

## To 3R Is Humane

*Heightened concern over toxic releases is increasing the need for better data about thousands of compounds. At the same time, new toxicology techniques may be able to pinpoint where chemicals interact at the cellular or genetic level to produce disease. Do these two trends mean a huge increase in animal testing? "Humane science" based on the principles of the "3Rs" can mean better scientific results. New techniques can reduce the need for animals and provide a focus for advancing science policy*

ALAN M. GOLDBERG and PAUL A. LOCKE

**E**ven before Earth Day — dating back at least to the publication of *Silent Spring* in 1962 — Americans have been concerned about the release of toxic chemicals into the environment, and the public health risks they create. Regulation of toxic substances became a part of environmental law in 1976, when the Toxic Substances Control Act was passed. TSCA was intended to protect against the introduction into commerce of new chemical substances that create health risks. It has not proven effective in generating the type and scope of information that society needs to evaluate risks about compounds already in commerce. It does require that manufacturers submit to EPA whatever toxicity data are available, but it does not require that safety testing be undertaken. It is very difficult for EPA to use TSCA to evaluate, control or, if needed, eliminate from commerce the thousands of compounds already in existence.

Today, almost three decades after the passage of TSCA, our knowledge about toxic substances in the environment is still deficient. We know less than we should about the extent of human and environmental exposure to chemicals, and little about their toxic effects. Laws such as the Emergency Planning and Community Right-to-Know Act, passed in 1986, have helped fill some of the gaps regarding exposure and potential exposure to compounds. But our knowledge about toxic effects has advanced only haltingly.

Since the 1980s, reports and investigations by several groups, including EPA, the Congressional Office of Technology Assessment, the General Accounting Office, The Conservation Foundation, Environmental Defense, and the American Chemistry Council, have demonstrated that we lack basic toxicology

data about the vast majority of chemicals produced and released in the environment. Absence of this information, in turn, means that we cannot adequately assess whether these compounds create unacceptable environmental and public health risks.

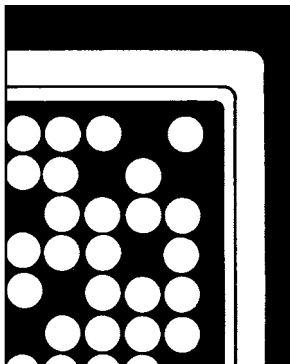
"Today in the United States, chemical safety is an illusion, and claims of chemical safety are based not on fact but on blind faith," wrote Environmental Defense's David Roe and William S. Pease in the May/June 1998 issue of this magazine, discussing ED's major study on "toxic ignorance" in the United States.

But in the last five years, there has been a renewed societal effort to obtain more information about the effects of exposure. The chemical industry has agreed to work with EPA and the environmental community to collect information about the toxicity of the approximately 3,000 compounds produced in high volume — greater than one million pounds per year. The High Production Volume, or HPV, program has obtained commitments from over 400 companies to test over 2,000 compounds. At about the same time, a new Voluntary Children's Chemical Evaluation Program has been launched, which will obtain health effects and exposure information on 23 pilot chemicals to which children are believed to be disproportionately exposed. EPA will use its authority under TSCA to demand testing if no companies agree to test voluntarily.

In Europe, the European Union is considering its own variant of a high volume testing and registration program called REACH, or Registration, Evaluation, and Authorization of Chemicals. REACH targets the same lack of knowledge about toxicity that the HPV and Children's Chemical Evaluation Program aim to correct. REACH would es-

**Alan M. Goldberg is Professor of Toxicology and Director of the Center for Alternatives to Animal Testing at the Johns Hopkins University Bloomberg School of Public Health. Paul A. Locke is a Visiting Scholar at the Bloomberg School of Public Health and a faculty member of the Center for Alternatives to Animal Testing.**





**We have moved from looking at overt disease endpoints — such as evidence of tumors in animals, observations about behavioral changes, or death — to the study of the actual processes that can cause such endpoints.**

establish a system for assessing both existing and new chemicals. It contains three elements. First, basic information for approximately 30,000 substances (new and existing substances being produced in excess of 1 tonne per year) would require submission of basic toxicologic information into a common database. Second, for compounds produced in excess of 100 tonnes per year or certain other compounds of concern (about 5,000 substances) a more extensive evaluation would be undertaken, looking particularly at long-term exposure. Third, compounds that are carcinogenic, mutagenic, or toxic to reproduction, and persistent compounds, would require authorization for use.

Extensive toxicology testing could eliminate the absence of basic toxicology information about the compounds these programs are concerned with. There is general agreement internationally about the type and nature of data needed. The Organization for Economic Cooperation and Development — the group of industrialized countries — has established six basic endpoints that should be studied to assess a chemical's toxicity. These endpoints are acute toxicity; chronic toxicity; developmental and reproductive toxicity; mutagenicity; ecotoxicity; and environmental fate.

This renewed focus on toxicology, and the toxicological information needed to make societal decisions about chemicals policy, reflects a theme that travels throughout the history of environmental law. It is, still and once again, important to improving public health and ecosystem health for the next generation of environmental lawyers, scientists, and policymakers. Many of the old questions, especially regarding the scope and nature of toxics in the environment, remain unanswered. But the practice of the science of toxicology, and the information it can yield, are changing.

Our understanding of biological processes has advanced. As we have learned more about how chemicals and other stressors affect the environment and humans, we have uncovered relationships between exposure and disease that are rarely straightforward and almost always perplexing. This has led to an effort at EPA and other federal agencies, such as the National Institutes of Health, to examine the cellular, molecular, and gene-level changes caused by chemical exposures that lead to disease. We have moved from looking at overt disease endpoints — such as evidence of tumors in animals, observations about behavioral changes, or death — to the study of the actual processes that can cause such end-

points. In other words, we have begun to focus our efforts on more subtle, precursor events that signal changes that lead to disease that occur before overt disease is present.

For preventing disease, and for designing more effective treatment, an understanding of the continuum from a healthy state to a diseased one is crucial. If we can understand this progression, we can intervene earlier along this continuum. The earlier the intervention, the better the chance of preserving or restoring health. This change toward studying the "mechanism" or "mode" of action of compounds has been reflected in the latest risk assessment guidelines used by EPA and other federal agencies.

Where we were once comfortable using information about death or obvious signs of toxicity in regulatory decisionmaking we now face complex inquiries about the interaction among environmental contamination, health, and other stressors. At the same time, our toxicology toolbox has gotten much more sophisticated. Our national effort to decode the human genome and genetic code of other species is beginning to bear fruit, and is being applied to environmental toxicology. The National Institute of Environmental Health Sciences has initiated several ambitious projects that highlight the potential value of these tools. In 1998, NIEHS started an Environmental Genome Project. The mission of the EGP is to improve understanding of human genetic susceptibility to environmental exposures. This project has focused its efforts on the molecular level to look at how individuals with slightly different genetic sequences, called genetic polymorphisms, could be differentially susceptible to disease. In 2000, NIEHS created the National Center for Toxicogenomics. Toxicogenomics is a combination of traditional toxicology with genomics — the investigation of genetic make-up and how it translates into biological functions. The center is coordinating nationwide research to develop a toxicogenomics knowledge base.

Toxicogenomics uses a technique called microarrays (also called DNA chips or gene chips), which contain many hundreds or thousands of short DNA strands, each in its own compartment. By washing a toxic substance in solution over the whole chip at once, any section of DNA affected can be made to glow or fluoresce, indicating which genes are "turned on" or "turned off" by the substance. This change can indicate that the gene has been altered. It may begin to make a specific protein or fail to make a protein correctly (or

## ANOTHER VIEW

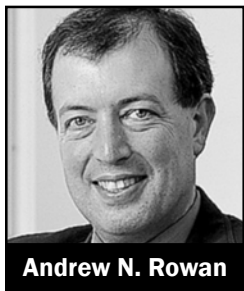
**Green NGOs, U.S. And EU Agencies In Humane Convergence**

A little over a decade ago, I organized a discussion among representatives of environmental and animal protection groups on alternatives to animal testing. The meeting was prompted by a perception that environmental activists were pressing for more safety testing and, hence more animal use, and that this was leading to unnecessary conflicts between the two groups. Not much was resolved that day, but I still remember one moment from the meeting.

The discussion ranged over the scientific challenges to animal testing and the political problems in getting regulatory agencies to accept data from non-animal tests. Then one of the environmental activists interrupted to ask, "When are we going to get to the animal rights stuff?" For some reason, she had expected to spend the afternoon arguing about the moral status of animals. The representatives of the animal groups pointed out that the case against animal testing was not going to be won or lost on the philosophical arguments, but on the social and technical costs and benefits of the new alternative test systems.

Ten years later, there was another clash between animal and environmental interests when EPA announced its intention to fill in the basic data gaps for about 2,800 High Production Volume chemicals. This program was the result of an Environmental Defense initiative. ED had demonstrated that the available toxicity data for these HPV chemicals accounted for only about 30 percent of what would be needed to report on 5 very basic toxicities (including acute, genetic, and reproductive toxicity). Animal groups were unhappy because EPA was now proposing that the data gaps be filled by performing traditional animal tests. It was estimated that these tests would require a total of one million animals. In the end, the clash between EPA,

the animal groups, and ED led to modifications of the proposed program, resulting in an estimated reduction in demand for new animal tests of about 75 percent. The animal groups accepted this reduction as the best that could be gained but, as a result of the arguments over the HPV program, ED also supported the call for quicker non-animal tests that would provide adequate data for hazard assessment.



Andrew N. Rowan

This was the first time that an environmental group had supported the development and use of non-animal tests, demonstrating how widespread the support for such alternatives has become. In the past two decades, major companies in the consumer and chemical industry have incorporated the public demand for alternatives to animal testing into their safety testing strategies. Also, significant political support for alternatives has developed in Europe and is growing elsewhere.

The European Union is, through the seventh amendment to the Cosmetics Directive, committed to phasing out animal tests for cosmetic products. Support for phasing out animal tests for other product categories is also growing. Funding for the European Center for the Validation of Alternative Methods has just been increased to 9 million euros per annum. At the same time, the EU is significantly expanding its demand for toxicity data on chemicals in the environment — the REACH program. To address this potential conflict, it has been proposed that an Alternatives Foundation be established, funded in large part by industry, to provide a major part of the 150 million euros over the next 10 years projected as the amount needed to develop, validate, and implement a range of non-animal alternatives to animal tests.

Scientists are looking at such new and developing technologies as quantitative structure-activity relationships and toxicogenomics, as

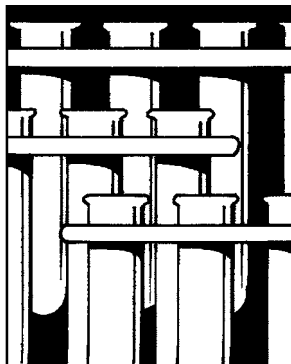
well as the older technologies such as tissue culture, to come up with the new non-animal test systems.

In the United States, political support for these new technologies is not as well developed. Regulators are reluctant to switch from a system in which the survival of an animal dosed by a drug or industrial chemical provides empirical and emotional support that humans would not be significantly harmed by the compound. While the United States, as a result of a political alliance of animal organizations and some large companies, established its own Interagency Coordinating Committee for the Validation of Alternative Methods within the National Toxicology Program about seven years ago, ICCVAM has neither the funding nor the political support that ECVAM enjoys in the EU. Nonetheless, the multinational companies that have to provide safety data to both the EU and the United States are not going to accept two different testing paradigms on either side of the Atlantic.

In the next decade, we will begin to see a major change in the way that hazard assessment and risk evaluation is conducted. The new approach will result in far less animal testing as non-animal test systems are developed and validated. The more progress that is made in this regard, the greater will be the pressure to replace what animal testing remains. The drive to develop non-animal systems will also lead to better understanding of toxicity and toxic mechanisms as we move away from the purely empirical approach of dosing an animal with a chemical to see what happens.

I predict that the drive to develop and implement animal alternatives will lead to better toxicology, because the new tests will usually be cheaper, faster and based on a better understanding of how the test agent causes toxicity — and hence better protection of humans and the environment.

*Andrew N. Rowan is Chief of Staff of the Humane Society of the United States, in Washington, D.C.*



**Where we once used death or signs of toxicity in lab animals in regulatory decisionmaking, we now face complex inquiries about the interaction among environmental contamination, health, and other stressors.**

at all). Further investigation about whether the new or changed protein (or a needed one that is absent) causes adverse effects in living tissues would be warranted.

Our ability to reach further down into the inner workings of molecular machinery has, frankly, outpaced our understanding of the meanings of the information we obtain. Nevertheless, these new techniques are exciting because of the potential information they can yield. Advanced scientific techniques have the potential to illustrate the modes of action, and mechanisms by which compounds in the environment cause or contribute to disease. And right now, few, if any, are used in regulatory toxicology testing. Today most basic tests rely on toxicology techniques that have not been updated for many years. This is especially true of tests that have been accepted by the EPA and OECD and are used to make regulatory judgments.

**A**t the same time as the new multinational movement to vastly expand testing of chemicals for toxicity gains speed, and promising advances in toxicologic methods develop, there is growing societal concern about how animals are used in toxicology testing, including a recognition of the ethical issues associated with the use of animals in science.

In Europe, animal welfare issues have been routinely addressed in testing programs and proposals. The number of research animals used in places like the United Kingdom has halved since the 1970s, in spite of increasing testing. Animal use has dropped in the United States and the world since 1976. One of the seven objectives of the REACH program is to promote the use of non-animal testing. But toxicology, especially the tests set out in U.S. testing protocols for basic toxicity information, requires high numbers of animals, especially rodents such as mice and rats.

Research animals can feel pain and suffer distress. Ethicists have long studied the circumstances under which animals are used for cosmetic, pharmaceutical, and biomedical testing. Some have questioned whether it is acceptable, from a utilitarian perspective — the greater good argument. The more aggressive philosophers have argued that animals should be eliminated as biomedical research subjects, further asserting that animals should have legal rights that deserve protection under the law.

Applying today's approved regulatory testing protocols, widespread testing for chemical toxicity would require large numbers of animals. A basic battery of tests on one pesticide can involve more than 10,000 animals across multiple species. The United Kingdom's Medical Research Council estimated that to fully implement the REACH program might involve testing as many as 30,000 compounds. This testing could cost approximately \$11 billion and sacrifice 13 million animals. EPA estimated in 1998 that the full battery of basic toxicology tests could cost up to \$205,000 per compound. And such testing could reveal the need for more detailed testing.

Today, at least for biomedical research, about 70 percent of Americans support the use of animals for necessary studies. This high level of support is understandable based on the belief that animal experimentation is necessary to eliminating human disease and suffering. It is likely that this rationale carries over to animal testing associated with chemicals in the environment, because the ultimate aim of this research is to prevent disease and protect health. But more and more the public is expressing concern about the numbers of animals used, seeking non-animal testing, and demanding that when animals are used, they are treated humanely and that their pain and distress is eliminated or at least minimized. It makes good sense, then, that environmental lawyers and policymakers become familiar with animal welfare issues in toxicology testing.

We believe that understanding animal welfare issues — coupled with an appreciation for the challenges and opportunities in regulatory toxicology — is critical for a much more basic reason. Although ethical and economic arguments are important, applying good animal welfare to toxicology research — practicing humane science — can help produce high-quality, reliable data for regulatory decisionmaking. It is also key to bridging the gap between traditional, animal intensive toxicology and the new technologies that toxicology is rapidly developing. Bringing these new toxicological tests online has the potential to jointly reduce animal use and produce information that can aid in regulatory decisionmaking. In addition, animal based toxicology research is improved when principles of humane science are applied. To explain why humane science yields robust and reliable results, we need a fuller understanding of what "humaneness" is and how

## ANOTHER VIEW

## Protecting Well-Being Of Humans, And Non-Humans Too

The United States pours out over a trillion dollars every year for the medical treatment of chronic diseases. Many of these, including asthma, autoimmune diseases, neurodevelopmental disorders, and neurodegenerative diseases, are clearly rising in incidence. Increases in disease incidence that occur faster than our genome can evolve point to environmental factors.

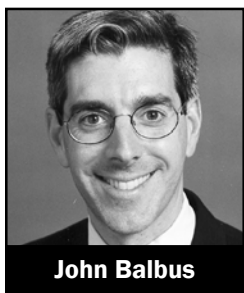
Among the many environmental factors causing or contributing to diseases, exposure to synthetic chemicals is one of the least understood. This is primarily because the burden of proof is on the government and citizens to demonstrate harm from chemical exposures, rather than on those who profit from the sale of chemicals to demonstrate that their products do not cause harm.

We thus live in a world with two types of laboratories: one is the set of institutions that conduct experiments using laboratory animals and other biological systems like cultured cells to determine which chemicals cause harm; the other laboratory is the world itself, in which all living creatures, including humans, are inadvertent subjects of poorly monitored chemical exposure experiments.

In the first type of laboratory, experiments are generally designed to maximize the likelihood of discovering a harmful effect if there is one. The second type, where the vast majority of chemicals are tested, is far sloppier, with only short odds of identifying the links between specific diseases and specific chemicals.

Improving the situation requires improvements in the use of both types of laboratories. In our 1997 *Toxic Ignorance* report, Environmental Defense showed that even basic screening-level toxicity data were not publicly available for the great majority of widely used chemicals. Spurred by those findings and similar studies by the chemical industry and EPA, all

three joined to launch the High Production Volume Chemical Challenge as an initial step to ensure that hazard data for chemicals exist and are publicly available. Under this program, producers of HPV chemicals were invited to identify and fill gaps in the Screening Information Data Set, a set of basic hazard data established by the OECD. Several lessons can be gleaned from this program and the OECD's parallel international initiative:



John Balbus

First, it is feasible for the chemical industry to generate basic screening data on a large number of chemicals. Data for several thousand HPV chemicals are being developed and made publicly

available through these programs. Second, such data can be generated with far less use of traditional animal testing than was projected, by using previously unpublished data, structure-activity relationship estimation models, and read-across methods within categories of related chemicals. Indeed, sponsors under the HPV Challenge have proposed to use methods *other than* new animal-based tests to fill more than 90 percent of the required data elements (though, as noted below, some proposals were challenged on scientific grounds).

Third, while judicious application of SAR analysis and category-based methods can reduce both costs and the need for animal testing, our review of companies' proposals has frequently identified an overreliance on such approaches, which would lead to the generation of poor quality data if these methods were pursued.

These lessons are relevant to the European Union's REACH proposal, which seeks to shift the burden of proof of safety from society onto manufacturers, ultimately covering most existing chemicals, not just HPV. REACH seeks to move beyond screening-level to more-sophisticated data for chemicals used in medium to high quantities.

The point is not just to do more testing, of course, but to do whatever

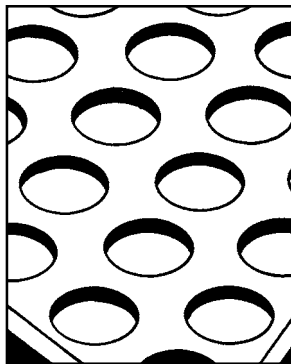
testing is necessary in a smarter fashion. New "toxicogenomic" methods appear to have great potential to increase the amount of information obtained from animal testing, while ultimately reducing the extent of testing in mammals.

While improving testing and shifting the burden of proof is essential to preventing inadvertent harm from chemicals, we must recognize that — with tens of thousands of existing compounds in use — the resources necessary to understand chemical toxicity through laboratory testing will be large and progress incremental. Steps need to be taken to improve our understanding of exposures and resulting harm in the laboratory of the world as well.

Since chemical risk involves not just hazard potential but actual exposure, improving the availability and quality of exposure data can help prioritize the development of hazard data. Expanding the current, modest scope of the human biomonitoring program conducted by the Centers for Disease Control is one means of providing an objective basis for determining which chemicals people are actually exposed to. Biomonitoring data also facilitate the epidemiologic study of environmental factors as causes of chronic disease. Requiring an expanded set of use and exposure data from manufacturers is also essential to improving our understanding of exposures. Last, investment in tracking chronic diseases is woefully inadequate. Without knowing who is suffering from particular diseases and where they live and work, efforts to understand and prevent these ailments are thwarted.

Whether within laboratory walls, or within the larger laboratory of the world, the well-being of humans and other living things must be of primary concern. A constant focus on refining our test methods and policies regarding chemical hazards will ensure we prevent harm rather than continue to uncover it after the fact.

*John Balbus, M.D., M.P.H., is Director of the Health Program at Environmental Defense in Washington, D.C.*



**Widespread testing for chemical toxicity would require large numbers of animals. A basic battery of tests on one pesticide can involve more than 10,000 animals across multiple species.**

it applies in the laboratory. In short, we need to recognize and appreciate a series of concepts known as the “3Rs.”

**I**n 1959, two British scientists, William Russell and Rex Burch, published *The Principles of Humane Experimental Technique*, a book that defines and explains humane science. Humane science is captured in the concepts of refinement, reduction, and replacement, which are referred to as the 3Rs. Alternatives to animal tests are a key part of the 3Rs framework. The 3Rs are based equally on ethical consideration of animals in the laboratory setting and the recognition that when the researcher in experimental design and implementation appropriately applies these principles, they result in a situation that is likely to produce more robust scientific results.

The first of the 3Rs, refinement, is defined as any method that reduces or eliminates pain and distress in animals during experiments. To implement the refinement prong of the 3Rs, it is not enough to simply administer analgesics or anesthesia to animals in pain. Every procedure in the experimental protocol must be considered from the perspective of the need to reduce or eliminate pain and distress. Thus, noninvasive imaging — the use of MRI, PET scanning, x-ray techniques, or biophotonic imaging — is finding its way into the laboratory. Biophotonic imaging occurs when luciferase, a compound obtained from fireflies or glow worms, is attached to cells, bacteria, viruses or specific genes. When these genes are “turned on,” indicating a molecular change of toxicological significance, a luminescence reaction takes place. Since light is produced, the reaction can be measured without invasive procedures. Toxicogenomics and related techniques fall squarely in this area.

Refinement also encompasses the substitution or use of species lower on the phylogenetic (evolutionary) scale for species that are higher in phylogeny. For example, using rodents instead of primates in an experimental protocol to obtain the same or more scientific information is an example of refinement, as is the use of *c. elegans*, a worm, or zebra fish instead of rodents. Finally, designing experiments that study humane endpoints, such as early tumor growth or changes in biological or genetic markers, rather than death, embrace refinement because the animals that are studied are likely

to be healthier, and in less pain and distress, during early stages of disease.

The second of the 3Rs, reduction, is defined as a method that seeks to use fewer animals in an experimental protocol to obtain the same or similar information of scientific value, or use the same number of animals to obtain more scientifically valuable information. For example, using a methodology such as biophotonic imaging can reduce the numbers of animals used because each animal can serve as its own control. In addition, reduction can be achieved when animal tissues or samples are used in more than one scientific protocol.

Russell and Burch point out that “one general way in which great reduction may occur is by the right choice of strategies in planning and performance of whole lines of research.” Thus, statistics, computational methods, and design considerations are critical tools in an overall approach to humane science.

Replacement, the third of the 3Rs, is defined by the use of techniques that do not use living animals. Replacement strategies use alternative tests. Sophisticated methods being developed at NIEHS substitute in vitro (essentially “test tube”) protocols for animal testing. So-called structure-activity relationships and computational methods use information about the molecular structure of a compound to determine its potential for harm. The rapid advances in genomics and proteomics (which deals with the proteins individual genes cause to be manufactured) hold great promise as replacement strategies. Databases containing genomics information have yet to be widely utilized, but their value to toxicology in general and alternatives in particular are not in doubt. In addition, the ability to study a very specific genetic change that is known to be, or suspected of being, important in the development of disease, or a change that indicates that a movement toward disease has, or is beginning, to take place (as can be examined in microarrays or DNA chips), can be powerful in showing the early effects of exposure to compounds.

By focusing on humane scientific techniques, Russell and Burch also made explicit what biomedical researchers had long recognized in practice: that good animal welfare sets the stage for better scientific results. Since publication of their book, there is a growing recognition that high standards of animal welfare result in scientific research that is more reliable, relevant, and reproducible — better science, in other words.

Consider, for example, the role that stress can play in animal-based toxicology. Physi-

ologically, vertebrate animals under stress exhibit elevated heart rates, higher blood pressure, and increased body temperature. Crudely put, their physiological engines are revved up as a result of the stress they are under. When data are obtained from animals under stress, it is fair to ask whether such data are truly representative of the responses to exposure that they would experience in everyday settings. If the answer is no, it is harder to argue that this information can be extrapolated to the exposures that are of interest to the regulator.

Another example of this principle is spelled out in some of the experimental work in animal behavior, including research into stereotypy. Stereotypy, defined as repetitive, functionless, non-varying actions, is fairly common, especially in laboratory animals raised in a non-enriched laboratory environment. Behavioral research in this area is growing rapidly, and seems to indicate that stereotypy can result in physiological changes, especially in neurological chemistry and pathways. Such changes are hypothesized to have an unknown but potentially relevant effect on data generated using such animals.

It is not yet possible to determine with certainty whether these changes could affect underlying experimental results and conclusions. Nevertheless, given the possible risks associated with incorrect conclusions due to suboptimal data, researchers should see the benefit in eliminating, to the extent possible, the conditions that lead to excess or unnecessary stress or stereotypic behavior. As we study toxicologic phenomenon that are more complex and mechanistic, treating experimental animals humanely becomes even more important.

In the United States, the Animal Welfare Act defines the relationship between the laboratory researcher and certain, but not all, experimental animals. The AWA requires that certain animals in research are protected by regulations that have requirements for the humane handling, housing and feeding of animals; contain provisions minimizing pain and distress in experimental procedures; review alternatives to any procedure that can produce pain and distress to animals; and provide veterinary care to animals so that they have the benefit of tranquilizers, analgesics, and anesthetics when necessary.

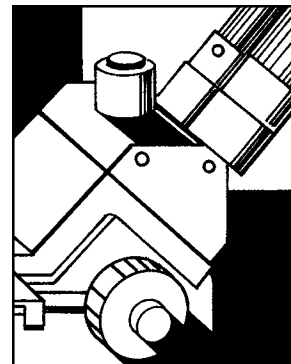
In addition, the AWA calls for the establishment of an Institutional Animal Care and Use Committee to monitor animal research and self-police the provisions of the AWA at

facilities covered by the act. Among other things, IACUCs must appoint a minimum of three members, one of whom is a veterinarian, and one of whom is not affiliated with the institution and represents community interests. IACUCs are also required to inspect, at least semiannually, all animal study areas and animal facilities with a specific eye toward practices involving pain and distress and provide training for scientists in humane practice and procedure. Perhaps most importantly, IACUCs must review and approve research protocols involving animals to assess compliance with the AWA.

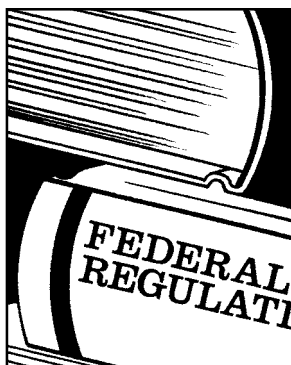
Rats, mice and birds — which make up close to 90 percent of all animals used in biomedical research — fall outside the requirement of the AWA. However, the United States Public Health Service (of which the National Institutes of Health are a part) has adopted an animal protection policy that has much in common with the AWA but with a broader reach. The PHS policy covers all institutions that receive PHS support and applies to all vertebrate animals. Between the AWA and the PHS policy, it is likely that the majority of research facilities that use vertebrates in research are covered. It is not possible to determine with precision the percent or number of facilities covered, however, because such statistics are not available for the United States.

**W**e believe that society's need for more toxicological information and the growing sophistication of toxicological science complement and embrace the practice of humane science. We need a proactive science policy, taking advantage of these advances in toxicology and based on the highest of ethical standards, that serves environmental law, policy, and decisionmaking. The 3Rs and a humane science approach to research chart the path for accomplishing these important societal goals.

Humane science is ethically appropriate and has the added advantage that it can result in the generation of more robust and reliable scientific information. In the near future, it is not likely that non-animal tests will completely replace animal tests in regulatory toxicology. When replacement alternatives cannot be developed, or do not provide needed information, or when non-animal tests will not suffice for other reasons, animal testing will occur. In such cases, the animal tests should



**Replacement strategies use alternatives to animal testing. Sophisticated methods substitute in vitro protocols or use information about the molecular structure of a compound to determine its potential for harm.**



**A proactive science policy, taking advantage of these advances in toxicology and based on the highest of ethical standards, will serve environmental law, policy, and decisionmaking.**

(For more information about the Center for Alternatives to Animal Testing, please visit <http://caat.jhsph.edu>. For more information about alternatives, please visit AltWeb (<http://altweb.jhsph.edu>).

be carried out in as humane a way as possible, recognizing that the humane approach fosters good scientific data.

Bringing online better science by developing and using more alternatives and practicing humane science faces certain challenges. Validation of alternatives is the first major hurdle. There exists today a series of animal-based toxicology tests that are traditionally used to make regulatory decisions. Some of these tests are codified in regulations, such as EPA test rules under TSCA or under OECD testing protocols, or both. If the intent of alternatives is to replace these tests, alternatives must demonstrate that they produce predictive and repeatable results. In regulatory toxicology, validation is defined as the establishment of the reliability and relevance of an alternative method for a specific purpose. In other words, for a test to be validated, it must show that it can consistently reproduce results and that these results are predictive — that is, they provide information that is of value for assessing whether the compound is a hazard.

A validation process for alternatives has been established in the United States by the Interagency Coordinating Committee on the Validation of Alternative Methods, known more commonly as ICCVAM, and several international conferences have been devoted to validation. This process is complex, expensive and time consuming. For validation, among other things it must be shown that the endpoint of the alternative is related to the biological effect of interest (e.g., showing biological relevance to the toxic process in question); that the test is reproducible among different laboratories; and that the methodology has been published in (or prepared for publication in) a peer-reviewed journal.

Bringing an alternative through the validation process is a time consuming and expensive task. Based on experiences validating tests in Europe, validation studies can cost as much as \$1.6 million and take approximately 10 years from test development to final validation. To date, approximately 2 alternative tests have been validated by ICCVAM and 10 by ECVAM, its European counterpart. Clearly, this process needs to be streamlined so that it is faster and less costly, while delivering the same quality of information.

After a test has been validated, it must then jump a second major hurdle. Regulatory agencies must formally accept the test for use in their programs. ICCVAM guidance on regulatory acceptance states that such acceptance is predicated on several factors, including

whether the method has undergone independent scientific peer review; whether it contains a detailed protocol, with standard operating procedures; whether it is robust in methodology and transferable among laboratories; and whether it generates information that assists in risk assessment. Some of these criteria overlap with the ICCVAM criteria for validation.

In the United States, validation does not guarantee regulatory acceptance. The ICCVAM law does not require that agencies adopt validated alternatives; each agency can decide for itself whether a validated alternative test will be acceptable under its regulatory programs. In addition, agencies are under no obligation to replace the tests already used with the validated alternatives. In fact, it is possible that agencies could require both tests.

A third possible hurdle exists. In addition to having alternatives that are validated and accepted by U.S. regulatory agencies, international agencies, specifically ECVAM and OECD, must accept these tests if they are to have maximum impact in advancing toxicologic testing, regulating dangerous compounds, reducing animal use, replacing animals and minimizing pain and distress. In some cases, international acceptance proceeds contemporaneously with acceptance in the United States. International and U.S. agencies are also working to adopt and use the same protocols for testing, thereby harmonizing the toxicological requirements for alternatives. For example, EPA's Pesticides Office has been working with OECD on some common issues.

**D**espite these challenges, we believe that a humane science approach and the development, validation, and use of replacement alternatives (when feasible) hold great promise for advancing our toxicological knowledge base about chemicals in the environment. To assure that progress continues to be made in protecting against toxic substances exposures, we must bring on line new techniques and adopt a science policy agenda that fosters and encourages replacement alternatives.

A focus on replacement alternatives to animal tests, and the full implementation of a humane science agenda, will serve us well as a nation and participant in an international community dually concerned about the health hazards associated with chemicals in the environment and ethical treatment of animals in toxicological research. •