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Environmental Toxicology: Testing and Screening

Maurice Zeeman, Anne Fairbrother, and Joseph. W. Gorsuch

ABSTRACT: *In response to Congress, the Office of Technology Assessment (OTA) is preparing a study on the Toxic Substances Control Act (TSCA) to evaluate the Existing Chemicals Program. The purpose of the Chemical Testing and Screening Workshop was to identify the present and future methods of screening and testing of commercial chemicals using nine specific endpoints, one being environmental toxicology (i. e., ecological effects assessment). This chapter addresses the state of the science by responding to several specific questions asked by the OTA (e.g., "what are the best tests available to identify a chemical of concern and to evaluate its toxicity?'?. This chapter concludes that basic screening and testing methods are already being applied by EPA/OPPT, especially by the use of structure-activity relationships (SARs/QSARs) for ecotoxicity screening purposes, and by the use of rapid and inexpensive tests to actually assess ecotoxicity. Areas for improving existing methods include sorting priorities to assess chemical exposure information and SARs/QSARs for avian species, plants, earthworms, and sediment dwelling organisms.*

One of nine specified topics of interest addressed at the Office of Technology Assessment (OTA) Workshop was the testing and screening methods used by the U.S. Environmental Protection Agency (EPA) and others to assess environmental toxicology. Methods for "environmental toxicology" were understood to mean screening and testing methodologies used to assess potential ecological effects on organisms found in the environment from TSCA-regulated chemicals.

The Toxic Substances Control Act of 1976 (TSCA) provided the EPA Office of Pollution Prevention and Toxics (OPPT) with authority to require development of adequate data for assessing the risk to human health and the natural environment from industrial chemicals identified as having risk potential. "Protection of the environment" means different things to different people. To some it means maintaining a place where humans can live and be healthy. To others, it is tied to commodity production or extraction. Still others look for a system that looks and functions as it did prior to the arrival of Europeans in North America and that has the capacity to sustain all native plants and animals. Congress purposefully left this definition vague in almost all environmental legislation in order to allow continued public debate to frame the question. Nevertheless, implementation of TSCA requires the EPA to explicitly describe what "protection of the environment" means within this context, in order to request the proper information to evaluate whether a chemical has the potential to significantly degrade that environment.

Within OPPT, the Environmental Effects Branch (EEB) has provided the scientific and technical evaluation of environmental/ecological hazard of industrial chemicals, and has determined the type and adequacy of data needed to identify and assess their possible adverse effects. Over the past 15 years this group has provided significant direction to, and rationale for, how ecological hazard and risk assessment activities have been addressed under TSCA (26, 29, 30).

Environmental protection can occur at many different levels of ecological organization. Traditionally, wildlife and fisheries managers have protected populations while plant ecologists look for healthy, evolving communities. The Endangered Species Act (ESA) requires protection of the health of individual organisms. Animals,

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however, have differing strategies for population maintenance. Small mammals (rodents, etc.) have a large reproductive capacity to balance the high annual mortality (up to 80% of the population in some cases). In this instance, a little additional mortality from environmental contaminants would be inconsequential, while a reproductive inhibitor could have longer-term effects. Conversely, most large animals such as elephants and eagles have a long life span, relatively low annual mortality, and a low reproductive rate. Loss of one or two reproductive seasons would have little effect on these populations as the adults would survive to reproduce another year. However, increased mortality of adults due to an environmental contaminant would severely depress the population.

While species differ in their life history strategies, it is intuitively obvious that increased mortality and decreased reproduction will affect the population over the long-term. The amount of changes in these parameters that is "significant" depends on the species and the community with which it is associated. Compensatory changes in reproduction, predation, competition, etc., all affect the severity of the impact of chemical-induced effects. Thus, TSCA-related ecological risk assessments include measures of lethality (LC_{50}) and reproductive effects with an associated uncertainty ("assessment") factor to accommodate our imprecise knowledge of ecological systems (29). Sublethal effects (immune suppression, endocrine disruption, neurotoxicity, etc.) could potentially influence population demographics, but in more subtle ways that have not yet been clearly established. Therefore, their inclusion as endpoints upon which regulatory decisions can be based is still open for debate.

To assure that adequate ecotoxicity data are developed to assess the possible adverse ecological effects of industrial chemicals, screening methods, test procedures, and guidelines have been established by OPPT (26, 29, 30, 3 1). For example, several hazard assessment structure-activity relationship (SAR/(Q)SAR) screening methodologies have been developed and refined in OPPT specifically y for the data-poor new chemical assessment process (4, 5, 6, 13, 14, 15, 28). These (Q)SAR methodologies are now being applied to the hazard and risk screening of TSCA-regulated existing chemicals (3, 30). It should be noted that (Q)SARs have been used to predict toxicity, biodegradability, and bioaccumulation (18).

BEST AVAILABLE TESTS

The best available tests to identify and evaluate chemical toxicity will depend on the potential risks, the uncertainties, the natural resources at risk, and the resources available for analysis. No single test is "best" for all situations. The best tests to assess the ecological effects of a chemical of concern, i.e., the most ecologically relevant and producing the most accurate results, would most likely be field assessment tests. These should identify where the TSCA existing chemicals are being released or applied in the field and also assess the impacts on the numerous types of organisms that exist in the environments that are being exposed. Depending on the location and size of the area of concern and the level of biological focus, the number of species potentially exposed and impacted could vary from dozens, to hundreds, thousands, or even millions of species.

Although most meaningful ecologically, field testing seldom would be conducted without prior knowledge of the potential toxicity of the chemical to plants or animals, particularly at concentrations expected to be found in the environment. Field tests are very expensive (in the order of several million dollars) and are technically difficult to conduct. In addition, it is difficult to communicate the significance of such study results to chemical industries, regulatory decision-makers, and the public. Multiple stressors: chemical, biological, and physical, are often difficult to differentiate in populations/communities. However, *in situ* effluent biomonitoring frequently is done in aquatic situations as a bioassay for toxicity detection (10).

In fact, most ecological risk assessments of TSCA-regulated chemicals are oriented toward

the aquatic environment. This is because the majority of environmental releases are presumed to be aquatic releases. Air releases are another route of environmental exposure and may influence terrestrial systems as well, but with the exception of smelters and intentional applications to land (e.g., dioxins in sludges applied to forests and pastures), adverse terrestrial effects from this or any other source have not been well documented (17, 25, 26).

Another main reason is that seldom can an adequate regulatory case of significant exposure, hazard, and risk to organisms in the environment be provided to warrant field testing. Typically, the majority of cases where TSCA-regulated existing chemicals are known to be released into the environment and resulted in exposures of organisms, has often focused on chemical production releases into the aquatic environment. As a result of estimated environmental dilution, and adsorption to particulate, this frequently ends up in predictions of very low chemical exposures and risks. For the terrestrial environment, only a few examples of potential exposures and effects have even been assessed, let alone been considered for any form of field testing (17,25, 26).

As we move away from field testing, because they are so complex and expensive, to other more derived test methods, that may be less meaningful ecologically, our ability to accurately predict the overall effects of a chemical may be compromised. One of the more feasible surrogates for testing in the field is mesocosm or microcosm testing of chemicals. However, these tests can also be fairly lengthy, moderately expensive, and their results difficult to interpret and defend, as is the situation with field study results.

The next most ecologically realistic and important level of testing is long-term ecotoxicity testing performed in the lab. If such tests are of sufficient duration, they can be designed to evaluate the potential impacts of a chemical on the mortality, growth, development, and reproduction of field populations (or of appropriate surrogates for these species). Test durations long enough for whole life-cycle testing are preferred, but such test results are seldom available for industrial chemicals. More available, but still relatively uncommon, are industrial chemical results (e.g., maximum acceptable toxicant concentration (MATCs)) from different versions of the 30-90 day fish early life-stage test, or the 14-21 day partial life-cycle test for some aquatic invertebrates, such as *Daphnia*.

Short-term (e.g., 2-4 days) ecotoxicity testing results of acute lethality (i.e., LC_{50} or EC_{50} values) are usually the most readily available (but perhaps less ecologically meaningful) results found for existing industrial chemicals. From such limited test results, estimations of longer-term impacts can be made by using uncertainty factors to set potential exposure levels where ecological risks may occur **(31).**

Practically speaking, short-term testing of fish, aquatic invertebrates, and algae (the three basic trophic levels found in many aquatic food chains) represents most, if not all, of the testing performed for industrial chemicals (29). The primary reasons for this are the rapidity and inexpensiveness of these short-term tests. Performing a 48-hr daphnid EC_{50} test is quick, and if it is only used for internal chemical screening purposes (e.g., does not follow Good Laboratory Practices (GLPs) standards), would cost approximately $1,000 to accomplish. However, without chemical test concentration verifications, some test results might be of little value in predicting what would happen if the chemical were released into the environment. Similarly, inexpensive and quick tests are available for screening chemicals for toxic effects to plants. The germination and root elongation test (5-7 days in length) (11, 21), the vegetative vigor test (14-21 days in length) (20), or seedling growth tests (1, 9) could support the evaluation of the potential impact of a chemical in soils. Even limited acute ecotoxicity test data are preferred to no data at all.

Proposed cellular and molecular toxicity endpoint tests (e.g., promoter gene activation, stress protein induction, Ah receptor binding) may be useful for providing information about modes of action for a chemical and, therefore, direct concern towards particular species that may be most sensitive in this response. For example, a chemi-

cal that is shown to be induced through the Ah receptor would raise concern for mustelids (mink) and songbirds, but not for waterfowl. These suborganismal tests do not provide enough information about ecologically relevant effects (e.g., "significant" change in mortality or reproduction) to form the basis of a regulatory decision without additional information. It will take many years of research to develop relationships between gene induction and changes in population growth rates, and realistically, it may not be possible to do.

PRIORITY SETTING

OPPT SAR/(Q)SAR methodologies were developed for estimating ecotoxicity in order to screen thousands of chemicals per year in a very short time frame. Screening assessments typically occur with little or no ecotoxicity data being provided by chemical sponsors of the industrial chemicals submitted to EPA.

One primary use of (Q)SAR technology has been to set testing priorities by estimating how toxic a chemical may be to aquatic organisms. If this estimate results in a prediction of sufficient risks in the environment, the sponsor is encouraged to consider performing testing to define the actual toxicity of that industrial chemical. As discussed in detail elsewhere, these quick and inexpensive (Q)SAR methods have been used extensively in assessing the over 26,000 new industrial chemicals submitted to OPPT from 1979 through 1994 (1, 2,27,29, 30).

TRADEOFFS IN SCREENING

One potential problem is uncertainty about the accuracy of the ecotoxicity that is predicted by (Q)SAR. The (Q)SAR values themselves are only estimations of toxicity. They are only as good estimates as are possible based upon the ecotoxicity values present in the data set for the chemical class or biological activity being predicted (18). In general, the larger the number of chemical toxicity values that are present in the data set for an appropriate chemical class, the higher the chances are that the ecotoxicity predictions for that class are accurate.

OPPT SAR estimations can vary from simple similarities, such as using test data available for a similar chemical grouping or analogs, to being able to provide quantitative estimates of ecotoxicity. Quantitative estimates are possible when an empirical mathematical relationship has been established for a chemical grouping/class to which the new chemical also belongs. OPPT has developed over 120 (Q)SARs for about 45 classes of industrial chemicals (23, 24, 31).

Except for earthworms, the OPPT SAR database is limited to aquatic organisms. Similar models for terrestrial organisms (other than laboratory animals used for human risk assessments) need to be developed. Sufficient data exist for some classes of chemicals so that this could be done for plants, birds, and mammals. However, the database of toxicity information for reptiles and amphibians is too sparse to allow SAR models to be developed for herptofauna.

The aquatic (Q)SARs for some chemical classes result in a hazard profile of six ecotoxicity values that estimate both the acute and chronic toxicity of such chemicals to fish, daphnids, and algae, respectively (table 7-l). Typically not enough chemical ecotoxicity data exist to construct (Q)SARs for all parts of these hazard profiles (e.g., sometimes only one, two, or all three of the acute ecotoxicity values can be predicted). Some (Q)SARs may also be based upon data for only a few chemicals in the class.

Table 7-1: OPPT Standard Hazard Profile for Aquatic Ecotoxicity

Freshwater Test Descriptions
Fish Acute Toxicity (96hr LC_{50})
Daphnid Acute Toxicity (48hr EC_{50})
Algal Toxicity (96hr EC_{50})
Fish Early Life Stage (28-90 day MATC)
Daphnid Partial Life Cycle (14-21 day MATC)
Algal Toxicity (96hr NOEC)

SOURCE: Zeeman, M., "Ecotoxicity Testing and Estimation Methods Developed Under Section 5 of the Toxic Substances Control Act (TSCA)," *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment,* Chapter 23, G. Rand (ad.) (Washington, DC: Taylor & Francis, 1995)

However, the vast majority (95-98%) of the discrete organic chemicals found on the TSCA inventory come from only 7-10 chemical classes (7, 28, 30). Therefore only a smaller subset of the OPPT (Q)SARs will need to be used. Furthermore, several of the most commonly used (Q)SARs are also for chemical classes with a relatively large amount of ecotoxicity data used to construct the model. Therefore, they are among the more reliable (Q)SAR estimation methods.

VALIDATION/ REPRODUCIBILITY

As discussed in detail elsewhere, the validation of these OPPT (Q)SAR ecotoxicity estimation methods is an ongoing process (3, 7, 14, 15, 30). The validation of the OPPT (Q)SARs used for assessing the aquatic toxicity of new industrial chemicals has been performed and results were published in the peer-reviewed literature (1 5).

In addition, a recent joint EPA/ European Union (EU) project independently assessed the accuracy of a variety of the SARs used by OPPT for estimating the physical/chemical parameters, environmental fate, human health, and ecotoxicity of industrial chemicals. This study compared the (blinded) U.S. predictions with the limited base set of test data received by the EU for their new chemicals (16, 22). (For example, only acute toxicity data for fish and daphnids are required as the ecotoxicity base set by the EU at the time of this study). In this "Structure Activity Relationship/Minimum Premarketing Dataset" (SAR/MPD) study, the European Union experts concluded that the EPA/OPPT ecotoxicity (Q)SAR methodologies "performed extremely well in predicting acute toxicity to fish and Daphnia" (16, 30).

Significant attempts have been made to make these OPPT ecotoxicity screening methods available to the public. The 1988 version of the OPPT (Q)SAR Manual (4) was widely distributed, both nationally and internationally. It has been updated and currently contains about 120 OPPT SAR/QSARs available for assessing the ecotoxicity of about 45 classes of chemicals (23). A computer program was also developed that incorporates the revised OPPT (Q)SAR Manual and it was recently released as a PC Version, called ECOSAR. ECOSAR is publicly available (24), has been widely demonstrated and distributed, e.g., in national and international fora, such as at trade association meetings, scientific meetings, and public meetings.

RECEPTOR AND MECHANISM-BASED ASSAYS AND SAR

Knowing the mechanism(s) by which a chemical impacts specific receptors of organisms and thereby causing adverse effects is a highly desirable scientific goal. In human health risk assessments, extrapolations of toxic effects are made from several species to one species. In ecological risk assessment, on the other hand, extrapolations must be made from data on a few species to many thousands of species, and from individuals to populations. Information on mechanisms of action of new chemicals (e.g., inhibition of the enzyme AChE essential to nerve conduction), coupled with knowledge of the comparative physiology of various plant and animal classes would allow toxicity estimates to be made to a wide variety of species without the need for empirical testing.

However, such mechanistic approaches are not currently feasible as the information on which to base them is lacking. Moreover, the technical expertise required to make such comparative physiological-based toxicology interpretations is scarce. This can prove especially difficult when it is necessary to rapidly screen and assess very large numbers of chemicals that also have widely different structures. Pragmatically speaking, that is why the development and use of chemical class specific (Q)SARs have been such a priority for OPPT in its need to routinely assess the ecotoxicity of the thousands of industrial chemicals reviewed each year from industries.

INTEGRATED SCREENING AND TESTING STRATEGIES

A comprehensive evaluation of an industrial chemical would require not simply (Q)SAR estimations of ecotoxicity, but also data from acute,

subchronic, and chronic toxicity testing in a variety of appropriate environmental species (or their lab surrogates). Where feasible, microcosm and mesocosm studies should be performed and, when significant exposures are anticipated, even field testing should be considered.

The types and utility of several diverse ecotoxicity testing methods that are readily available to assess industrial chemicals have been determined for aquatic and terrestrial environments and have already been implemented by OPPT (19, 26). OPPT has also developed a tier-testing strategy (19, 26, 31) that allows for a sequencing to move from the quick and simple ecotoxicity tests to the test methods that are more long-term in duration and, therefore, more expensive.

It should therefore be relatively simple to integrate new test methods and results into an overall screening and testing strategy for a comprehensive evaluation of an industrial chemical.

NEW DEVELOPMENTS

During the next decade several different types of new test technologies will certainly be developed. It is very important to keep in mind that there needs to be a reality check on the direct utility of such test technologies for ecological effects assessment, including their applications (e.g., relevance, cost, and exposure routes). Because we are, or will be, capable of performing specific tests does not make them useful or meaningful for the purpose of determining their ability to detect the significant effects of a chemical on organisms in the environment. The basic issue is whether a technology will help in determining if the chemical of concern can affect the mortality, growth, development, and/or reproduction of the populations of organisms that exist and interact in the environment.

Because ecotoxicity test data are becoming available for earthworms, OPPT has developed and is starting to use, a (Q)SAR for neutral organic industrial chemicals for these terrestrial species. However, the need for additional ecotoxicity data for other terrestrial and sediment-dwelling species is well documented. The places where such additional terrestrial (Q)SARs could be most useful are for plant and avian species. Also needed are (Q)SARs for sediment-dwelling species, such as burrowing worms and crustaceans. Mammalian SARs should also be broadened beyond the laboratory animal data to integrate information on carnivore and ruminant species, as well as information available on wild rodents. This will broaden the basis for the SAR and may confound the model used for human health risk assessment, but it will become much more helpful for ecological risk assessments. It may be that two mammalian SARs can be developed: one that utilizes all the data and one that uses a subset specifically directed toward making extrapolations for humans only. The EPA/OPPT (Q)SAR program can be used to help direct the current controversy about chemicals that are endocrine disrupters both in humans (e.g., reduced sperm counts) and wildlife (e.g., abnormal breeding behaviors of gulls) (8), and has increased our awareness that the types of adverse impacts that some believe have occurred for many years to several species in the Great Lakes may also be happening to humans.

CONCLUSION

One of the main reasons that society should care more about what happens to organisms in the environment is that these organisms serve as monitors of what chemicals are capable of doing to other living organisms, such as humans (10). It is very easy to think that significant impacts to nonhumans, which may mean nothing at all, will happen to us.

The screening tools and test methods that have been developed by OPPT and other researchers to assess ecotoxicity are reasonable and cost-effective. Society decides how much it is willing to spend to generate ecotoxicity data. The reasonableness to adequately assess the potential impacts of industrial chemicals should be based upon what quality of data science indicates can be reasonably expected and needed to derive a specific level of certainty around risk or safety assessments. Excellent ecotoxicity screening tools

already exist (e.g., (Q)SAR) and are being used by OPPT to screen and prioritize several thousands of the discrete organic existing chemicals on the TSCA inventory for their potential to persist, bioconcentrate, and be highly toxic to organisms in the aquatic environment (6, 28, 30, 31).

Similar screening methods are still needed for organisms in the terrestrial environment. However, it must be recognized that exposure scenarios in terrestrial environments are much more complex than those in aquatic systems and may not be amenable to incorporation into (Q)SAR models in a similar fashion. Furthermore, our knowledge of actual long-term ecosystem effects of chemicals in the environment will remain rudimentary unless well-designed monitoring studies can be put in place. This type of "adaptive management" would allow us to verify our predictions and alter our management strategies accordingly, while allowing chemicals to remain in commerce.

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