Priorities for Research and Standardization of Soil Toxicology Methods for Assessing the Effects of Contaminants in Soil

Rick Scroggins, Environment Canada
Kristin Becker-van Slooten, EPFL
Gladys Stephenson & Lesley Novak, ESG International
Steve Siciliano, University of Saskatchewan
Roman Kuperman, U.S. Army
Doug Bright, UMA Engineering

Workshop to Identify Priorities

- **Held in February 2003 at Environment Canada's Pacific Environmental Science Centre, North Vancouver**
- **Combination of key plenary presentations, breakout group discussions and periodic reporting back to all participants by working group chair person**
- **Three breakout groups chaired by Kristin Becker (WG 1), Gladys Stephenson (WG 2) & Doug Bright (WG 3)**
- **Workshop funding from the Program of Energy Research and Development (PERD)**

Workshop Objective

Identification of 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

Working Groups

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**

Participants

- 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

Workshop Participants

Europe:

Kristin Becker, Jorg Rombke, Paulo Sousa, John Jenson

United States:

Roman Kuperman, Roman Lanno, Mike Simini, Anthony Hay

Canada:

Jan Addison, Janet McCann, Craig Buday, Mary Moody, Ken Doe, Rick Scroggins, Jennifer Miller, Graham vanAggelen, Murray Dixon, Gladys Stephenson,Suzanne Visser, Ping Gong, Manon Bombardier, Steve Siciliano, Jeff Wilson, Mike Zemanek, Debbie Chan, Richard Johnson, Doug Bright, Graham Osler, Darwin Anderson, Juliska Princz, Ted Nason, Miles Tindel, Julie Roy, Bill McGill, Kathryn Bessie, Natalie Feisthauer, Lesley Novak

Environment Canada Soil Toxicity Test Methods

- **Method for measuring emergence and growth of terrestrial plants exposed to contaminants in soil** (alfalfa, barley, corn, cucumber, radish or Northern wheatgrass)
- **Method for measuring survival and reproduction of springtails exposed to contaminants in soil** (*Onychiurus folsomi* or *Folsomia candida)*
- **Method for measuring survival, avoidance, and reproduction of earthworms exposed to contaminants in soil** (*Eisenia andrei, E. fetida, Lumbricus terrestris)*

Plant Method

Test unit for definitive plant test

Collembola Test

Test species:

Onychiurus folsomi

Folsomia candida

Test duration - **28 days for *Folsomia candida***

- **35 days for *Onychiurus folsomi***

Three Earthworm Test Options

1. **Acute (14-day) Lethality Test**
2. **Acute (48- or 72-hour) Avoidance Test**
3. **8-Week Test for Effects on Survival, Reproduction, and Growth**

Lumbricus terrestris, Eisenia andrei, or Eisenia fetida

Working Group #1

General Recommendations

- WG #1 started discussion with focus on the initial three EC test methods and possible additional species for future consideration
- Current EC standardized methods – a great start
- Species selected for future test method should be culturable in the laboratory
- Reference toxicant test of shorter duration can be used if a similar dose response curve with the same endpoint is obtained
- Recommend the establishment of a national database on organism health criteria or test performance criteria

Working Group #1

General Recommendations

- Five natural soils similar to those for EC method validation are adequate for future test method development
- Recommend the establishment of a national database on organism health criteria or test performance criteria.
- Recommend standardization of a pH measurement method using KCl or $CaCl_2$
- Soil moisture measurement and expression should be standardized using either the ISO Water Holding Capacity or water filled pore space methodologies
- Regression analysis of toxicity test data is recommended

Working Group #1

Recommendations for Soil Invertebrate Methods

- Research on current methods should focus on the collembola method and verifying a second sexually-reproducing species (i.e. *O. folsomi* or another sexually reproducing species)
- Top priority for new method development is a test using predatory mites
- For reproduction tests with invertebrates, it is recommended to always feed regardless of soil type
- Priority to establish selection criteria to help identify, collect and culture invertebrate species for testing non-agricultural lands

Working Group #1

Recommendations for Plant Methods

- The six plant species currently proposed in the EC test method are a good start
- Three species should always be tested during definitive testing and include at least 1 monocot and 1 dicot
- Conduct a study to explore the development of methodology for boreal, native non-agricultural, perennial, and recreational grass species
- Consider developing a life-cycle plant test
- Nutrient deficiency must be considered

Working Group # 2

Accomplishments

- Incorporated functional assays into a risk assessment framework by linking assessment endpoints with measurement endpoints and then recommending specific assays for generating data for determination of these measurement endpoints
- Test methods were evaluated and discussed in terms of their limitations and advantages in light of established selection criteria
- A consensus was reached as to the selection of specific tests for the various tiers of a risk assessment and when or where they should not be applied

Working Group # 2

Assess/Meas. Endpoint	Standard Method	Further Development
Soil Fertility/Respiration	SIR ratio (ISO 17155)	
Soil Fertility/Nitrification	Soil nitrification (ISO 15685)	
Feeding Activity		Bait lamina
OM Breakdown		Litter bag
Productivity Below Ground	Fumigation-extraction (ISO 142420; OECD 217)	PLFAs
Functional Diversity		DGGE

Working Group # 2

Recommendations:

1. Functional assays should be part of the test battery used to develop soil quality criteria
2. Functional assays should be used in conjunction with single species assays and NOT be used as stand-alone tests
3. Environment Canada should develop microcosm test methods (intact and disturbed) - BUT further research needed
4. Bioreporters and biomarkers require more research to link these test results to ecological measures
5. Assessment of structural of diversity is important – BUT interpretive and quantification guidance is lacking
6. Environment Canada should develop guidance for the interpretation of functional assays in risk assessment context

Working Group # 3

Recommendations

- For selecting the appropriate test battery, the number of species tested is less relevant than route of exposure and functional groups. Focus on species representative of the important ecotypes
- Better collaboration across scientific disciplines is urgently needed. For example, with regard the issue of declining dose, the combined effort of soil scientists, chemists, and toxicologists is required. For selecting new species for ecotypes not covered, the combined effort of ecologists, taxonomists, soil scientists and toxicologists is required
- There is overwhelming support for the need to assess not just mineral soils, but also the overlying LFH (or similar organic layers) where this is an important element of site soils

Working Group # 3

Recommendations

- Researchers should be cognizant of the fact that toxicity tests may not capture dietary exposures
- Appropriate guidance such as decision trees and limitations should be developed for challenging substances as an addendum to standardized methods
- First preference is to utilize the pre-existing concentration gradients from the site to investigate concentration – response
- Field validation studies are needed of single-species laboratory toxicity data
- Can't start a toxicity test until you have your sample – many outstanding questions on sample collection, preparation, storage – need a Guidance Document

Working Group # 3

Recommendations

- The issue of data adequacy is not so much whether the existing methods are adequate, but rather how confide we are that the data is communicated in an open and transparent way
- Priority should be given to chronic and non-lethal versus acute and mortality-type tests
- Forestry perspective was missing from workshop. Should be pursued
- Predictive modelling is a good idea but we are not there yet
- If species is not appropriate to the soil type, do not use them

Workshop Output

- The main output from the workshop will be a proceedings document that captures critical discussion and key recommendations
- Proceedings will identify new opportunities, remaining challenges and research priorities including:
  - issues and potential obstacles to advancing ecologically-relevant soil toxicity testing; and
  - identification of priorities for method research, validation and standardization that would lead to the development of a second generation of standardized soil toxicity test methods
- Proceedings should be available in approximately 2 months
- SETAC session planned and three peer-review papers