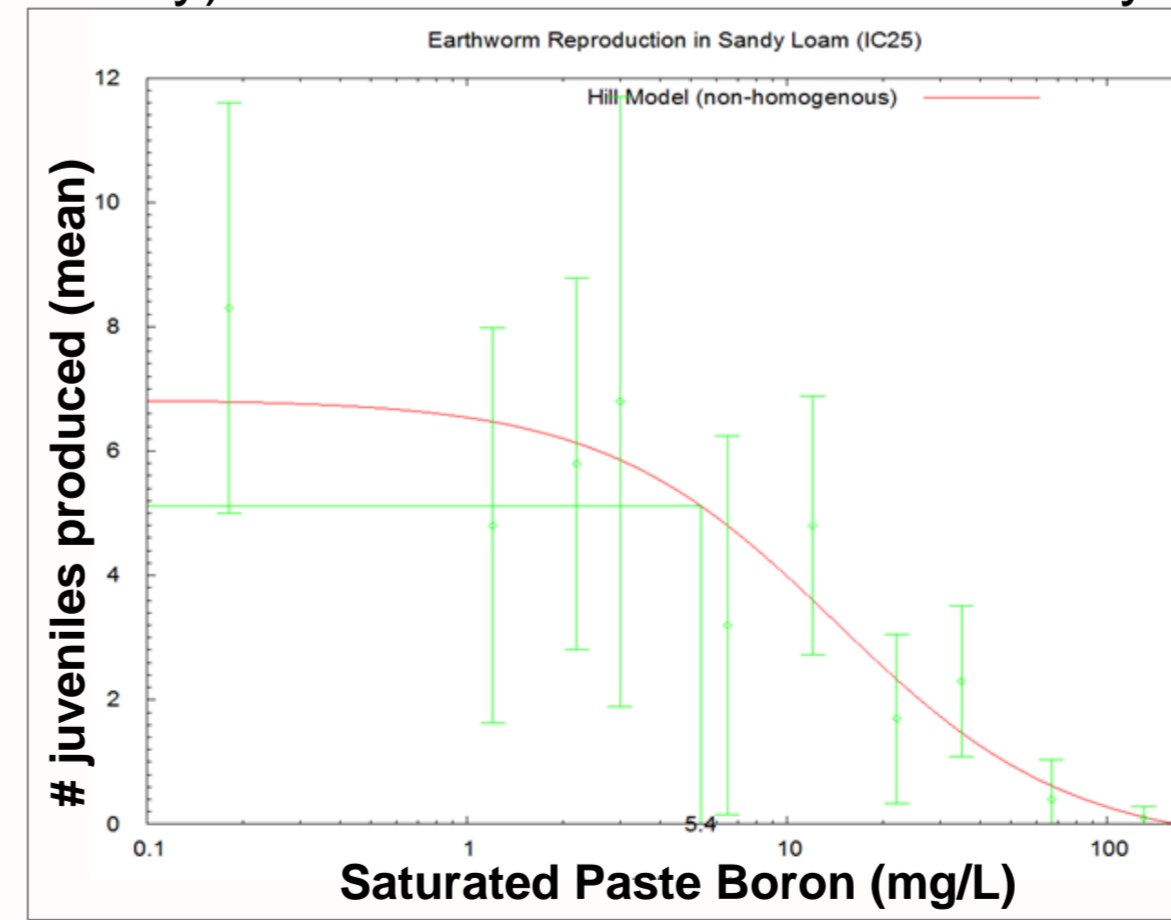
3. Boreal Plants

- Method development boric acid toxicity tests previously performed by Environment Canada and Saskatchewan Research Council for a range of seven boreal plant species (mainly in artificial soil)
- Updated Exova/PTAC toxicity tests ongoing for various boreal species (eg, Jack Pine, White Spruce)
- Three soil types tested: peat, sandy mineral soil (~sand), and slightly acidic artificial soil
- Four endpoints measured: shoot length (SL), shoot biomass (SB), root length (RL) and root biomass (RB)
- HWS vs sat paste relationships differ by soil type; slope (related to K_d) ranges from 0.66 in sand to 16.1 in peat (thus, substantially higher sorption in organic peat)
- Saturated paste B toxicity response is more consistent across soil types than HWS B, as shown for White Spruce in the figures (left side) below
- In White Spruce, artificial soils yield lower IC₂₅'s than peat (mineral soil is intermediate for White Spruce)
- In Jack Pine IC₂₅'s have a lower range than White Spruce IC₂₅'s. No clear trends between endpoints or by soil types regarding sensitivity for Jack Pine



4. Soil Invertebrates

- Method development boric acid toxicity tests previously performed by Environment Canada for range of soil invertebrates (earthworms, springtails, mites) in artificial and field soils
- Updated toxicity tests carried out by Exova (supported by Husky) in 2013 on earthworms in sandy loam (allowed measurement of sat paste B rather than regression estimates from spike levels for previous tests)
- Four endpoints measured: adult survival, adult dry weight, juvenile survival and juvenile dry weight. Focus on juvenile (reproductive) endpoints since more sensitive
- The IC₂₅ for juvenile earthworm survival in sandy loam is 5.39 mg/L sat paste B, and for juvenile biomass is 26.38 mg/L (indicates variability between endpoints)
- This new toxicity data compiled with older data below:

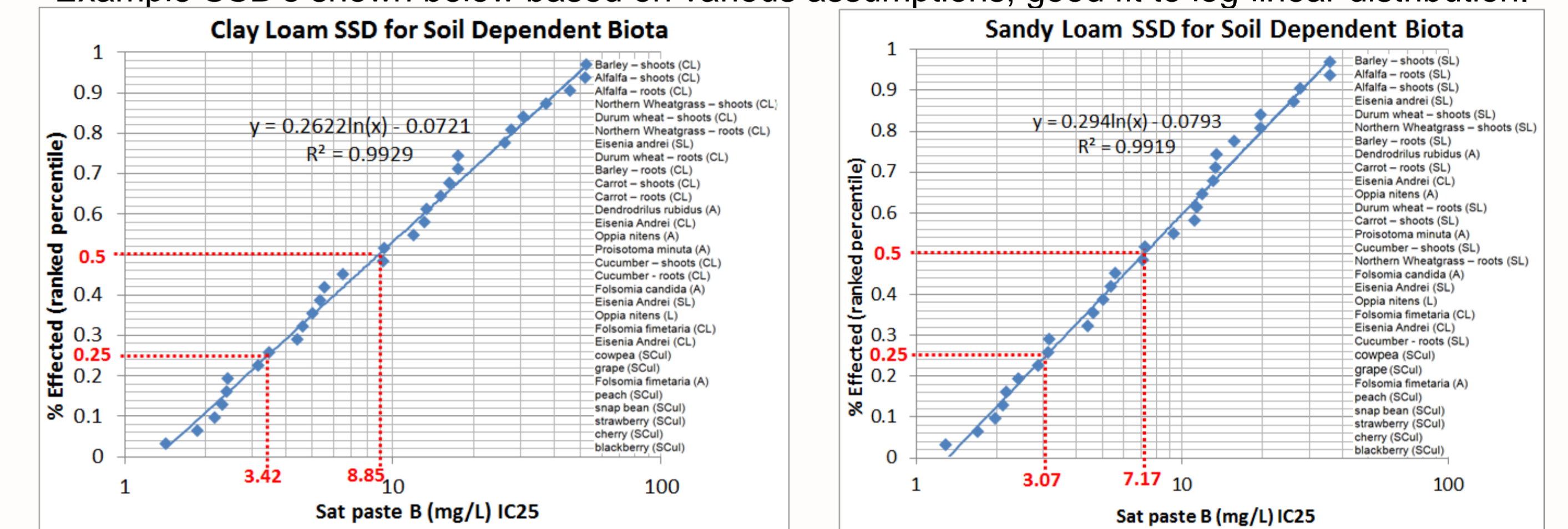


Species	Duration	Soil	Measure	sat paste Boron (mg/L)	Reference	Notes	
Earthworm	Eisenia andrei	63 days	sandy loam	IC25	26.4	* Exova, 2013	mass of juveniles
Earthworm	Dendrodrilus rubidus	56 days	artificial	IC25	13.5	Environment Canada, 2008-2010	geomean of four separate 56 day studies in 2008-10
Earthworm	Eisenia andrei	84 days	clay loam	IC25	13.1	Ingraldi, 2004	number of juveniles
Oribatid mite	Oppia nitens	28 days	artificial	IC25	12.0	Environment Canada, 2008-2010	geomean of all four trials (2008-2007, 2010)
Springtail	Protosoma minuta	21 days	artificial	EC25	9.4	Environment Canada, 2010-2012	geomean of 3 separate studies (2010-2012)
Earthworm	Eisenia andrei	63 days	sandy loam	IC25	5.4	* Exova, 2013	number of juveniles
Springtail	Folsomia candida	28 days	artificial	IC25	5.6	Environment Canada, 2007c	geomean of 2 extraction methods
Oribatid mite	Oppia nitens	28 days	loam with 2-7% OM	IC25	5.1	Princz, 2010	geomean of amended and unamended soils
Springtail	Folsomia fimetaria	21 days	clay loam	IC25	4.7	CECOTOX, 2005	number of juveniles (geomean of two 21 day trials)
Earthworm	Eisenia andrei	84 days	clay loam	IC25	4.4	Ingraldi, 2004	mass of juveniles
Springtail	Folsomia fimetaria	21 days	artificial	IC25	2.4	CECOTOX, 2005	number of juveniles (geomean of two 21 day trials)

* Note: 2013 Exova study directly measured saturated paste B, thus estimations from spiked boron levels are not required. All other studies had saturated paste boron estimated from spiked boron levels using soil regressions

5. Combined Soil-Dependent Biota SSD's

- IC₂₅'s of the agricultural plant toxicity data (Exova and literature) and soil invertebrate toxicity data (Exova and literature) were combined into one overall soil-dependent biota SSD (one for fine soils, and one for coarse soils)
- Toxicity data which are available for solely one soil texture were included in both SSD's (reasonable since sat paste B), while those with data for both separated into fine and coarse
- Example SSD's shown below based on various assumptions, good fit to log-linear distribution.



- The fruit species from sand culture experiments are the most sensitive in both soil textures, followed by soil invertebrate species, then cucumbers. Barley and alfalfa are generally the least sensitive soil dependent biota across both soil types
- Example 25th percentile for fine soils is ~3.4 mg/L sat paste B, and for coarse soils is ~3.0 mg/L sat paste B. The overall average is ~3.2 mg/L based on this set of assumptions

6. Livestock, Wildlife, Irrigation, DUA, FAL

- Other receptors also considered include livestock/wildlife food and water ingestion, irrigation from groundwater, human water consumption from a DUA, and freshwater aquatic life
- Risk to livestock and wildlife from boron in food is greater than from soil or water ingestion. Boron in food can be estimated from vegetation boron data (such as from Exova tests).
- Risk to aquatic life (FAL) and other groundwater pathways such as irrigation and domestic use aquifer (DUA) can be estimated using various Tier 1 equations and saturated paste B in mg/L.

7. Conclusions and Next Steps

- Sensitive fruit and vegetable species from literature have been added to the agricultural plant dataset for additional protection and regional relevance
- Boreal toxicity testing and guideline development is underway, confirming that saturated paste boron is more suitable than HWS boron for developing guidelines applicable to a range of soil types (from sandy to organic) with less variability in toxic response based on texture
- New earthworm toxicity data falls within the range of invertebrate IC₂₅'s from the literature and provides a strong overall soil invertebrate dataset to be combined with plants
- Combining updated Exova plant and invertebrate toxicity data with literature plant and invertebrate toxicity data allows generating overall SSD's for soil dependent biota, a key step in overall guideline development

8. References

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9. Acknowledgements

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