

# **Development and Standardization of Soil Test Methods for Assessing Toxicity in Contaminated Soils**

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**ESG International**

# Project Objective

- To develop and standardize three soil toxicity tests using terrestrial plants, earthworms and springtail species relevant to Canadian soils
- To valid the toxicity methods using various substances spiked into reference soils and contaminated soils
- To publish methods as part of Environment Canada's biological test method series

# Project History

- **Four project phases:**
  - **1994** - **assessment of possible method options**  
(Bonnell Environmental report)
  - **1995 to 1998** - **method development research**  
(Aquaterra Environmental reports)
  - **1999 to 2002** - **method validation using different soil types and contaminants**  
(ESG International reports)
  - **2000 to 2004** - **inter-lab. validation and national method preparation, peer review, & publication**

# Project Supporters

- **Direct Funding**

- ✓ Program of Energy Research and Development (PERD)
- ✓ Canadian Association of Petroleum Producers (CAPP)
- ✓ Environment Canada

- **In-Kind Support**

- ✓ University of Guelph
- ✓ ESG International
- ✓ Canadian Network of Toxicology Centres (CNTC)
- ✓ Environment Canada and private sector laboratories

# Test Methods

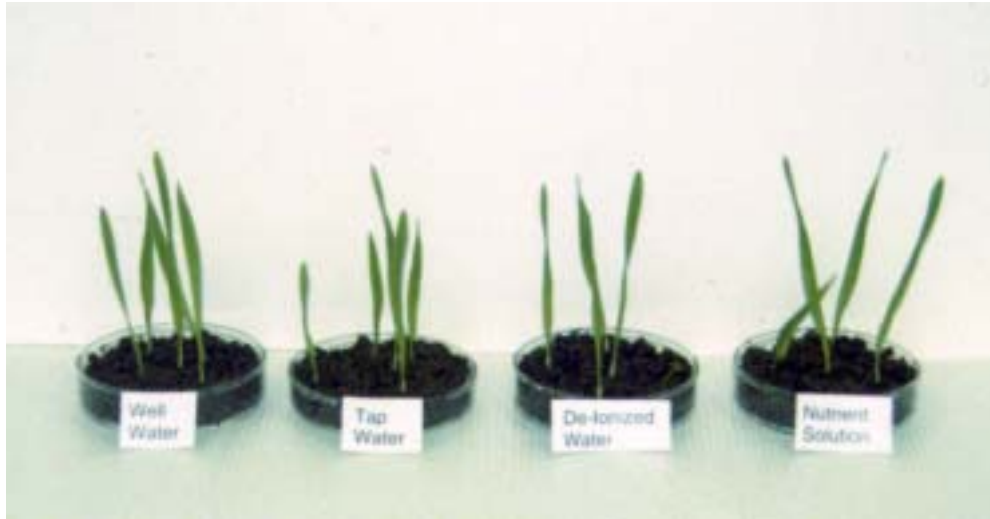
- Method for testing early seedling growth and plant vigor using terrestrial plants (6 species: northern wheatgrass, alfalfa, barley, corn, cucumber or radish)
- Method for testing survival, avoidance and reproduction using earthworms (3 species: *Eisenia andrei*, *Eisenia fetida* or *Lumbricus terrestris*)
- Method for testing survival and reproduction using springtails (2 species: *Onychiurus folsomi* or *Folsomia candida*)

# Terrestrial Plant Method

- Test duration: 14 days - barley, corn, cucumber & radish  
21 days - alfalfa & northern wheatgrass
- Test endpoints: shoot/root length & shoot/root dry mass  
(wet mass optional; observation of % emergence for test validity)
- Statistical endpoints: for multi-concentration tests, a regression design will be used to calculate an IC50 point estimate
- Reference toxicant: boric acid

# Plant Method

**Testing unit for preliminary range-finding**



**Testing unit for definitive plant test**



# Toxicity of F3 Fraction to Barley



**14-d Shoot Length  
IC50 = 29.7 mg/g**



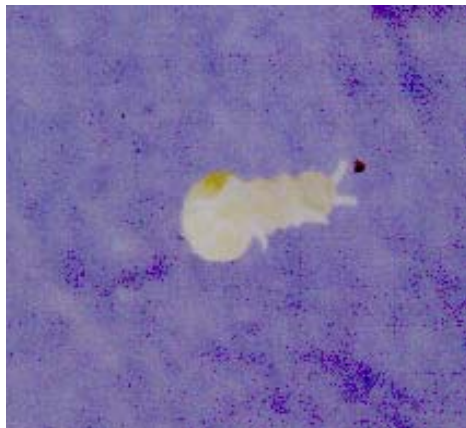
# Earthworm Methods

- Test durations: 14 day adult survival  
72 hr adult avoidance behaviour  
56 day reproduction & growth (including 28 day survival)
- Test endpoints: survival (LC50) at 14 or 28 days;  
avoidance (EC50); no. of juveniles produced (IC50); mean dry weight of surviving juveniles (IC50)
- Reference toxicant: potassium chloride - 7 day-LC50  
(boric acid testing planned as possible 2<sup>nd</sup> reference toxicant)



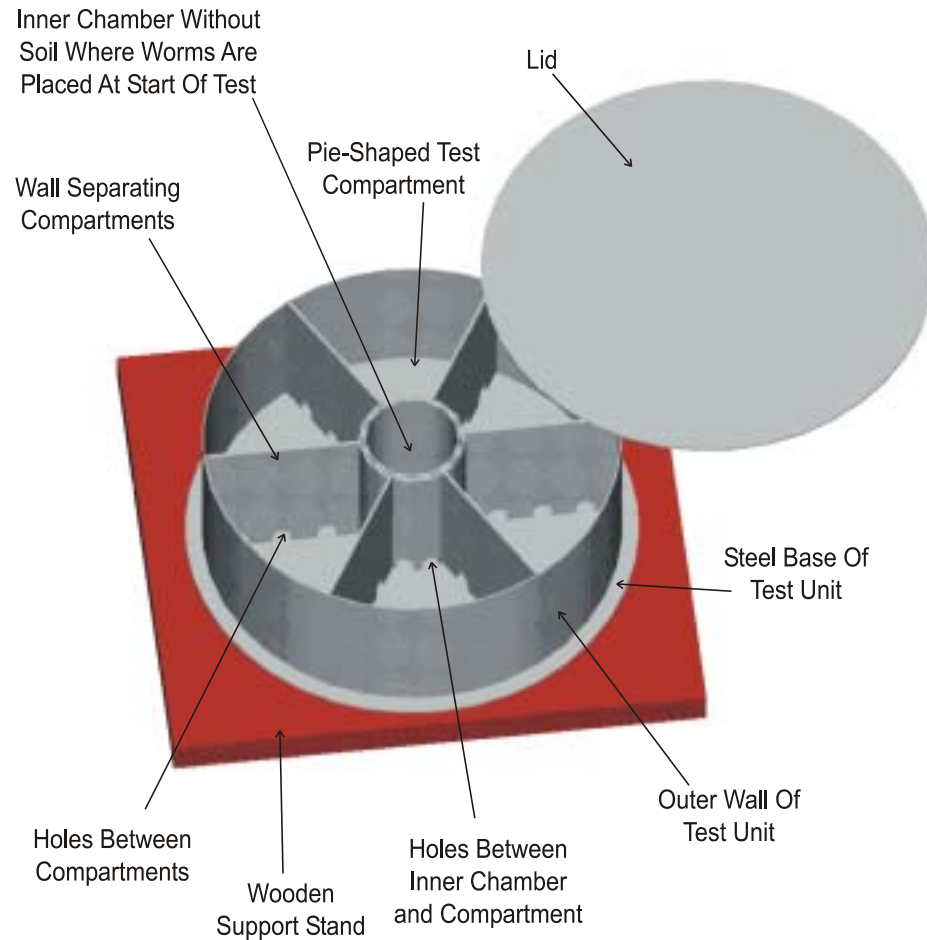
**Earthworm species**

*Eisenia andrei* and  
*Lumbricus terrestris*



**Collembola species**

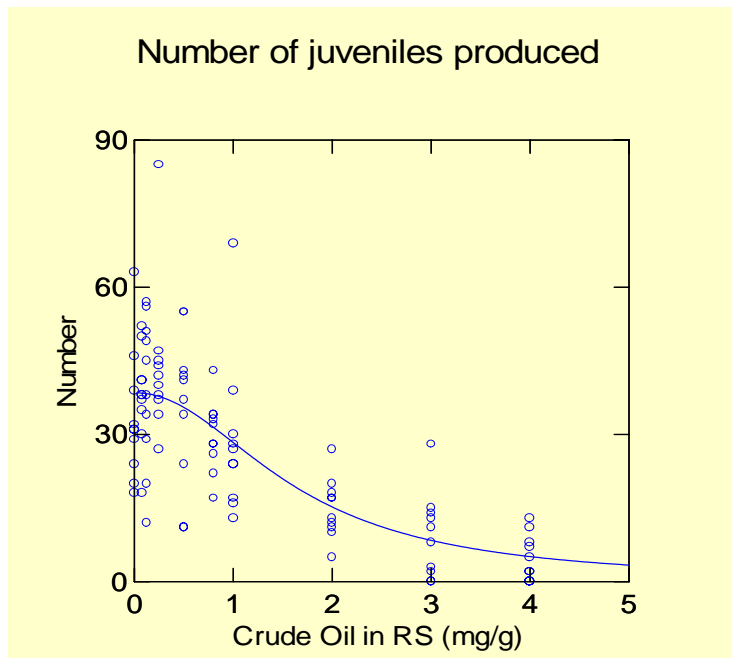
*Onychiurus folsomi*



## Test unit for earthworm avoidance behaviour

# Crude Oil Toxicity to *Eisenia andrei*

**14-d adult survival LC50 = 4.0 mg/g**



**56-d IC50 (no. of juveniles)  
= 1.6 mg/g**

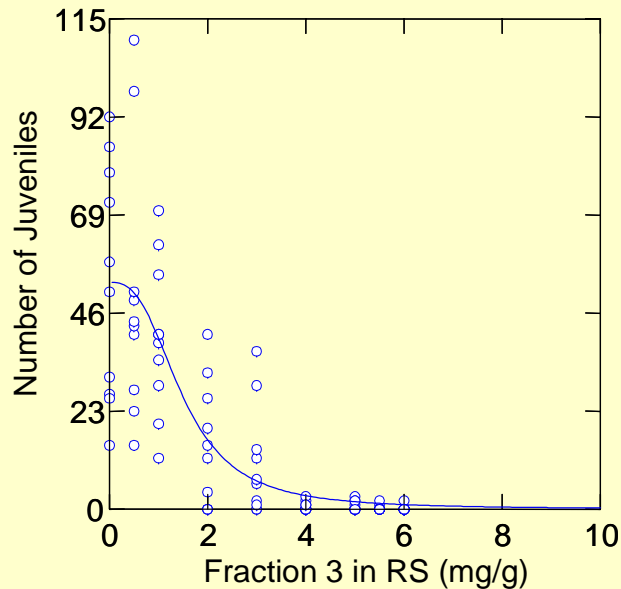
**Avoidance response threshold  
for adults between 1 and 2 mg/g**

# Springtail Methods

- Test duration: 28 days - *Folsomia candida*  
35 days – *Onychiurus folsomi*
- Test endpoints: adult survival (LC50); adult fecundity (EC50); no.of juveniles produced (IC50)
- Reference Toxicant: testing still underway

# Toxicity of F3 Fraction to *Onychiurus folsomi*

Number of Juvenile *O. folsomi* Produced



**35-d Adult LC50 = 4.3 mg/g**

**35-d IC50 (no. of juveniles) = 1.5 mg/g**

# **Standardization Process for Environment Canada Test Methods**

**Preparation of first draft method document**



**Critical review of 1<sup>st</sup> draft by Scientific Advisory Committee**



**Preparation of external review draft method document**



**International peer review of proposed standard method**

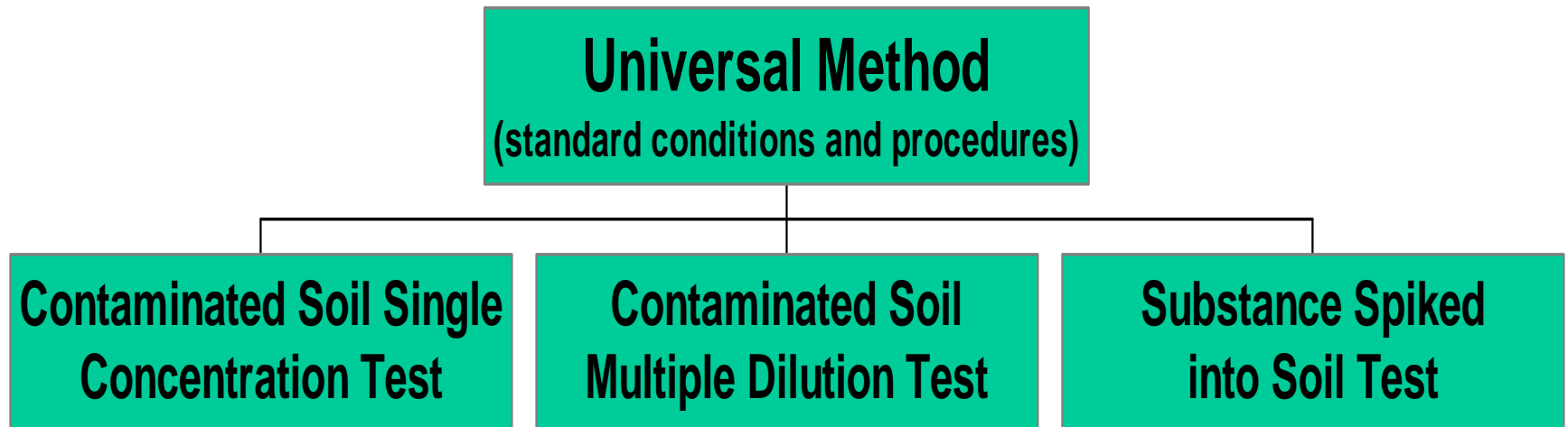


**Inter-laboratory validation of final draft test method**



**Finalization of method and formal publication as an  
Environment Canada test method document**

# Different Applications of Standardized EC Soil Toxicity Methods





# Method Validation Efforts

- **Test Substances and Contaminated Soils**
  - hydrocarbon fractions, gasoline, copper, fungicide, insecticide
  - amine waste in soil, condensate waste in soil, metals in soil originating from smelter air emissions
- **Reference Soils**
  - clay loam, sandy loam, silt loam & boreal forest soils
  - OECD artificial soil

# Status of Standard Methods

- **Earthworm method:** Expert Advisory Committee comment received; revised method to be sent for international peer review shortly
- **Terrestrial Plant method:** second draft in preparation and will be sent to Expert Advisory Committee shortly
- **Springtail method:** additional method development testing with both species underway; formal test method writing delayed to Spring 2002
- **Inter-laboratory validation:** testing rounds planned for the fall 2002 and winter 2003