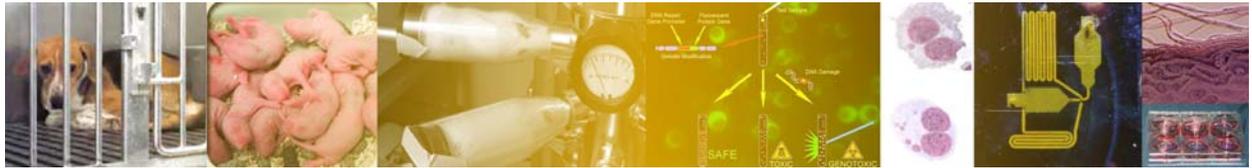

Regulatory Testing: *Why is the U.S. So Far Behind Europe?*



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Introduction

Tens of millions of animals are killed annually throughout the world in the name of regulatory testing.¹ Regulatory testing is performed to allow chemicals (industrial chemicals, pesticides, and pharmaceuticals) to be sold and traded within the U.S. and internationally. The stated goal of this testing is to provide some measure of the toxicity to humans that may result from exposure to a given chemical.

However, the overwhelming majority of toxicity testing is based on science that is decades old and has no relevance to public or environmental health protection.

Awareness of this problem has been increasing for decades, and is now widely recognized by scientists, regulators, and other stakeholders including the animal protection community. Recent highly publicized debacles have emphasized the inadequacies of current regulatory testing paradigms, particularly with respect to pharmaceuticals.²

The overwhelming majority of toxicity testing is based on science that is decades old and has no relevance to public or environmental health protection.

Retrospective analyses also suggest a general failure of the current paradigm: a striking example of this is the fact that 94% of all cancer drugs that have been thoroughly tested for efficacy and toxicity in animals fail in clinical trials due to toxicity or lack of therapeutic value in humans (Okie, 2006).

In Europe, some progress has been made in overhauling both regulatory policies and testing methods to take advantage of scientific advances that make regulatory testing more relevant. The trend toward the elimination of animals in regulatory testing and other kinds of experimentation began over 20 years ago in Europe as a social commitment to the principle of the "3Rs": the *reduction* and *replacement* of animals used for experimentation, and *refinement* of animal tests to cause less suffering. This principle was first outlined in 1959 (Russell and Burch, 1959), and was written into legislative policy in Europe in 1986 (see below, *Council Directive 86/609/EEC*).

¹ In the U.S. alone, it is estimated that more than 15 million animals are used annually in regulatory toxicity testing. Because the animals most commonly used in these tests – namely birds, rats, and mice – are excluded from the minimal protections of the U.S. Department of Agriculture's Animal Welfare Act (AWA), no records are required to be kept on their numbers.

² One example is the unanticipated risk of cardiovascular complications associated with the arthritis medication Vioxx (<http://www.drugs.com/vioxx.html>) even though the drug was shown to be heart protective in six different animal species. Another example is the dramatic multi-organ failure experienced by volunteers in clinical trials of TG1412 (http://news.bbc.co.uk/2/hi/uk_news/england/london/4807042.stm).

Introduction (cont'd)

While the U.S. leads the world in cutting-edge science, including the non-animal science that could be applied to regulatory testing, the regulatory situation in the U.S. has been stagnant for decades. A major cause of this discrepancy is a lack of legislative initiative.

world in cutting-edge science, including the non-animal science that could be applied to regulatory testing, the regulatory situation in the U.S. has been stagnant for decades. A major cause of this discrepancy is a lack of legislative initiative.

To gain a perspective on this problem, a comparison between legislation in Europe and the U.S. is provided below.

The National Research Council just released a lengthy report, entitled “Toxicity Testing in the Twenty-first Century: A Vision and a Strategy,” that recommends a shift away from the current animal tests toward more modern approaches that are largely based on non-animal methods.

on non-animal methods (NRC, 2007). In its report, the NRC recognizes that “A revolution is taking place in biology,” and now is the time to capitalize on that revolution.

Interestingly, as “alternative” or non-animal-based methods were developed, scientists also realized that these methods can be more sensitive, reproducible, and more relevant to predicting human health effects than the standard animal methods. Consequently, as a result of its active implementation of the 3Rs, Europe has been making noticeable progress in the realm of effective regulatory testing.

However, during this same time period, the U.S. has failed to make any substantive changes to its regulatory testing policies.

The result is that, while the U.S. leads the

This issue is timely because two pieces of European legislation - Cosmetics Directive (7th Amendment) and REACH (see below) - that involve regulatory testing are coming into effect over the next five years; both will have a great impact on regulatory testing involved in international trade.

At the same time, the U.S. National Research Council (NRC) has just released a lengthy report, entitled “Toxicity Testing in the Twenty-first Century: A Vision and a Strategy,” that recommends a shift away from the current animal tests toward more modern approaches that are largely based

1. The Situation in Europe

Legislation

Council Directive 86/609/EEC

In Europe, there are legislative mandates covering the replacement of animals dating back as far as 1986. Council Directive 86/609/EEC on the approximation of the laws, regulation, and administrative provisions of the member States regarding the protection of animals used for experimental and other scientific purposes, contains two articles promoting the replacement of animals with non-animal methods (EC, 1986):

- Article 7.2: "An experiment shall not be performed if another scientifically satisfactory method of obtaining the results sought, not entailing the use of an animal is reasonable and practicably available" and
- Article 23: "The Commission and Member States should encourage research into the development and validation of alternative techniques which could provide the same level of information as that obtained in experiments using animals, but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field."

A direct result of this legislation was the creation of the European Centre for the Validation of Alternative Methods (ECVAM) that is responsible for the development and validation of non-animal testing methods (see below, *The European Center for the Validation of Alternative Methods*).

Cosmetics Directive 76/768/EEC 6th and 7th Amendments

Europe has long sought to reduce, and eventually eliminate, the use of animals in cosmetics testing. In order to facilitate the development and use of alternative methods, specific provisions were introduced in 1993 by the 6th Amendment of the Cosmetics Directive. The 6th Amendment introduced a clause prohibiting the marketing of cosmetic products containing ingredients or combinations of ingredients tested on animals beginning in 1998 (EC, 1998). This date was subsequently postponed twice until its deadlines were superseded by the 7th Amendment, which became law in 2003.

The 7th Amendment to the Cosmetics Directive, issued in 2003, contained much stronger provisions for replacement of animals in cosmetics testing (EC, 2003). The result of this amendment is a complete ban on animal testing of finished cosmetic products, and a phased-in ban on animal testing of cosmetic ingredients in the European Union (E.U.).

1. The Situation in Europe (cont'd)

This Amendment also banned the marketing of cosmetic products and ingredients tested using animals in the E.U. from other countries. In 2009, a testing ban goes into effect for the majority of tests irrespective of the availability of non-animal tests; in 2013, the remaining tests are scheduled to be banned (with the possibility that this deadline may be postponed). This legislative initiative has translated into an enormous economic incentive for industry to develop non-animal methods for common cosmetic tests such as skin and eye irritation and corrosion, and skin sensitization.

Registration, Evaluation, and Authorization and Restriction of Chemicals (REACH)

The REACH legislation, adopted by the European Parliament in December 2006, requires the registration of all chemicals produced or sold in Europe in quantities in excess of one ton per year (both old and new chemicals, totaling about 30,000 chemicals) (EC, 2006; DG Environment, 2006). Registration includes recording safety data for these chemicals; eventually data gaps will need to be filled, potentially requiring a vast array of animal tests. From an animal welfare standpoint, this legislation is devastating; however, it has had some beneficial repercussions. First, REACH requires data sharing by industry and the assignment of companies that manufacture similar chemicals into consortia to minimize redundant testing. Second, the legislation mandates that the European agency responsible for the administration of the program, and the European Commission itself, explore options and develop strategies for the use of alternative methods. The legislation makes reduction of animal testing a “key consideration” in the development of guidance on the implementation of the law (Recital 47). Finally, for manufacturers, animal testing on the scale proposed by REACH is prohibitively expensive and time consuming.

From an animal welfare standpoint, this legislation is devastating; however, it has had some beneficial repercussions.

The result has been to create an economic incentive for industry to develop cheaper, faster non-animal methods. In addition, the REACH legislation has provided momentum for ECVAM and others to develop a broader range of *in vitro* methods than those required by the cosmetics industry (i.e., for developmental and reproductive toxicity).

1. The Situation in Europe (cont'd)

The Sixth Framework Program for Research and Technological Development (FP6)

The FP6 (2002- 2012) is a collection of activities at the E.U. level to fund and promote research to serve two main strategic objectives: strengthening the scientific and technological bases of industry and encouraging its international competitiveness, while promoting research activities in support of other E.U. policies (DG Research, 2007). Through this program, several initiatives have been undertaken whose goal is the development and regulatory acceptance of non-animal test methods. For example:

- ReProTect (E.U., 2006) is an assembly of 35 different European partners from academia, governmental institutions, and industries focused on developing a testing strategy for reproductive/developmental toxicity testing that involves the development and validation of non-animal methods.
- The ACuteTox (E.U., 2005) project has the overall objective of developing an *in vitro* test strategy that can replace *in vivo* testing of acute toxicity of chemicals (i.e., lethal dose testing in which groups of animals are force-fed different concentrations of chemical until the concentration at which half of the animals die is determined).

Government Organizations

The Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET)

In response to Directive 86/609 EEC, ZEBET was established by the German government in 1989, as part of the German Federal Institute of Risk Assessment (BfR) (BfR, 2003). The goal of this scientific institution is to bring about the replacement of legally prescribed animal experiments with alternative test methods, to minimize the number of animals used, and to alleviate the pain and suffering of animals used in experiments.

All toxicology at the BfR will...concentrate on in vitro methods. Almost all animal experiments will therefore stop at the BfR.

ZEBET is responsible for assessing and promulgating regulatory acceptance of non-animal methods and also maintains a database of alternative methods. Currently, ZEBET has a staff of 12, including six scientists and four technicians. ZEBET's annual budget for funding research projects in Germany is 370,000 €.

1. The Situation in Europe (cont'd)

ZEBET is currently expanding. The BfR is establishing a new department entitled "Experimental Toxicology – ZEBET." All toxicology at the BfR will be conducted within this unit and it will concentrate on *in vitro* methods. Almost all animal experiments will therefore stop at the BfR. The new department will include the "National Reference Laboratory for Alternative Methods," which will provide additional funding for in-house research (Spielman, 2007).

The European Centre for the Validation of Alternative Methods (ECVAM)

Also as a direct result of Directive 86/609 EEC, ECVAM was created by a Communication from the Commission to the Council and the Parliament in October 1991 (JRC, 2007). ECVAM was established in 1992 as a unit of the Environment Institute, part of the Joint Research Centre (JRC).

As defined in the Communication of the European Commission to Council and the European Parliament in October 1991, the duties of ECVAM are:

- To coordinate the validation of alternative test methods at the E.U. level.
- To act as a focal point for the exchange of information on the development of alternative test methods.
- To set up, maintain, and manage a database on alternative procedures.
- To promote dialog between legislators, industries, biomedical scientists, consumer organizations, and animal welfare groups, with a view to the development, validation, and international recognition of alternative test methods.

Moreover, ECVAM seeks to promote the scientific and regulatory acceptance of alternative methods. ECVAM has its own Scientific Advisory Committee (ESAC), with participation from all E.U. member states and relevant stakeholders including industry, academia, and animal welfare associations.

ECVAM is currently organized into priority areas determined by the specific animal tests to be replaced and based on expected needs due to the Cosmetics Directive and REACH.

ECVAM receives approximately 50 million € per year...has 60 staff members, roughly half of whom work directly in laboratories.

Working groups have been established in 15 key areas, including: systemic toxicity, topical toxicity sensitization, carcinogenicity, reproductive toxicity, ecotoxicity, biologicals, nanotoxicology, computational toxicology, and a database initiative on alternative methods.

1. The Situation in Europe (cont'd)

ECVAM receives approximately 25 million € per year from the E.U. Directorate General on Research (ICCVAM, 2006). ECVAM is a division of the EC's Joint Research Council's Institute for Health and Consumer Protection (IHCP) and is housed in IHCP facilities in Ispra, Italy. It has 60 staff members, roughly half of whom work directly in laboratories. ECVAM is currently involved in the evaluation of 170 methods (Hartung, 2007). Last year, the Commission decided to create a reference laboratory within ECVAM, which will strengthen ECVAM's active contribution to validation of *in vitro* methods.

National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)

In 2004, the U.K. parliament established the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs; NC3Rs, 2007). NC3Rs provides funding for research that focuses on refinement, reduction, or replacement of animals in experiments, provides education and outreach to legislators and others about progress in these developments, and works to gain regulatory acceptance of alternative methods. NC3Rs received about £800,000 from the government and £150,000 from industry for specific projects in the 2005-2006 fiscal year. The U.K. government just announced plans to award NC3Rs £2.4 million for 11 new projects. NC3Rs plans to award approximately £1.5 million in extramural grants in 2007.

Fund for the Replacement of Animals in Medical Experiments (FRAME)

Although not an official governmental institution, FRAME, which is currently associated with the University of Nottingham, has over the years received much funding from the U.K. government and has become an integral part of alternative method development in Europe. FRAME's research is based on the 3Rs principles and its initial focus was to raise awareness and raise funds for the development of alternative methods. The first director of ECVAM, Dr. Michael Balls, had been the head of FRAME for more than 10 years before moving to ECVAM. In 2000, three replacement alternatives were accepted by E.U. regulators. One of these, a phototoxicity test, had been validated in a 1997 study in which the FRAME Laboratory was a participant (FRAME, 2006). FRAME continues to develop alternative methods and participate actively in validation studies.

2. The Situation in the U.S.

Legislation

The NIH Revitalization Act of 1993

The first piece of legislation in the U.S. covering the development of alternative methods is the National Institutes of Health (NIH) Revitalization Act of 1993 (Public Law 103-43).

This Act does not mandate the replacement of animal methods with non-animal methods when those are available, nor does it provide the impetus for an organized commitment to the development of non-animal methods.

This act required the National Institute of Environmental Health Sciences (NIEHS) to establish criteria for the validation and regulatory acceptance of non-animal toxicological testing methods and to recommend a process to achieve the regulatory acceptance of scientifically valid alternative test methods.

Specifically, the Act authorized the NIH to conduct or support research into:

- methods of biomedical research and experimentation that do not require the use of animals;
- methods of such research and experimentation that reduce the number of animals used in such research;
- methods of such research and experimentation that produce less pain and distress in the animals used; and
- methods of such research and experimentation that involve the use of marine life (other than marine mammals).

The Act also stipulated that the NIH should prepare a plan to:

- establish the validity and reliability of the method(s) described above;
- encourage the acceptance by the scientific community of such methods that have been found to be valid and reliable; and
- train scientists in the use of methods that have been found to be valid and reliable.

2. The Situation in the U.S. (cont'd)

This Act is significantly different from European legislation. It does not mandate the replacement of animal methods with non-animal methods when those are available, nor does it provide the impetus for an organized commitment to the development of non-animal methods.

The ICCVAM Authorization Act of 2000 (Public Law 106-545)

This Act authorized the Director of the National Institute of Environmental Health Sciences to establish the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) as a permanent committee under the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) (ICCVAM, 2003). The Act instructs ICCVAM to carry out the following functions:

- Coordinate the technical review and evaluation of new and revised test methods
- Submit ICCVAM test recommendations to appropriate U.S. Federal agencies
- Facilitate interagency and international harmonization of test protocols that encourage the reduction, refinement, and replacement of animal test methods
- Facilitate and provide guidance on validation criteria and processes
- Facilitate the acceptance of scientifically valid test methods
- Facilitate awareness of accepted test methods
- Consider petitions from the public for review and evaluation of new and revised test methods for which there is evidence of scientific validity
- Make ICCVAM final test recommendations available to the public
- Prepare reports on ICCVAM progress and accomplishments under the Act and make these available to the public

...ICCVAM is...composed of representatives from 15 government agencies, with no provision of infrastructure with which to carry out research or validation studies itself.

It is important to note here that ICCVAM is a "coordinating committee," composed of representatives from 15 government agencies³ with no provision of infrastructure with which to carry out research or validation studies itself.

³ The agencies are: Agency for Toxic Substances and Disease Registry, Consumer Product Safety Commission, Department of Defense, Department of Energy, Department of the Interior, Department of Transportation, Environmental Protection Agency, Food and Drug Administration, National Cancer Institute, National Institute for Environmental and Health Sciences, National Institutes of Health, National Institute for Occupational Health and Safety, National Library of Medicine, Occupational Safety and Health Administration, U.S. Department of Agriculture.

2. The Situation in the U.S. (cont'd)

Government Organizations

The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285) established ICCVAM – originally created in 1997 to aid in the government-wide adoption of non-animal testing methods – as a permanent interagency committee of the NIEHS under NICEATM, which is located in Research Triangle Park, N.C. Along with the mandate to ensure that any new or revised toxicity testing methods – both animal and non-animal – were scientifically validated for use, Congress clearly believed that an overarching government entity to meet the demands of Federal regulators was crucial in order to reduce the number of animals used by industry.

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) provides advice to ICCVAM and NICEATM on priorities and activities related to the development, validation, scientific review, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods.

ICCVAM is composed of representatives from 15 U.S. Federal regulatory and research agencies that generate, use, or provide information from toxicity test methods for risk assessment purposes. NICEATM and ICCVAM depend upon the Federal agencies for development and support of all ICCVAM activities.

National Center for Computational Toxicology (NCCT) – US EPA

The NCCT is a part of EPA's Office of Research and Development (ORD) and coordinates and implements EPA's research in the field of computational toxicology. NCCT scientists serve as scientific reviewers and advisors by providing technical assistance to other Laboratories and Centers within ORD, to EPA Program Offices and Regions, and to the states. The stated objectives of the computational toxicology initiative are to improve understanding of the adverse biological consequences from environmental exposure to toxic chemicals, provide predictive models for screening and testing, and improve quantitative risk assessment. The EPA's FY 2007 budget for research in computational toxicology in the program area of human health and ecosystems is approximately \$15 million.

2. The Situation in the U.S. (cont'd)

The EPA has created the ToxCast™ program to evaluate *in vitro* methods for the purpose of prioritizing chemicals for toxicity testing. Currently, the EPA has allocated approximately \$6 million of its research budget for Phase I testing, in which over 300 chemicals are being evaluated in various versions of several different non-animal tests.

NTP High Throughput Screening Initiative

In August 2005, the NTP began a formal collaboration with the NIH Chemical Genomics Center (NCGC) to test substances of interest to the NTP across a spectrum of HTS assays. NTP has supplied six cell-based assays and 1408 test chemicals. The program is currently testing 14 concentrations of each compound in each of the assays using a variety of human and non-human cells. The NTP HTS program is also collaborating with the EPA's Chemical Prioritization Community of Practice, a consulting group that advises ToxCast™ (Tice, 2007). In March, 2008, a Memorandum of Understanding was issued formalizing these collaborations.⁴

Currently, the HTS initiative is not part of a formal branch of NTP; however, the current reorganization of NTP includes the creation of a Biomolecular Screening Branch that will have a budget and permanent staff (Bucher, 2007).

The Status of Testing in the U.S.

The U.S has not been implementing available non-animal tests and technology to reduce and eliminate tests on animals, as Congress clearly intended by the ICCVAM Authorization Act.

The U.S. is not implementing available non-animal tests and technology to reduce and eliminate tests on animals, as Congress instructed.

ECVAM and ICCVAM are similar in acronym only and each reflects its respective government's commitment to the development of alternative methods. ECVAM is an integrated part of a large research institution, with scientific staff, laboratories, and significant government funding for carrying out basic validation and translational research. ECVAM is integrated with other European government initiatives

to develop alternative methods (e.g., ZEBET, ReProTect, ACuteTox). By contrast, the members of ICCVAM are appointed to the Committee by their respective agencies using unknown criteria.

⁴ National Institutes of Health, National Human Genome Research Institute, Newsroom:
<http://www.genome.gov/Pages/Newsroom/CurrentNewsReleases/ntpncgcepamou121307FinalV2.pdf>

2. The Situation in the U.S. (cont'd)

It has been the experience of the U.S. animal protection community over the past decade that ICCVAM appointees are either apathetic to the issue of non-animal test development or actively antagonistic toward it.

It has been the experience of the U.S. animal protection community over the past decade that ICCVAM appointees are either apathetic to the issue of non-animal test development or actively antagonistic toward it. The non-profit Physicians Committee for Responsible Medicine (PCRM) filed a Freedom of Information Act lawsuit in an attempt to obtain documents revealing qualifications of agency

representatives and the manner in which they are chosen. After losing a court battle, NIEHS has provided some documents, but none relevant to these issues.

With its nominal budget, ICCVAM must hire outside “experts” for validation studies, and is dependent on outsiders to nominate methods for consideration (which it then often ignores). Consequently, ICCVAM’s review panels frequently include academicians with no regulatory or practical experience in the application of the science and who impede the validation process.

Even though ICCVAM is responsible for “encourag[ing] the use” of validated methods, ICCVAM has taken years to make recommendations after the reviews are completed, and has no authority to ensure that the recommended methods are used by agencies or by industry.

In addition to the obvious differences in political commitment to the 3Rs between the U.S. and Europe, ICCVAM is clearly not capitalizing on the limited capabilities it does have. For example, one of ICCVAM’s tasks is to facilitate harmonization of international regulatory test methods. Yet, of the over two dozen methods that have been accepted for regulatory purposes in Europe, ICCVAM has reviewed 15, only four of which have been recommended for use by ICCVAM and accepted by U.S. regulatory agencies.

Further, despite the fact that ICCVAM members are on the ECVAM Scientific Advisory Council and are therefore privy to ECVAM validation proceedings, subsequent ICCVAM review of those methods approved by ECVAM usually results in either an outright rejection of the validation or recommendation of the method only in severely limited applications.

Another aspect of failed harmonization is in validation criteria used by ICCVAM and ECVAM. ICCVAM’s criteria are similar but more stringent than those used by ECVAM (arguably too stringent to be practical in many cases). This results in added difficulties when attempting to harmonize the validation and use of alternative

2. The Situation in the U.S. (cont'd)

methods. To make matters even worse, the criteria are applied differentially to *in vitro* and *in vivo* methods. An extremely relaxed set of these criteria are applied to *in vivo* methods, if applied at all, and none of the *in vivo* methods currently used by regulatory agencies has ever been validated using the current criteria applied to *in vitro* methods.

The lack of harmonization is especially troubling since the U.S. is a member country of the Organization for Economic Co-operation and Development (OECD). The OECD is currently made up of more than 30 member states that include all members of the E.U., the U.S., Canada, and Japan. Since 1961, its mission has been to promote economic growth of member countries and aid in the development and implementation of regulations governing trade, including chemicals. The OECD has several programs focused on the development and validation of toxicity testing methods, including the development and validation of alternative methods.

ICCVAM committee members sit on a number of OECD review panels. As an OECD member, the U.S. is legally bound by an OECD Council Decision on the Mutual Acceptance of Data in the Assessment of Chemicals (MAD) which states that "data generated in the testing of chemicals in an OECD Member country in accordance with OECD Test Guidelines and OECD Principles of Good Laboratory Practice shall be accepted in other Member countries for purposes of assessment and other uses relating to the protection of man and the environment." This decision has no binding authority on a country's internal decision making, but it does require each member country to accept valid OECD data for international applications.

By not accepting the validation recommendations of the OECD, in which its representatives played an integral part, U.S. regulatory agencies are violating at least the spirit of the OECD Mutual Acceptance of Data agreement.

By not accepting the validation recommendations of the OECD, in which its representatives played an integral part, U.S. regulatory agencies are violating the spirit of the OECD Mutual Acceptance of Data agreement, if not the letter.

The animal protection community recently sent a letter to the head of each U.S. agency documenting the lack of harmonization. In its response, the

NIEHS pointed out that ICCVAM does not have the staff or funding or laboratories by which to conduct studies, and depends on other organizations to provide results and evaluate methods. The fact that the only governmental entity charged with modernizing regulatory testing in the U.S. lacks these basic elements is a disgrace.

2. The Situation in the U.S. (cont'd)

Due to a demonstrated lack of progress during its first decade, ICCVAM was recently charged by the U.S. Congress to draft a five-year plan. The draft was submitted to the public for comment, and was reviewed internally by a working group of ICCVAM's scientific advisory committee. Interestingly, the criticisms brought forth by the working group were nearly identical to those put forth by the animal protection community (FYPWG, 2007; Amundson, 2006; Amundson et al., 2007). Major gaps identified in the plan included the identification of regulatory endpoints that are ripe for modernization, an organized approach for identifying priority areas, and a plan for the translation of validation to regulatory use.

These are all areas that are clearly within ICCVAM's mandate and are essential to the development of a useful path forward.

In response to the criticism received, ICCVAM has consistently responded with excuses or exaggerated claims of progress. ICCVAM claims to have reviewed "over 185 alternative test methods," but closer inspection of their documented activities demonstrates this to be a gross exaggeration.

Of the over two dozen methods that have been accepted for regulatory purposes in Europe, ICCVAM has reviewed only 15 and only four have been recommended for use by ICCVAM and accepted by U.S. regulatory agencies.

For example, ICCVAM claims to have reviewed 23 alternatives to the use of animals in "biologics testing." However, the footnote to that claim states that this "review" consisted of one workshop in which several people were invited to present different approaches to address a single purpose — botulism toxin testing. In this case, it appears that ICCVAM is equating listening to a talk with a "review." Similarly, 138 of the 185 "methods" are for testing endocrine disruption and are actually variations of the same four

methods. Additionally, ICCVAM claims that it has reviewed 95 estrogen receptor (ER) transcriptional activation methods to assess endocrine disrupting chemicals. Since 95 entirely unique ER transcriptional activation assays do not exist, one can only assume that ICCVAM is using an extremely narrow definition of the word "method" (e.g., changing a minor component of the protocol constitutes a new "method") in an effort to make it appear as though they have accomplished vastly more than reality would indicate.

2. The Situation in the U.S. (cont'd)

The fact of the matter is that, after 10 years, only four methods that have been processed by ICCVAM have received regulatory acceptance by U.S. agencies (and this fact is clearly stated on its own Web page) (ICCVAM, 2007b). This number compares poorly to the more than two dozen methods that have been accepted for regulatory purposes in Europe (see Appendix A).

In addition, of these methods, only one is a non-animal method originating in the U.S. (Corrositex for skin corrosion). Three of these methods still use animals or animal derivatives (Up/Down for acute toxicity, LLNA for skin sensitization and BCOP/ICE for eye corrosion), including one that still involves poisoning animals until they die (Up/Down).

While the legislative differences between the U.S. and Europe are a significant factor in this differential progress, the lack of commitment on the part of the regulatory agencies themselves is also extremely problematic. As mentioned in the SACATM working group's review of ICCVAM's five-year plan, "it is incumbent upon the...agencies themselves – as critical stakeholders - to fully embrace the 3Rs and exert the leadership needed to assure that the validated methods...are actualized into regulatory testing frameworks as soon as practicable." This commitment has been sorely lacking, as evidenced most clearly by the U.S. EPA.

This agency, which requires more chemical toxicity testing than any other Federal agency, has become known for "check-the-box" toxicity testing programs that consume hundreds of thousands of animals, while officials remain adamantly opposed to the incorporation of non-animal test methods.⁵

If the problems with the current animal based paradigm and the advantages of an alternative, non-animal based strategy are so clear, why are regulatory agencies so resistant to change? An important answer was articulated in the NRC report mentioned above: "[C]urrent toxicity-testing practices are long established and deeply ingrained in some sectors," the report warns. "Thus, some resistance to the vision [of a modern non-animal, science-based approach] proposed by this committee is expected."

Legislative mandates such as those that exist in Europe are needed to provide the incentive for regulatory agencies to progress.

⁵ For example, in 1999 the EPA fought the incorporation of the cost-effective, sensitive, and internationally-accepted non-animal test method for genetic toxicity into its High Production Volume chemical testing program. The agency finally relented when the White House intervened at PETA's urging (<http://www.stopanimaltests.com/u-hpv.asp>).

2. The Situation in the U.S. (cont'd)

A new report from the National Research Council concludes that an effective toxicity testing program rests on a foundation of non-animal, science-based methods.

“eventually eliminate the need for whole-animal testing.” The report goes on to detail the components needed for such a program, how the components fit together, the technology and tools required and how to develop them, and describes an approach for validation and regulatory decision making based on this new paradigm.

The NRC report also discusses funding, administration, and whether such a program should be housed within an existing institution such as the NTP or EPA, or whether an entirely new entity should be created. The NTP has advantages in that it already has several multi-agency projects, and houses ICCVAM and the future Biomolecular Screening Branch. However, as detailed above, ICCVAM’s progress thus far has been dismal, which suggests that housing a large and complex new entity within

The necessary elements are in place for modernizing regulatory testing in the US: Science has provided the means and the NRC has provided a roadmap for follow-through.

The way forward has been clearly articulated by the NRC report. The “vision” described in this report includes a foundation based on non-animal methods that rely on knowledge of biochemical mechanisms and human biology. An essential feature of this approach is a tiered paradigm, in which a comprehensive array of *in vitro* methods “provides a stronger, mechanistically based approach for environmental decision-making” and will

“eventually eliminate the need for whole-animal testing.”

NIEHS would not bode well for its success.

Similarly, while there would be an advantage in housing such a program in a regulatory agency, such as the EPA, which may facilitate translation of the science into practice, the scale and budgetary requirements of this project may be far outside the scope of the EPA. Further, there are serious concerns, as expressed above, regarding the EPA’s commitment to the implementation of non-animal methods.

We therefore support the creation of an entirely new entity to manage this program, with funding and oversight that are separate from existing institutions, with intra- and extra-mural components and interagency input.

Because of the current situation, it has been left to various non-profit laboratories to fill some of the gaping holes in non-animal test development in the US. However, only one such entity – the Maryland-based Institute for In Vitro Sciences – has been successful in both developing the methods and in working with regulators to facilitate use of the methods. There is no U.S. entity that is remotely similar to FRAME or ZEBET.⁶

⁶ The Johns Hopkins Center for Alternatives to Animal Testing is known only for its database of alternative methods and has not contributed to the development and incorporation of non-animal test methods.

Conclusion

The NRC report warns: “[C]urrent toxicity-testing practices are long established and deeply ingrained in some sectors. Thus, some resistance to the vision [of a modern non-animal, science-based approach] proposed by this committee is expected.”

promising avenues of interdisciplinary research and making a concerted commitment to implementing new paradigms.

While our initial interest in toxicology was prompted by our desire to protect animals from cruel and useless experiments, the fact that these animal tests do not

adequately protect public health or the environment, represent shoddy science, and waste taxpayer funds, should be of the utmost concern to everyone.

The necessary elements are in place for modernizing regulatory testing in the US: Science has provided the means; some efforts through the EPA and NTP have begun, although they are far from satisfactory; and the NRC has provided a roadmap for follow through.

A program of the scale envisioned by the NRC is not unprecedented. The U.S. has rallied to solve seemingly intractable issues in the past, such as sending a man to the moon and sequencing the human genome, by focusing resources on the most

adequately protect public health or the environment, represent shoddy science, and waste taxpayer funds, should be of the utmost concern to everyone. At stake is no less than the protection of human health and the environment. All that is needed to modernize regulatory testing in the U.S. is the political will to put science into action. The longer the legislature and U.S. agencies wait, the further behind we as a nation will be relative to the rest of the industrialized world, and the longer animals will continue to suffer needlessly in laboratory experiments.

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Appendix A

Despite consistently prompt reviews and endorsements of US-approved alternative methods by ICCVAM's European counterpart—the ECVAM Scientific Advisory Committee, or ESAC (**Table 1**)—ICCVAM has yet to even consider the great majority of alternative methods and/or testing strategies pioneered in the E.U. and endorsed by ESAC (**Table 2**).

Table 1: History of ESAC acceptance of ICCVAM-endorsed test methods

Endpoint	Name of Test	ICCVAM Final Rec. ^a	ESAC Stmt. ^b
Skin allergy	Local lymph node assay	March 1999	October 1999
Acute oral toxicity	Up-and-down procedure	March 2000	— ^c
Skin corrosion	CORROSITEX™	December 2000	December 2000

^a <http://iccvam.niehs.nih.gov/methods/methods.htm>

^b http://ecvam.jrc.it/f_home.cfm?voce=m&idvoce=3

^c ESAC statement unnecessary given international acceptance of the UDP as OECD Test Guideline 425 since September 1998

Table 2: ESAC-endorsed alternative methods/testing strategies awaiting ICCVAM review, acceptance and formal testing recommendations

Endpoint	Name of Test	ESAC Stmt. ^{a-b}
Antibody production	<i>In vitro</i> monoclonal antibody production	November 1997
Photoirritation	3T3 neutral red uptake (NRU) phototoxicity test	May 1998
Vaccine potency	Toxin binding inhibition (TOBI) test	December 2000
Vaccine potency	ELISA test for human tetanus vaccines	December 2000
Embryotoxicity	Embryonic stem cell test (EST)	May 2002
Embryotoxicity	Micromass assay	May 2002
Embryotoxicity	Whole rat embryo assay	May 2002
Vaccine potency	ELISA test for erysipelas vaccines	June 2002
Acute toxicity to fish	Upper threshold concentration (UTC) approach	March 2006
Acute neutropenia	Colony forming unit granulocyte macrophage assay	March 2006
Genotoxicity	<i>In vitro</i> micronucleus test	November 2006
Skin corrosion	Skinethic™ human skin model	November 2006
Chronic toxicity	Ending 1-yr dog studies	November 2006
Skin irritation	EPISKIN™-SIT	April 2007

^a http://ecvam.jrc.it/f_home.cfm?voce=m&idvoce=3

^b <http://ecvam.jrc.it/page.cfm?voce=s&idvoce=27&idmm=4&idsm=27>

Appendix A (cont'd)

Moreover, the handful of instances in which ICCVAM has undertaken reviews of ESAC-endorsed methods (**Table 3**) can hardly be claimed to “make best use of existing resources and scientific expertise,” “maximize the efficiency of test method validation efforts and evaluations,” and/or “minimize duplication of effort.” For example:

- Whereas ESAC and the National Coordinators of the OECD Test Guidelines Program⁷ endorsed a strictly non-animal testing strategy for skin corrosion testing based on the ECVAM-validated human skin models EPISKIN™ and EpiDerm™, ICCVAM and its U.S. agency members continue to require that chemicals testing negative (i.e., non-corrosive) *in vitro* be subject to “confirmatory” animal testing. In addition, ESAC has endorsed the use of EPISKIN™-SIT for skin irritation. Thus, while the E.U. and other OECD member countries have moved toward 100% replacement of animal use for skin corrosion and irritation testing, ICCVAM’s position allows for only a modest reduction in animal use and only for skin corrosion.
- Nearly a year after ESAC endorsed the validity of five *in vitro* human blood-based tests for pyrogenicity, ICCVAM undertook a second, full peer review of these methods, despite the stated policy that “it is inappropriate for ICCVAM to conduct such reviews for methods where there is no **substantive** disagreement with the ECVAM assessment.”⁸ Nearly two years after ECVAM first submitted the test method information and validation studies, the ICCVAM-selected Peer Review Panel found fault with the new background documents ICCVAM had prepared and to date has failed to recommend even the minimal use of these methods originally proposed by ICCVAM, additionally recommending extensive parallel *in vivo/in vitro* validation studies.
- On the basis of a retrospective ECVAM validation study, ESAC endorsed the conclusion that “the *in vitro* micronucleus test (MNT) is a scientifically valid alternative to the *in vitro* chromosome aberration (CA) assay for genotoxicity testing.”⁹ This endorsement led to almost immediate regulatory acceptance of the MNT under the E.U. REACH chemicals regulation,¹⁰ as well as a proposal from the E.U. that a new OECD Test Guideline be created for the MNT. Despite this overwhelming endorsement of the MNT by E.U. regulators, ICCVAM’s comments¹¹ regarding the draft OECD MNT Test Guideline did *not* reflect support for ESAC’s position, calling instead for substantial additional work (i.e., expanded data sets on indirect-acting chemicals requiring metabolic activation, the inclusion of an optimized test protocol and performance standards, and an additional commenting round) before the MNT is accepted at the OECD level.

⁷ <http://caliban.sourceoecd.org/vl=3371732/cl=15/nw=1/rpsv/ij/ocedjournals/1607310x/v1n4/s30/p1>

⁸ <http://iccvam.niehs.nih.gov/docs/expedite.pdf>

⁹ http://ecvam.jrc.it/publication/ESAC25_statement_MNT_20061128.pdf

¹⁰ http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_396/l_39620061230en00010849.pdf

¹¹ <http://iccvam.niehs.nih.gov/methods/genetox/genetoxdoc/DraftRevMn30Jan07v4.pdf>

Appendix A (cont'd)

- Most recently, ESAC endorsement of the validity of a variant of the Local Lymph Node Assay (rLLNA), under which animal use can be reduced by as much as 50%.¹² ICCVAM's response has again been to propose a second peer review.

Given the extent to which international validation and regulatory acceptance criteria have now been harmonized, it is incomprehensible that ICCVAM persists in carrying out redundant peer reviews of test methods that have already been independently reviewed and endorsed according to substantially equivalent criteria.

Table 3: History of ICCVAM acceptance of ESAC-endorsed test methods

Endpoint	Name of Test	ESAC Stmt. ^a	ICCVAM Rec.	Notes
Skin corrosion	EPISKIN™	April 1998	June 2002 ^b	Recommended use only as "positive screens," with <i>in vitro</i> negatives being subject to "confirmatory" animal testing
	EpiDerm™	May 1998		
	Rat transcutaneous electrical resistance (TER) assay	April 1998		
Pyrogenicity	Human whole blood IL-1	March 2006	— ^c	Subject to second peer review in Feb. 2007; final ICCVAM recommendations have yet to be transmitted to Federal agencies
	Human whole blood IL-6	March 2006		
	Human cryopreserved whole blood IL-1	March 2006		
	PBMC IL-6	March 2006		
	MM6 IL-6	March 2006		
Eye corrosion/severe irritation	Bovine corneal opacity permeability (BCOP) test	April 2007 ^d	Oct 2007 ^e	Conducted a second peer review; the report published in Nov. 2006.
	Isolated chicken eye (ICE) test	April 2007 ^d		
Skin sensitization	Reduced local lymph node assay (rLLNA)	April 2007	— ^f	ICCVAM currently proposing a second peer review and other evaluations

^a <http://ecvam.jrc.it/page.cfm?voce=m&idvoce=3#1>

^b <http://iccvam.niehs.nih.gov/methods/dermal/dermal.htm>

^c <http://iccvam.niehs.nih.gov/methods/pyrogen/pyrogen.htm>

^d Regulatory acceptance by E.U. Competent Authorities since November 2002:

http://ecb.jrc.it/DOCUMENTS/New-Chemicals/Manual_of_decisions.pdf

^e <http://iccvam.niehs.nih.gov/methods/ocutox/ocutox.htm>

^f http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

¹² http://ecvam.jrc.it/ft_doc/ESAC26_statement_rLLNA_20070525-1.pdf