



## Project Summary

# AEERL Procedures Manual: Level 1 Environmental Assessment Terrestrial Ecological Tests

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Some test methods designated for EPA/AEERL's Level 1 environmental assessment biological testing program are sufficiently new that little or no published literature is available describing specific application of the procedures. This manual provides detailed procedures for EPA/AEERL's Level 1 terrestrial bioassays. The manual supplements the "IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests" (second edition, EPA-600/8-81-024, NTIS PB 82-228966) which provides the basic procedural steps. The protocols described in this manual are the same as those in the supplemented manual, but are expanded in those areas of methodology which involve culturing methods, materials and supplies, and specific scoring procedures.

The three tests included in this manual are: (a) Plant Stress Ethylene Test, to assess the toxic effects of gaseous samples on plant foliage; (b) Root Elongation Test, to determine the toxic effects of liquid samples and aqueous leachates of solid samples on seed germination and root elongation in a number of plant species; and (c) Insect Toxicity Test, to measure lethality and reproductive toxicity in *Drosophila melanogaster* to both liquid and some solid samples.

*This Project Summary was developed by EPA's Air and Energy Engineering Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).*

## Introduction

This bioassay manual supplements EPA/AEERL's latest procedures manual for Level 1 environmental assessment biological tests.\* There is little published material on the application of the Level 1 terrestrial bioassays for environmental assessments. Thus, this manual provides a more detailed description of the terrestrial bioassays than are presented in the Level 1 biological testing manual. The protocols described in this manual are the same as those in the supplemented manual. Details and references to assist laboratories in implementing Level 1 terrestrial ecological effects testing have been added.

The Level 1 terrestrial ecological effects tests include assays for determining toxicity of complex industrial process streams in plant and insect test organisms. The tests are able to detect sublethal toxic response to stress in plants (Plant Stress Ethylene Test—PSE), sublethal and lethal toxic responses in germinating seeds (Root Elongation Test—RE), and acute toxicity and reproductive impairment in insects (Insect Toxicity Test—IT).

These tests provide a range of terrestrial organisms for assessing the relative effect of effluent streams on the environment. Test organisms include maturing plants, germinating seeds, and insects. These tests offer testing capabilities for all sam-

\*Brusick, D.J., and R.R. Young. "IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests," EPA-600/8-81-024, NTIS No. PB 82-228966, Litton Bionetics, Inc., Kensington, MD, October 1981, 150 pp.

ple types (including gases) with the advantages of low cost, reproducibility, and relatively rapid performance. A future goal for the Level 1 program is to include a test procedure for assessing the impact of effluent samples on soil microorganisms (decomposers).

Terrestrial ecological tests are used to determine the concentration of test material that produces a defined toxic effect on a specified percentage of the test organisms in a fixed period of time. The PSE test is designed to assess and rank the toxic effects of gaseous effluents on plants by measuring the stress ethylene of plant response and by assessing relative foliar injury in exposed plants. The RE test measures the inhibition of root elongation and seed germination; although both are observable toxic responses and are reported, root-elongation inhibition is the preferred end point. The concentration which inhibits root elongation by 50% of the control ( $EC_{50}$ ) is estimated and used to rank effluent samples. The IT assay measures the acute toxicity and reproductive capacity of fruit flies treated with environmental samples. The dose lethal to 50% of the flies ( $LD_{50}$ ) compared to the control is calculated and used to rank test samples. In the optional fertility test, the effective concentration which reduces the fecundity of surviving dosed flies to 50% of control flies  $EC_{50}$  is calculated. Characteristics of the Level 1 terrestrial bioassays are given in Table 1.

### Level 1 Plant Stress Ethylene (PSE) Test

The EPA Level 1 PSE test is designed to assess the toxic effects of gaseous effluents on plants by employing the stress ethylene plant response. Under normal conditions plants release low levels of ethylene which function hormonally to regulate growth and development. In response to various stresses, ethylene production increases substantially. Viable plant tissue is necessary for ethylene production. Ethane evolution may also increase in response to some stresses and is thought to indicate more severe damage and tissue autolysis. Critical to the effectiveness and applicability of the PSE test is that induction of stress ethylene is proportional to the intensity and duration of the stress over a wide range of stresses. However, the stress ethylene response has yet to be documented for a large number of pollutants and complex mixtures.

The test procedure minimizes laboratory manipulation to provide a cost effective bioassay. The procedure involves culturing

**Table 1.** Characteristics of Level 1 Terrestrial Ecological Effects Bioassays

Characteristic	Plant Stress Ethylene Test	Root Elongation Test	Insect Toxicity Test
Species	Bush bean	Cucumber, wheat, red clover, radish, lettuce	<i>Drosophila melanogaster</i>
End point(s) measured	Metabolic stress evidenced by ethylene production, foliar damage	Root length, germination	Lethality, reproductive capacity
Amenable to sample type	Gases	Liquids, solids (leachates)	Liquids, solids
Data expression	Positive or negative	$EC_{50}$	$LD_{50}$
Special features	Only validated Level 1 bioassay for gases; sensitive	Detects toxicity to terrestrial producers; multiple species	Detects lethality to terrestrial consumer; can also provide data on fertility

of test plants, collection of gas samples, exposure, incubation, ethylene analysis, and visual examination. Bush bean, *Phaseolus vulgaris* L., cultivar Harvester, has been selected as the bioassay plant because it develops rapidly and is easy to grow. The plants are cultured under controlled conditions and are subsequently exposed to the test or control (chlorine) gas. After a period of dark incubation in sealed containers, ethylene and ethane are analyzed by gas chromatography. Three days after the assay, the plants are examined visually for injury.

Chapter 1 of the full report contains detailed procedures for gas sampling, design and construction of exposure chambers, and methods for exposing test plants to control and test gases. Also discussed in detail are plant culturing techniques, post-exposure incubation, and ethylene and ethane analysis. Visual inspection of the test plants is also discussed as an adjunct method for assessing test material toxicity. Finally, criteria for assay acceptance, interpretation, and evaluation are presented.

### Level 1 Root Elongation (RE) Test

Assessing phytotoxic effects requires the selection of a stage in plant development that is sensitive to a broad range of toxicants and is important physiologically. Seed germination and root elongation, critical links in plant development between a dormant embryo and a period of rapid growth with the formation of essential plant structures, were selected for phytotoxicity testing. Toxic substances that prevent or reduce germination or root elongation will decrease plant populations and reduce crop yields. In natural systems, affected species are less able to compete

with other species, and tolerant species may be selected, resulting in changes in species diversity, numbers, and population dynamics.

The RE/seed germination bioassay, in Chapter 2 of the full report, is a rapid and simple test. Germination and root elongation can be observed after 115 hours of incubation, and the test does not require large expenditures for equipment and facilities or complicated techniques. The RE test is suitable for testing liquid sample or solid samples from which aqueous leachates can be prepared.

A toxicant may cause responses at different doses in different plant species. To detect an effect from chemicals of unknown toxicity, several plant species are selected. The species used in this test are lettuce (Butter Crunch), *Lactuca sativa* L.; radish (Cherry Belle), *Raphanus sativus* L.; wheat (Stephens), *Triticum aestivum* L.; cucumber (Hybrid Spartan Valo), *Cucumis sativus* L.; and red clover (Kenland), *Trifolium pratense* L. The species are representative of economically important plants and different plant families. The seeds chosen germinate and grow rapidly, contain no natural inhibitors, and require no special pretreatment. The test organisms are grown under identical environmental conditions (constant 25°C temperature constant dark, and enclosed to maintain uniformly high relative humidity) and are exposed to several effluent concentrations. Although inhibition of both RE and germination are observed in toxic responses, in this bioassay RE inhibition is the preferred end point. Usually inhibition is observed at lower concentrations of toxic substances than is seed germination. Testing approaches are described, and criteria for interpretation are included.

Chapter 2 of the full report gives a detailed description of the methodology for the RE test. General procedures are discussed concerning the test organisms, test chambers, reagent and material preparation, and the preparation of test material and control solutions. The procedures for planting the seeds, exposure to test solutions, incubation, and data collection in both range-finding and definitive tests are also presented. The assay acceptance, interpretation, and evaluation criteria are discussed in detail.

## **Level 1 Insect Toxicity (IT) Bioassay**

*Drosophila melanogaster* is a common insect species in nature; many features of this organism make it attractive as an *in vivo* test system for detecting environmental toxicants. These features include: 1) a short life cycle of 10-12 days; 2) minimal space, monetary, and manpower requirements to maintain stocks; 3) the ability to detect toxic effects at specific life cycle stages (adult, germinal, and developmental); 4) a well-defined genetic system which makes the detection of specific genetic end points possible; 5) the ability to biotransform genotoxic chemicals via *in vivo* metabolic enzyme systems; and 6) amenability to exposure to liquids, soluble solids, gasses, and small particles. Feeding via solution is the most common route of exposure.

*Drosophila* is included in EPA Level 1 environmental assessment testing not only because of its application as a toxicity screening test, but also because it can be expanded into testing for additional end points. For Level 1 applications, *Drosophila* will be used to assess acute toxicity in the adult flies and reproductive capacity among surviving flies following exposure to, or treatment with, environmental samples. The acute toxicity data will be used to rank effluent streams and identify sources for further testing. The fertility response will be recorded and may be used to provide possible direction for additional testing.

Details of the Level 1 IT protocol are presented in Chapter 3 of the full report. The source of and culture methods for *Drosophila* are presented. The methods to perform a basic feeding study are discussed. The design of range-finding tests, definitive tests for LD<sub>50</sub> determination, and fertility tests are presented as modifications of the basic feeding-study test design. Methods for data collection and interpretation and the criteria for test material ranking are also included in Chapter 3.

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*The complete report, entitled "AEERL Procedures Manual: Level 1 Environmental Assessment Terrestrial Ecological Tests," (Order No. PB 87-102 398/AS; Cost: \$11.95, subject to change) will be available only from:*

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