

Incorporating the 3Rs into Regulatory Scientific Practices

Sherry Sterling and Amy Rispin

Abstract

The Office of Prevention, Pesticides, and Toxic Substances (OPPTS) of the US Environmental Protection Agency (EPA) grapples with testing issues on a daily basis. In this discussion, the current practices within OPPTS that relate to refining, reducing, and replacing (the 3Rs of) animal use are explained, based on the authors' experience. Pertinent background about EPA and OPPTS is first described, and then some broad opportunities for implementing the 3Rs are reviewed. Finally, information about how the programs in OPPTS are making progress with regard to the 3Rs is presented.

Key Words: animal welfare; endocrine disruptors; EPA; ICCVAM; pesticides; toxic substances; toxicity testing

US Environmental Protection Agency (EPA¹) and Its Programs

It all starts with EPA's "mission," which is to protect human health and the environment. The Office of Prevention, Pesticides, and Toxic Substances (OPPTS¹) is unique among the various offices in that the statutes administered in this office provide the authority to require testing of chemicals for toxicological effects. For the industrial chemicals, the Toxic Substances Control Act (TSCA 1976) requires the Agency to make a case that testing is needed. In contrast, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA 1947) is in practice a licensing authority for pesticides. This means that industry must fulfill data requirements depending on the anticipated use of the compound to register the compound for marketing.

The goal for toxicological testing under both programs is to develop credible scientific information for assessing

the hazards and risks of chemicals to humans, wildlife, and the environment in general. In other words, good science is the foundation of the programs; and a commitment to refine, reduce, or replace animal use in our test methods whenever possible is a part of that good science.

Broad Considerations with Regard to 3Rs Practice

Some of the best opportunities for making progress with the 3Rs may relate to the way test methods have developed over the years. Historically, EPA test method compendia have developed in a piecemeal fashion. New toxicity tests have been developed in response to toxic events in humans. Testing for adverse effects started with development of the acute oral toxicity test. Tests for the evaluation of cholinesterase inhibition, and separate tests for dermal irritation, dermal sensitization, and dermal acute toxicity, were developed and used as respective needs arose. Subchronic and chronic tests and certain specialty determinations like mutagenicity were also added to the list.

Although this form of test method development has served the agency well, there has been little attention to the development of testing strategies for toxicity testing. There are opportunities for employing the 3Rs here, including both the concepts of tiers and batteries of tests. More attention to combining endpoints from several different tests into a single protocol may open yet more opportunities for employing the 3Rs. For example, a project that has just commenced under the International Life Sciences Institute is intended to rethink the ways pesticide toxicity testing may be conducted. More emphasis may be put on whole-life examinations, metabolism, and toxicokinetics. This broad picture approach abounds with opportunities to promote good science while embracing the 3Rs.

Another broad opportunity for change has its roots in the way regulatory programs have developed. Each regulatory program has had its own testing requirements. Multiple protocols of the same general test were used by different offices within the same agency. Yet different protocols were used by different agencies within a country, and certainly there are protocol differences among countries. There have been major steps forward in recent times to decrease the redundancy and to harmonize methods across authorities. For example, Congress asked the National Institutes of Environmental Health Sciences to develop guidance for the validation and regulatory acceptance of test methods and later

Sherry Sterling, M.S., is Associate Director for Policy and Communication; and Amy Rispin, Ph.D., is a Senior Scientist at the Office of Prevention, Pesticides and Toxic Substances, US Environmental Protection Agency, Washington, D.C.

¹Abbreviations used in this presentation: ECVAM, European Centre for the Validation of Alternative Methods; EPA, US Environmental Protection Agency; ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods; LLNA, local lymph node assay; OECD, Organization for Economic Cooperation and Development; OPPTS, Office of Prevention, Pesticides, and Toxic Substances.

established a standing federal committee, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM¹) (ICCVAM 1993).

Scientific and regulatory agencies have joined forces to support ICCVAM, which facilitates the movement of new and revised test methods through the processes of validation to regulatory acceptance. A similar group, the European Centre for the Validation of Alternative Methods (ECVAM¹), serves the needs of the European Union. These efforts go far in developing test methods that are useful to multiple regulatory authorities, thereby eliminating multiple tests.

ICCVAM members recognized that Europe's ECVAM was assessing the validation status of new test methods. Because it seemed wasteful to re-review each method in great detail, ICCVAM adopted procedures for an abbreviated review process for some test methods that have been found to be acceptable by ECVAM. This approach should aid in gaining agreement on test methods in two different regions of the world and enhance acceptance of methods internationally through the Organization for Economic Cooperation and Development (OECD¹).

Two other ICCVAM activities deserve mention here. The first is the mouse local lymph node assay (LLNA¹), which scientists from industry developed and validated as a means of assessing skin allergy. The scientists worked with ICCVAM to prepare background materials for an assessment of the status of validation, a tedious but eventually worthwhile task. A peer review committee was assembled to review the materials. The group noted that the LLNA ably predicted responses in the traditional test of skin allergy in the guinea pig. In addition, they found that the LLNA was as good a predictor of human skin allergy as the guinea pig. In 1999, ICCVAM concluded that the LLNA could be a valid stand-alone test for skin allergy for most chemicals (ICCVAM 1999). Compared with the guinea pig maximization test, the LLNA may result in savings both in animals committed for testing and in cost and will decrease both animal distress and testing time.

Numerous US regulatory agencies and programs, including EPA, have embraced the LLNA. The test was submitted to the OECD for consideration. At a December 2000 ICCVAM-sponsored instructional workshop on the conduct and interpretation of the LLNA, consensus was achieved on a few remaining issues. The LLNA was essentially approved by the OECD national coordinators in June 2001 after only one cycle of review.

The second noteworthy ICCVAM activity is work on in vitro acute toxicity testing. With input from EPA, ICCVAM sponsored a October 2000 workshop on the status of in vitro systems to predict acute toxicity (ICCVAM 2001a,b). The participants thought that in vitro systems were not well enough developed at that time to enable prediction of in vivo toxicity; however, they noted that existing data supported the use of in vitro cytotoxicity as one of the predictors of the starting dose for in vivo acute toxicity testing. These findings could result in greater efficiencies in animal

use and also aid in generating sound estimates of acute toxicity. EPA staff will consider the recommendation to use in vitro systems to predict a starting dose for in vivo testing even though the method has not been fully validated. Thus, during 2002, an in vitro alternative may be used in acute testing practices.

OPPTS Regulatory Action in Response to the 3Rs

In 1998, EPA launched the high production volume testing challenge in which EPA invited industry to volunteer in making publicly available a baseline set of environmental and health effects data on approximately 2800 industrial chemicals (EPA 1998). Selection of the chemicals was based on their production—in excess of one million pounds per year. Data that would be made publicly available included screening information on acute and repeat dose toxicity, reproductive and developmental toxicity, and mutagenicity.

In developing this initiative, EPA took a number of important steps to reduce the number of animals that would be needed for testing, to reduce the pain and suffering of animals in testing, and to replace animal tests as quickly as possible. These initiatives include the following key steps:

1. The Agency asked industry to identify existing information on the chemicals, evaluate it for adequacy, and avoid unnecessary testing or retesting of chemicals.
2. The Agency reviewed existing test methods and selected those that used fewer animals and those that combined toxicity endpoints and formed new policy positions that further reduced animal usage. Depending on the data needed, animal use was reduced by 60 to 80% in comparison with not including these considerations.
3. The Agency encouraged industry to consider the formation of chemical classes, with selection of a few representatives for testing and extrapolation of results to other members of the class.
4. The program also delayed the start for any new testing on individual chemicals until November 2001 in anticipation of the validation of nonanimal test alternatives. The ICCVAM report on the use of in vitro methods to predict acute toxicity starting doses may affect this determination.

Another EPA program with 3Rs potential is the Endocrine Disruptor Screening Program. This program is required under the Food Quality Protection Act, in response to finding adverse effects on fish and birds in the environment and to concern with potential effects in humans. The new legislation required EPA to develop a program to screen chemicals that may disrupt the endocrine system (FQPA 1996). This area of regulatory endeavor is totally new.

Instead of dealing with all hormones, EPA limited its

attention to agents that may affect male and female sex hormones and thyroid hormone. By simply limiting the scope of this action, the EPA has reduced the number of tests needed for the screening program. To ensure stakeholder involvement in the process of validating test methods for screening endocrine disruptors, EPA has established a public advisory committee to gain broad input in the direction, design, and validation of methods, and in the screening system in general. The advice from this committee will not only help OPPTS build a scientifically sound approach to endocrine disruption testing but will also identify opportunities where the 3Rs can be implemented.

Concern about endocrine disruption has extended beyond EPA. As a result, test method assessment activities are under way at OECD, where there are ongoing validation efforts involving two *in vivo* tests—one for androgen and one for estrogen. In the United States, ICCVAM is reviewing the status of the *in vitro* tests for androgen and estrogen receptor binding. The hope is that this work will result in a concise and accurate set of screens for endocrine disruption that will be acceptable to regulators in many regions of the world.

EPA is also exploring ways of using existing information on registered pesticides to make preliminary judgments about potential endocrine disruption. The agency plans to test the hypothesis that subchronic, chronic, and reproductive toxicity studies along with carcinogenicity studies may provide important clues as to whether agents may induce potential disruption. The concept is that data from these studies may negate the need to carry out all the endocrine screens on chemicals. Instead, the agency may be able to go to more definitive assays or, hopefully in some cases, directly to risk assessment without the need to perform preliminary screens.

Other examples of how regulators are implementing the 3Rs come from the pesticides program in which most pesticidal products are mixtures of various ingredients. Formulas are frequently modified by industry, often simply by changing the proportion of ingredients or making other minor adjustments. EPA has devised a set of bridging rules to be used by industry in testing new mixtures that are substantially similar to already tested products. For instance, if a product is categorized in the most toxic hazard class and a new product has the toxic ingredient as a greater percentage of the total, the hazard of new material will also be in the most toxic class. Likewise, if the median lethal dose of a mixture is 500 mg/kg and the product is diluted in half with water, the new product would be assumed to have an LD₅₀ of 1000 mg/kg. The United States facilitated the acceptance of these bridging rules at the OECD as part of the Globally Harmonized System for Classification (OECD 2001). These techniques significantly limit the need for re-testing of mixtures that are similar to tested materials.

Instead of testing non-food use pesticides with a full spectrum of toxicity tests, EPA uses a tiered testing approach. Results from subchronic and developmental toxicity and mutagenicity studies are reviewed before decisions are made as to whether higher tiered testing is required. Be-

cause the higher tiered tests may require significant numbers of animals, as in chronic toxicity and cancer studies, this EPA policy significantly limits the amount of testing while maintaining the ability to identify and test for certain health hazards when needed.

Finally, a new effort is under way throughout OPPTS in response to the fact that at any one time there may be scores of ongoing test method activities. To help integrate and coordinate the number of test method activities within OPPTS, and with other EPA programs, OPPTS has set up a Test Methods Group. The goal of the Test Methods Group is to facilitate interaction, coordination, and movement of test methods into the testing guidelines used by OPPTS. The Test Methods Group will establish test guidelines and strategies that generate credible scientific information for conducting risk assessments. The Group will also stay abreast of new technologies, like genomics, that may become potent test method directions in the future.

Conclusion

This overview of current practices of OPPTS to implement the 3Rs confirms that progress has been made in this area. Nevertheless, there is room for even more work to refine, reduce, or replace animals in testing further. There are many challenges ahead for EPA, and OPPTS in particular, in this area. To meet these challenges, scientists and policy makers must continue to work together.

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