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**Predicting ecological
impacts from
laboratory toxicity tests
SETAC Technical Workshop
May 1-5, 1999
Cornwall, Ontario**

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SETAC Technical Workshop

Predicting Ecological Impacts from Laboratory Toxicity Tests

Executive Summary

by

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The Society of Environmental Toxicology and Chemistry (SETAC) held a Technical Workshop May 1-5, 1999 in Cornwall, Ontario, to evaluate the state-of-the-science regarding the utility of standard laboratory aquatic toxicity tests in ecological risk assessment. Thirty-one scientists from North America and Europe participated on the basis of their experience, training, and interest in areas of environmental toxicology and chemistry related to aquatic toxicity testing and ecological risk assessment. Workshop participants broke into working groups that:

- ! Reviewed the ecological consequences of chemical toxicity and the data commonly used in ecological risk assessments;
- ! Examined the utility of current lab tests for predicting ecological effects, using the pulp and paper industry as a model;
- ! Determined whether the lessons learned about testing pulp and paper effluents applied to tests of effluents from other industry sectors;
- ! Recommended approaches for developing ecologically relevant tests for current or future effluents or compounds.

The working groups summarized their findings in a five chapter report to be published by SETAC in the year 2000. The participants affirmed that standard aquatic toxicity tests, as currently used for routine testing and monitoring of effluent quality, can be effective tools for assessing chemical hazards. Test procedures for monitoring reproduction, growth, and survival of aquatic biota in aquatic ecosystems describe the toxicity of effluents and chemicals released to aquatic environments. They provide data for predictive models and support ecological risk assessments, water quality criteria, effluent regulation, surface water quality monitoring, and assessment of contaminated sediments. The current tests have been used with success to direct major improvements in effluent quality, to reduce emissions of toxic substances to aquatic environments, and to direct remediation efforts and assess their effectiveness at contaminated sites.

Despite these successes, important ecological effects may still be missed. Field observations of unexpected effects and recent advances in our understanding of the mode of action suggest that aquatic ecosystems may be affected through mechanisms and pathways not addressed by current tests. Hence, research is needed to develop new laboratory and field methods to improve risk assessments. Improved approaches are also required to ensure that existing tests and endpoints are appropriate for the expected effects, and that they are calibrated through field studies and environmental monitoring.

To determine whether the current battery of toxicity tests meet the needs of environmental protection, the workshop examined in depth the history of toxicity testing of pulp and paper effluents. This sector was chosen because it has been the focus of recent studies in support of revisions to effluent regulations in both Europe and North America. Experience with pulp mill effluents has demonstrated many strengths and a few weaknesses of the current toxicity testing paradigm, many of which are applicable to other industrial sectors:

- ! Toxicity Identification and Evaluation (TIE), using standard acute lethality toxicity tests or new tests of sublethal responses, was a practical approach for isolating toxic components of effluents,

provided that rapid and practical test methods were available. For pulp mills, an example was the identification of resin acids as major contributors to lethality of untreated effluent;

- ! Toxic Equivalent Factors (TEFs) provided a practical way to assess risks of mixtures of compounds with similar modes of action, such as the chlorophenols;
- ! Effluent monitoring with standard invertebrate and larval fish tests were used to demonstrate the success of process changes and effluent treatment in improving effluent quality; particularly for the resin acids and for biological oxygen demand;
- ! Some sublethal effects were first noticed in field studies, highlighting the importance of field monitoring as a means of validating risk predictions. For example, important effects on fish reproduction were not predicted by laboratory studies, primarily because existing fish life cycle tests were not routinely implemented for effluents. In addition, tests with sufficient sensitivity to detect sublethal effects can be lengthy and expensive, and related physiological effects, such as impaired regulation of sex hormones, could not be assessed using standard tests;
- ! For marine brown algae (*Fucus vesiculosus*), tests using existing lab species (e.g. duckweed, *Lemna* spp.) did not assess sensitivity to chlorate, nor the enhancement of toxicity due to site-specific interactions of chlorate with low concentrations of nitrate. The rapid solution to the *Fucus* puzzle demonstrated the value of integrating field and lab studies, of multiple lines of evidence, of site specificity, and of appropriate effluent treatment;
- ! The ecological significance of some physiological responses of fish (e.g. immunodeficiency) are not immediately evident and still poorly understood, as are some reproductive responses (e.g. reduced egg number). Not all Aresponses@ to pulp mill effluent exposure have been associated with ecological Aeffects@.
- ! As improved treatment reduced toxicity and oxygen depletion by pulp mill effluents, eutrophication became more evident, with associated changes in benthic community structure and function. Nutrient effects, and implications of reduced nutrient discharge are not currently assessed as part of standard effluent compliance testing. They are not considered as Atoxicity,@ and standard tests (e.g. of primary productivity) are not routinely implemented.

The overall conclusion was that the strengths and weaknesses that are apparent when current standard tests are applied to pulp mill effluents are equally evident when these tests are applied to other discharges. For example, the potential reproductive effects of municipal discharges has emerged as an issue following the development of new tests for estrogenicity of effluents. Reproductive impairment in marine benthic fish exposed to PAH-contaminated sediments has also been recognized recently.

While there is an important and on-going role for existing toxicity tests, additional tests which evolve from newly-recognized modes of toxicity will improve ecological risk assessments. Useful tests will be those measuring function or performance, such as the physiology of fish or the productivity of ecosystems. These tests will be based on known mechanisms of toxicity and will integrate multiple chemical and non-chemical stressors.

The workshop attendees noted a particular lack of tests that measure, or that can be explicitly linked to, ecosystem level functions. Uncertainties in ecological risk assessment can only be reduced by developing new tests of ecosystem-level responses, and by confirming predictions through direct

measurements in the environment. Inferences about the ecological significance of toxicity to single species or to individuals will also require modelling and subsequent studies to validate or calibrate predictions. Small-scale experimental ecosystems are a stepping stone between lab and field, allowing the testing of hypotheses developed from laboratory tests or field observations in controlled environments.

Toxicity tests and field studies will only be of value if chemical exposure is well characterized. Characterizing exposure in both lab and field studies is critical for interpreting biological results. However, biological effects should drive chemical investigation of causation and exposure, and assessment of exposure should be hypothesis driven, so that analyses support the interpretation of biological testing.

Progress in implementing these recommendations will result in a greater variety of tests for assessing chemical hazards, a greater use of multiple lines of evidence linking laboratory and field studies, and a more site- or chemical-specific approach to testing. To take the maximum advantage of these new tests, more planning and research will be needed. The ecological risk assessment framework emerged as a key element for designing successful programs of laboratory testing, because it imposes a systematic identification of clear and specific objectives, based on the ecosystem values to be protected. Successful identification of ecological effects will involve an iterative approach that integrates lab and field studies. It will require additional research on previously untested species, with increased predictive accuracy of tests as conditions are modified to incorporate site-specific factors.

While we have been successful in testing and protecting some economically important species, there is much to learn about the remaining components, how ecosystems function, and how stressors interact with components of ecosystems to cause environmental change.

Overall, the workshop concluded that there are characteristic ecological effects of industrial effluents that dictate a broadly applicable strategic approach to studying sublethal responses. Not all biological responses to effluents constitute a negative effect, but the understanding of the relative importance of responses, and an awareness of responses outside the realm of standard tests, will only be achieved through studies of the environments to be protected, integrated with new and appropriate tests.

The workshop identified specific, high priority research needs. There is a need to:

- ! develop tests to measure the consequences of cumulative stressors;
- ! establish the ecological significance of sublethal changes in fish reproductive parameters (e.g. gonad-somatic index (GSI), changes in hormone regulation);
- ! develop rapid tests to measure the effects of chemical classes not detected by current standard tests (e.g. endocrine disruptors);
- ! develop better short-term tests of fish reproductive end-points that reflect effects on the complete fish life cycle;
- ! model population responses to chemical stress to understand consequences of single organism responses;
- ! develop sensitive and reliable tests of functional responses at the population, community and ecosystem level (e.g. meso- and micro-cosms approaches)

- ! develop experimental approaches to scale responses of microcosm and mesocosms to real ecosystems;
- ! develop standardized protocols for *in situ* toxicity testing under relevant exposure scenarios (e.g. on-site community bioassays, mesocosms);
- ! develop guidelines for collecting data on exposure to ensure that laboratory and field toxicity tests contribute useful data to ecological risk assessments;
- ! develop biomarkers of exposure and calibrate to measures of effect in laboratory and field studies;
- ! better integrate lab and field studies to ensure that lab toxicity tests measure ecologically-relevant effects.

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