



**Current Environment Canada Soil Methods  
and Priorities for Future Research and  
Standardization of Biological Test Methods  
for Assessing Contaminants in Soil**

# Soil Method Development History

- **1994** - identification of possible soil toxicity method options
- **1995 to 1998** - method development research
- **1998 to 2004** - method validation using different soil types and contaminants and inter-laboratory testing studies
- **2000 to 2004** - standard method preparation, peer review and publication

# Environment Canada Soil Toxicity Test Methods

- Method for measuring survival, avoidance, growth and reproduction of earthworms exposed to contaminants in soil (3 test species options)
- Method for measuring emergence and growth of terrestrial plants exposed to contaminants in soil (12 test species options)
- Method for measuring survival and reproduction of springtails exposed to contaminants in soil (2 test species options)

# 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 for international peer review



Inter-laboratory validation of final draft test method



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

# Earthworm Avoidance Behaviour Test



10 adult  
earthworms



- 48 or 72 hours
  - *E. andrei*
  - *E. fetida*
  - *L. terrestris*
- 3 replicates
- Percent avoidance and EC50 or EC20



# Earthworm Reproduction Test



2 adult  
earthworms /  
test vessel



56 to 63 d reproduction



28 d acute lethality



No. of live adults



No. of juveniles  
and weight



No. of cocoons  
(optional)

# Plant Tests



Seed Sorting and Selection

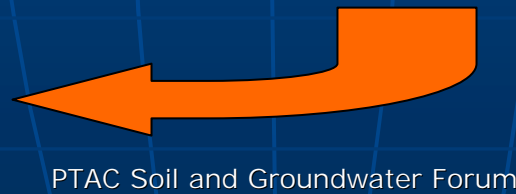
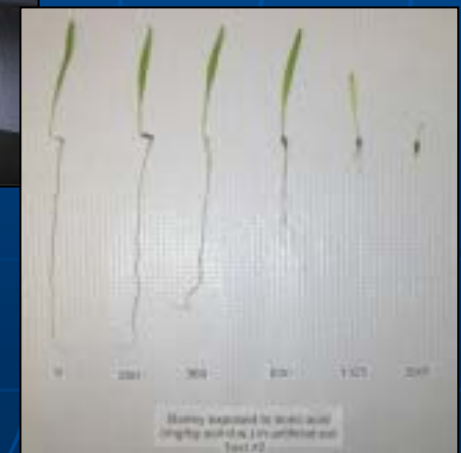
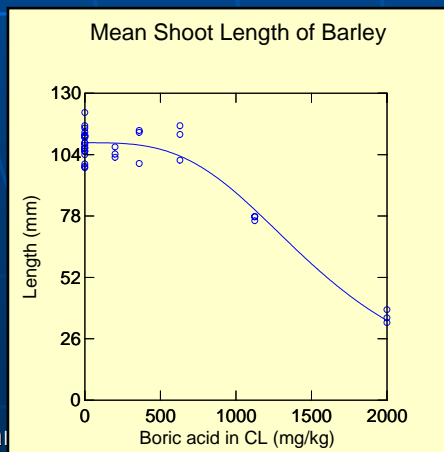


Seed Planting



Growth and Processing

## Statistical Analysis



Mal

PTAC Soil and Groundwater Forum

# Plant Species Options for Testing

| Species             | Variety                     |
|---------------------|-----------------------------|
| alfalfa             | common                      |
| barley              | Chapais                     |
| durum wheat         | AC Avonlea                  |
| carrot              | Royal Chantenay             |
| cucumber            | Marketmore 76               |
| tomato              | Heinz 1439                  |
| lettuce             | Buttercrunch                |
| radish              | Cherry Belle or<br>Champion |
| northern wheatgrass | common                      |
| red clover          | common                      |
| red fescue          | creeping                    |
| blue grama grass    | native seed                 |



# Collembola Survival & Reproduction Test



# Status Report on Test Methods

- **Earthworm Method:** Published English test method in April 2004
- **Terrestrial Plant Method:** Final English version of test method by April 2004 but formal published version by June 2004
- **Springtail Method:** Soils Workshop participants strongly recommended additional test method development to identify and confirm a third test species. However, writing of EC formal method is continuing.

# **Workshop to Identify Priorities for Research and Standardization of Toxicology Methods for Assessing the Effects of Contaminants in Soil**

**Held in February 2003 at the Environment Canada's  
Pacific Environmental Science Centre, North Vancouver**

# Workshop Objective

To identify priorities for method development, validation and standardization that will lead to a second generation of Canadian soil toxicology test methods for assessing the effects of contaminant mixtures in natural soils

# Workshop on Future Soil Toxicity

## Method Development

Combination of plenary presentations and breakout group discussions over 3 days

### Working Group # 1

Alternative Species and Procedure Modifications of Existing or New Terrestrial Toxicity Test Methods

### Working Group # 2

Laboratory Functional Assays and Exposure Systems for Site Soil Assessment

### Working Group # 3

Physical or Biological Factors Influencing the Results of Soil Toxicity Tests

# Participation of Experts

- 33 respondents to pre-workshop questionnaire (opinion provided on 80+ issues and 130 pages of comment)
- 3 working group discussion documents (100+ pages of discussion material)
- 38 experts attending Vancouver workshop

|               |      |
|---------------|------|
| Europe        | (4)  |
| United States | (4)  |
| Canada        | (30) |

**Expertise:** soil toxicology, microbiology, ecology, chemistry, earth science, plant physiology, chemistry of contaminants in soil

# Issues and Current Research Related to Existing EC Methods

## WG Recommendation

- Reference toxicant tests of shorter duration can be used as long as there is a common test endpoint and similar dose response curves

## Post-Vancouver Workshop Progress

- **Environment Canada has sponsored research which verified that boric acid, the recommended reference toxicant, met the above recommendation for earthworm, plant and collembolan test methods**

# Issues and Current Research Related to Existing EC Methods

## WG Recommendation

- The six plant species currently proposed in the EC test method are a good start but more species should be added.

## Post-Vancouver Workshop Progress

- **Corn as a test species has been dropped from the EC test method due to lack of sensitivity.**
- **Research at the EC's Environmental Technology Centre has lead to the addition of seven additional crop, market garden and grassland test species to be included in the test method.**



# Issues and Current Research Related to Existing EC Methods

## WG Recommendation

- Soil pH measurement should be conducted using either the KCl or  $\text{CaCl}_2$  method
- Soil moisture measurement and expression of results should be standardized using either the ISO or USEPA Water Holding Capacity or water-filled pore space methodologies

## Post-Vancouver Workshop Progress

- **Research completed at the Ecole polytechnique federale de Lausanne (EPFL) toxicology laboratory has resolved the soil pH and moisture issues**

# Research Towards Future EC Test Method Amendment

## WG Recommendation

Further research should be commissioned to identify and assess the effectiveness of additional sexually-reproducing species in the springtail method (i.e. sexually reproducing species – *Folsomia fimetaria*)

# Priority Research for Future Soil Toxicity Method Development

- New species should be representative of different ecological functions, Canadian habitats, and exposure scenarios.
- Order of priority for new test methods for assessment of contaminants in agricultural soils:
  - 1<sup>st</sup>** Predatory mite reproduction test (e.g., using *Hypoaspis aculeifer* or *H. miles*)
  - 2<sup>nd</sup>** Oribatid mite or Coleopteran tests
  - 3<sup>rd</sup>** Isopod or Gastropod tests

# Priority Non-agricultural Habitats for Toxicity Test Method Development

| Habitat Type                  | Total No. of Questionnaire Respondents |
|-------------------------------|--|
| Boreal Forest                 | 13                                     |
| Wetlands                      | 8                                      |
| Northern Areas (taiga/tundra) | 6                                      |
| Grasslands                    | 5                                      |
| Saline/Arid                   | 3                                      |
| Industrial Sites              | 2                                      |
| Muskeg/Peat Bogs              | 1                                      |
| Coastal Lands                 | 1                                      |
| Riparian Zones                | 1                                      |
| Urban Green Zones             | 1                                      |

# Priority Research for Future Soil Toxicity Method Development

## WG Recommendation

- Conduct a study to identify candidate boreal forest, tundra/taiga and native grassland species and initiate test method development
- A study should be conducted to identify and locate representative boreal forest and tundra/taiga invertebrate species for laboratory culturing and test method development

# Working Group # 1

## General Recommendations

- Consider developing a life-cycle plant test method
- National database on organism health criteria or test performance criteria should be established
- Minimum test battery should include plant tests with two species (monocot and dicot), one or two invertebrate tests (earthworm or collembola) and at least one microbial test

# Working Group # 2

## Accomplishments

- Incorporated functional assays in a risk assessment framework by linking assessment endpoints with measurement endpoints and selecting specific assays for generating data to determine the measurement endpoints
- Existing soil microbial health methods were evaluated in terms of their advantages and limitations as functional assays in light of selection criteria established by the WG
- A consensus was reached on the selection of specific tests for various tiers of a risk assessment and guidance was developed on when or where to apply the tests

# Working Group # 2

| Assessment or Measurement Endpoint | Existing Standard Method                     | Methods Needing Further Development or Standardization |
|------------------------------------|--|--|
| Soil Fertility/Respiration         | SIR ratio (ISO 17155)                        |  |
| Soil Fertility/Nitrification       | Soil nitrification (ISO 15685)               |  |
| Feeding Activity                   |  | Bait lamina  |
| Breakdown of Organic Matter        |  | Litter bag   |
| Productivity Below Ground          | Fumigation-extraction (ISO 142420; OECD 217) | PLFAs  |
| Functional Diversity               | PTAC Soil and Groundwater Forum              | DGGE   |



# Working Group # 2

## Conclusions and Recommendations:

- Functional assays should be part of the test battery used to develop soil quality criteria
- Functional assays should be part of a test battery with single species tests and NOT as stand-alone tests
- EC should develop soil microcosm test methods for intact and disturbed soil cores BUT, further research is needed
- Bioreporter and biomarker techniques require more research to link test results to ecological measures
- Assessment of structural biodiversity is important BUT, guidance on interpretation and quantification of results is lacking
- EC should develop guidance for the interpretation of functional assays used in a risk assessment context

# Working Group # 3

## Conclusions and Recommendations

- When selecting the appropriate test battery, route of exposure and representation of functional groups is more important than the number of species to be tested
- Better collaboration across scientific disciplines is needed (e.g., use ecologists, taxonomists & toxicologists when identifying new candidate test species)
- Consensus on the need to develop methods for assessing biological effects in overlying LFH organic layers (not just mineral soils)

# Working Group # 3

## Conclusions and Recommendations

- **“You can’t start a toxicity test until you have a good sample.”** There is an urgent need for standardized guidance on sample collection, preparation & storage for the purpose of biological testing. A national guidance document should be developed
- Priority should be given to development of chronic and sublethal tests versus acute mortality tests
- More lab or field studies are needed to validate single-species laboratory toxicity data

# Principal Output

- A proceedings is the main output of the workshop and will identify:
  - 1) **Prioritize method research, validation and standardization activities that, if acted upon, would lead to the development of a second generation of EC soil toxicity test methods**
  - 2) **Issues and potential obstacles to advancing ecologically-relevant soil toxicity testing**
- Workshop proceedings will be available at the end of March 2004

**Thank You**