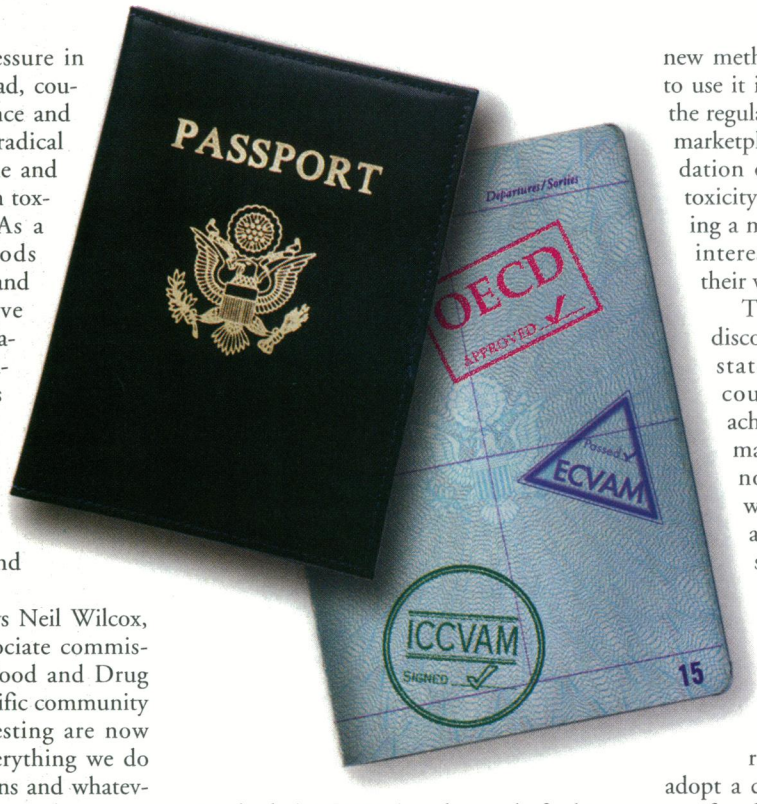
GLOBAL AGREEMENT ON ALTERNATIVE TESTING



In recent years, public pressure in the United States and abroad, coupled with advances in science and technology, have led to a radical reevaluation of the longtime and widespread use of animals in toxicological experiments. As a result, new testing methods designed to refine, reduce, and replace animal models have emerged in government, academic, and industry laboratories worldwide. But this sudden shift has left both researchers and regulators scrambling to determine when the results from alternative test methods are valid and acceptable and when they are not.

"It's a small world," says Neil Wilcox, special assistant to the associate commissioner for science at the Food and Drug Administration. "The scientific community and changes in toxicity testing are now international in scope. Everything we do has international implications and whatever we do impacts the international community. Harmonization will reduce the wasteful duplication of efforts by standardizing testing."

The search for a set of international standards began in earnest five years ago when the European Union (EU) created the European Center for the Validation of Alternative Methods (ECVAM). ECVAM's principal role is to coordinate the validation of alternative methods among the EU's 15 member states. As early as 1987, the global Organization for Economic Cooperation and Development (OECD) began to consider animal welfare issues in its international guidelines for the testing of chemicals. And in 1994 in the United States, the NIEHS established the Interagency Coordinating Committee of Criteria on the Validation of Alternative Methods (ICCVAM) with a goal of achieving domestic and international harmonization of criteria for the validation and acceptance of such methods. Today, the three groups are working together to bring scientific laboratories and regulatory agencies into agreement on alternative test guidelines.

"The challenge is to come up with useful criteria for scientists in any country who are developing new methods," says William Stokes, associate director for animal and alternative resources at the NIEHS and cochair of ICCVAM. "They must meet criteria that would be the same internationally. It wouldn't make sense for one country to consider a test method valid while another didn't. Each new method must be reliable, relevant, and reproducible in different laboratories. If you generate a new method, a company will be reluctant to use it if the data won't be acceptable to the regulatory agencies in the international marketplace. In the development and validation of alternative methods for acute toxicity, the Europeans have been pursuing a more focused effort and we're very interested in following the results of their work."

The EU issued a directive in 1986 discouraging scientists in its member states from using animals if they could reasonably and practicably achieve the same result without animals. "EU legislation requires that nonanimal test methods be used whenever possible, and that efforts are made to develop and validate such methods," says Michael Balls, head of ECVAM. "The main obstacles are scientific and administrative. [For example], the development and validation of relevant and reliable alternative methods is difficult [and] regulatory authorities tend to adopt a conservative stance and feel more comfortable with animal test data."

Because well-established animal tests have set the standard for decades, successfully replacing them will be a challenge. Alternative methods may feature the use of microbes, cells, tissues, and other *in vitro* methods, or rely instead on computer databases and mathematical models. Other new methods may involve use of genetically engineered animals or lower species. Revised methods may include modifications that require fewer animals or minimize or eliminate animal pain and distress. If international harmonization is achieved, each interested country's laboratories and regulatory agencies will accept the results of new or revised tests, including nonanimal tests.

For instance, as companies expand into overseas markets, their products must meet

the regulatory guidelines of each country to which they want to export. At the moment, many countries have their own guidelines, which may differ from those of other countries. With this *ad hoc* process, companies risk the loss of potential markets or the heavy expense of additional tests. For example, nonanimal test results of a product that might be acceptable in one country might be rejected in another for the same reason: because the test methods did not include animals.

"Harmonization helps us significantly," says Katherine Stitzel, associate director of the human safety department at Procter & Gamble. "In a global company that's working across borders, it saves us animals, time, and money. It's a drag on our resources to have to do things differently for different countries. It will make things so much easier when everyone is in agreement."

An International Platform

Founded in 1960 to stimulate economic progress and world trade and promote social welfare, OECD membership now includes 26 countries including most European countries, as well as the United States, Mexico, Canada, Australia, New Zealand, and Japan. With its global reach, the OECD has become a major player in the quest for international harmonization.

"The OECD is a unique forum for countries with advanced market economies," says Herman Koëter, principal administrator at the OECD's environmental health and safety division based in Paris. "Until the early 1990s, our role in alternative test methods was to accept applications and distribute them to our members for comments and approval. But we gradually began to realize that approaches to validation were not exactly the same in Europe and elsewhere. So in 1991, OECD decided to take a more active role in encouraging nonanimal testing by bringing members together to negotiate, discuss, and arrive at a consensus."

In 1987 and in 1992, the OECD issued guidelines for skin and eye testing that examined for the first time the possibility of using alternative, nonanimal methods for testing chemicals. At the time, however, the guidelines acknowledged that many tests were not ready to be conducted solely *in vitro*, especially those that assess the potential for skin sensitization. Accurate results, the guidelines said, could still only be achieved through standard *in vivo* testing.

In January 1996, the OECD organized a workshop in Stockholm, Sweden, on toxicological test alternatives to discuss international harmonization of validation and regulatory acceptance criteria for alternative tests. The ICCVAM draft report, ECVAM documents, and documents from other animal welfare centers were used as the basis for discussion. The draft ICCVAM report was developed by 15 U.S. federal scientific and regulatory agencies. At the workshop, participants agreed on test strategies for skin and eye tests "where schemes could be made mandatory," says Koëter. "When we discussed different approaches member-country-wide, there were no principal differences. Science and safety remain the key elements."

"One of the recommendations from the workshop was that developers of new methods should contact the appropriate regulatory agencies during the development and validation stages in order to optimize the usefulness of test methods," says Stokes. "This way, they can make changes in their method early on. You need a phase where you optimize the test protocol prior to formal validation studies."

Another workshop recommendation was to allow the use of patented methods, according to Stokes. Patented methods "may be necessary to stimulate creativity and innovation," he says. Currently, OECD guidelines do not permit patented methods because some countries may not be able to run the tests if such patented expertise and materials are difficult to acquire. In addition, Koëter said, adopting a patented test from one company as an OECD guideline may put the OECD in the position of providing that company with an unfair advantage over its competitors.

The OECD will present the workshop recommendations to the policy representatives or national coordinators of each of its member countries. The formal adoption of new methods, Koëter says, may take months because each proposed method will face legal, policy, and economic implications that must be addressed by each country's governing body. In order to be approved as an OECD guideline, each proposal must be unanimously approved by its member states.

After two years of discussions and negotiations, the OECD member states approved an alternative test method as an OECD guideline at the beginning of this year. Called the acute toxic class method, this alternative approach allows for a significant reduction in the number of animals required in acute oral toxicity tests. The test allows for the use of as few as three animals—"a fraction of the original amount," says Koëter. He also notes, "Sometimes it's easier to modify an existing guideline by adding new text in line with animal welfare. For example, an *in vitro* test could be recommended or even required before an animal test can be performed."

Modifying standard animal tests has opened many new questions about what constitutes an appropriate replacement. "While some *in vitro* methods appear to be useful for specific chemical classes, they are not generally useful for all chemicals," says Stokes. "You have to validate a method for a specific purpose, which is often more readily done in a stepwise fashion."

"Regarding the use of rodent models," adds Wilcox, "it's not always clear where it is adequate in different chemical classes. For example, in some classes, a rat might give 80% concordance or accuracy. But what do you do for the other 20%? Do you use a mouse model? Or should you use alternatives, such as a transgenic model? We still don't know if it is possible to take one rodent species and supplement it with an alternative. It's a very complex scenario, no question about it."

Harmony in Europe

Independent and neutral, ECVAM is part of the European Union Joint Research Center's Environment Institute in Ispra, Italy. The center's scientific advisory committee includes representatives from the member states of the EU as well as from the chemical, cosmetic, and pharmaceutical industries and from animal welfare groups. With no single EU-wide regulatory agency, harmonization must be approached industry by industry and country by country. But when agreements are reached in the form of EU directives, they are binding on all the member states, which must adapt their national legislation to comply with the directive's provisions.

Within the EU, there is "a communal commitment to the replacement of animal tests as validated nonanimal tests and testing strategies become available," says Balls. "However, the strength of feeling on this issue varies from country to country, as would be expected. Germany, the Netherlands, Sweden, and the UK are particularly committed to the reduction, refinement, and replacement of animal testing."

While ECVAM's principal role is to coordinate the validation of alternative methods at the EU level, its representatives are active in all worldwide discussions on harmonization. However, with 15 members, the EU is also in a position to make international progress on its own. In general, this could lead to tensions, says Balls, but as far as alternative methods are con-

cerned, "the existing good relations between partners such as ECVAM, ICCVAM, and the OECD should help to avoid that problem. There may be minor differences, but only because ECVAM's duty is to serve the European Commission and the EU, whereas ICCVAM's primary focus is the U.S. regulatory [and research] agencies."

Harmony in the United States

Similar in concept to ECVAM, ICCVAM also recommends processes acceptable to 15 U.S. scientific and regulatory agencies. Mandated by the National Institutes of Health Revitalization Act of 1993, ICCVAM consists of *ad hoc* representatives from those agencies. Though its principal mandate did not include international harmonization, ICCVAM approached the OECD to consider its criteria for validation and acceptance of new methods, knowing that all testing methods approved in the United States should also meet international criteria for approval.

"ICCVAM agreed OECD should be involved from the start to make it easier to get acceptance internationally," says Koëter. "Before finalizing its report, ICCVAM wanted OECD to take a look at it, not as a bible, but as a basis for discussion. As we determined at the Stockholm workshop there were no essential differences, OECD will now develop a document close to ICCVAM's. When it came to a way of thinking, there was widespread acceptance of alternative tests."

"We're in the business of developing and validating methods that would be more useful for predicting environmental health hazards," says Stokes. "As we learn more at the molecular and cellular levels about the mechanisms by which chemicals cause toxicity, we can transform our understanding into a mechanistic test method. As we develop better test methods, the hope is that we can better predict the toxicity of that chemical before it gets into the environment, the workplace, or the food supply, and before it can cause adverse effects on human health."

It is generally agreed that the process of harmonization will be lengthy, requiring ongoing meetings and workshops. It may take years to establish a universal set of guidelines agreed upon by scientists and regulators. But the results should dramatically reduce the need for animals in toxicological experiments while ensuring the validity and acceptance of such tests worldwide.

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