Society of Environmental Toxicology and Chemistry

Technical Issue Paper

Alternative Approaches to Aquatic Vertebrate Toxicity Tests

It is important to establish a safe concentration of all man-made or natural chemicals, regardless of use, in order to protect environmental health. With this goal, scientists have developed a tiered approach, which starts simply and develops into more sophisticated testing. Each level of the approach provides insight into the hazard that these chemical characteristics may pose to the environment, including the effects potentially toxic chemicals may have on biological organisms, especially at the population, community, ecosystem, and biosphere levels. The lowest tiers of testing are used to establish the acute or short-term potential hazard profile of chemicals to different aquatic organisms. Determining how the chemical can be safely used and released is essential to protect humans and ecosystems, including plants, insects, fish, amphibians, reptiles, and domesticated, companion, and wild birds and mammals.

Ecotoxicity Testing to Evaluate Potential Hazard and Inform Risk Assessment

While chemical management approaches may vary depending on the regulatory jurisdiction and the intended use of the chemical, most use data obtained from chemical toxicity assays, which measure how toxic a chemical compound is to the tested organism. Traditional toxicity tests, according to internationally accepted regulatory guidelines, are based on studies on model organisms. For example, in the field of aquatic ecotoxicology, researchers regularly use algae and aquatic plants, invertebrates, fish, and amphibians to evaluate the effects of chemicals and contaminants in water. Increasingly, society has placed pressure on legislative bodies, who have responded by enacting animal welfare legislation. Non-embryonic life stages of vertebrate species, such as fish and amphibians[1] are considered protected in certain geographic locations (e.g., Europe), which has made obtaining the necessary chemical hazard information for these organisms challenging. Subsequently, there is a need to identify alternative approaches for providing relevant and reliable data for environmental hazard and risk assessments of chemicals and waste.

Alternative Approaches to Animal Testing

Alternative approaches to animal testing are often referred to as the "3Rs":

- **R**educe the numbers of animals used in testing
- **R**efine any procedures to minimize pain, suffering, and distress
- **R**eplace the use of animals whenever possible

[1] Depending on the life-stage of the organism

Although the concept of the 3Rs was initially focused on animal tests with mammalian species for ethical reasons, there has been significant progress over the last two decades toward developing alternative approaches for non-mammalian classes of vertebrates used in ecotoxicity testing: fish, amphibians, and birds. To further ensure that any alternative approach is reliable, relevant, and most importantly gains regulatory acceptance, the 3Rs have been extended to include three additional "Rs" for a total of "6Rs," which encourage alternative approaches to be:

- **R**eproducible and Reliable
- **R**elevant
- **R**egulatory accepted

A variety of potential alternative approaches to animal testing in ecotoxicity studies exist. The approaches are generally described as in silico, in vitro, and in vivo test methods.

In silico approaches to obtaining toxicity data rely on mathematical or physiochemical structural models and computer simulations. Examples of in silico techniques include the use of quantitative structure–activity relationship (QSAR) models, read across, and scaling and extrapolation models.

Read-across and QSAR models are used to screen new and existing substances by comparing them to other chemicals with similar structures. The models compare characteristics such as persistence in the environment, chemical uptake, and bioaccumulation and toxicity in aquatic organisms, reducing the need for animal testing. Scaling and extrapolation models apply chemical toxicity data for one test species to another species, thereby reducing the need to test each species.

In vitro refers to studies performed with biological material such as cultured cells and not the whole living organism. For example, scientists are using cultured fish gill cell lines, to study the potential effects that chemicals may have on these cells since the primary interaction between chemicals and fish begins at the gill cell surface. It is then possible to extrapolate these effects to the whole organism without needing to test on fish.

In vivo studies are performed with living organisms, so the alternative approaches aim to use fewer animals, younger life stages, and shorter durations. For instance, embryos are not covered by animal protection legislations in some geographies. Where embryos are used as models for older life stages (juvenile fish, in particular), yet provide equivalent insight into toxicity, the use of fish embryos is considered an improvement to animal welfare. Therefore, in some regulatory jurisdictions they may be seen as replacements to fish tests but in others as refinements to traditional fish assays.

Applying Alternative Approaches for Second Set of 3Rs

For some regulatory purposes, the use of vertebrate organisms for environmental risk assessments has been banned; in other situations, the number of organisms tested has been dramatically reduced or the laboratory procedure refined; and, yet for others, the use of standard methods for fish, invertebrates, and plants are required for compliance testing and new chemical registration.

In addition to the ethical considerations, the development of animal alternatives can also reduce the cost of performing vertebrate ecotoxicity tests, and in some cases, provide better information that can improve environmental risk assessments.

Regulatory acceptance of the alternative approaches remains the major challenge, particularly with regards to assessing ecotoxicity and bioaccumulation of chemicals. Translating the results of an in vitro, in silico, or alternative in vivo method into the reporting endpoint(s) needed for regulation is critical, and models, data, and approaches to do this are an important and complementary piece of an alternative method. Scientists continue to promote the advancement of the 6Rs through appropriate stewardship and dialogue with regulators so that alternative approaches for generating high-quality ecotoxicity data for chemical management become the norm. In the interim, better access to existing toxicity data could lead to a substantial reduction in the number of animals used in regulatory ecotoxicity testing with cooperation and harmonization of testing at both national and international levels.

Resources

Alternative approaches to vertebrate ecotoxicity tests in the 21st century: A review of developments over the last 2 decades and current status. Environmental Toxicology and Chemistry 35: 2637–2646; DOI: (10.1002/etc.3603)

Acknowledgments

SETAC is grateful for the efforts and contributions of those involved in the publication of this document: Scott Belanger, Michelle Embry, Adam David Lillicrap and Teresa Norberg-King.

Copyright

When referring to this text, please use the following citation:

[SETAC] Society of Environmental Toxicology and Chemistry. 2019. Technical Issue Paper: Alternative Approaches to Aquatic Vertebrate Toxicity Tests. Pensacola (FL): SETAC. 3 pp.