Alternatives to the use of animals in household product and cosmetic testing

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Considerable effort has been directed toward developing and validating alternatives to the use of animals in household product and cosmetic safety testing. This research area provides a veterinary scientist the opportunity to solve some challenging problems. Developing useful alternative methods is difficult. Scientists working in this area often find themselves trying to develop alternative methods for procedures when there is little understanding of the mechanisms underlying the end points measured in the tests being modeled. Assays must be found that can correctly evaluate a vast array of materials. Alternative procedures must also provide information that allows a toxicologist to make a proper safety assessment. This is all done in the context that currently used animal-based tests have generally done an excellent job protecting society from harm.

If this is true, why is so much effort being expended to develop alternative methods? Over the years, biomedical science has made great progress. New understanding of biological systems and new technology has made it possible to measure the effects of test substances in ways never dreamed of even a few years ago. With this new information, it may be possible to begin applying new knowledge to develop methods that will aid in the risk-assessment process. As these new methodologies become available and start to provide useful information, it will be possible to begin decreasing the need for the animal-based tests now required. Furthermore, introduction of new methodology holds the promise that toxicologists will be able to increase the accuracy of their safety assessments, decrease the cost of safety testing, and decrease the time required to obtain important safety data.

The purpose of this report is to describe an overall approach that can be used to develop and introduce alternative methods into the safety-assessment process. Veterinary researchers may contribute substantially to the progress that will be made in this area.

Product safety testing—Safety testing of household products and cosmetics is necessary for 2 reasons. First, nearly every man, woman, and child at one time or another comes in contact with a wide range of household cleaning products or cosmetics. In fact, it is not uncommon for individuals to come in contact with several of these products in any given day. Household products include laundry detergents, hard surface cleaners, dishwashing products, and a wide array of other household cleaning products. Personal care products such as bar soaps, toothpastes, mouthwashes, antiperspirants and deodorants, skin lotions, shampoos, hair conditioners, hair styling products, and an enormous number of make-ups are also included in the consumer products/cosmetics group. Because of the wide distribution of these items, it is critically important that producers assure the products distributed will cause no harm to people, animals, or the environment under normal use and misuse situations. The only way to do this is to perform safety tests.

Second, the public demands environmentally friendly and efficacious products. Advances in chemistry and formulation sciences have allowed continued introduction of substantially improved

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products. These new products not only offer better performance, but they have other benefits. For example, the introduction of concentrated products that require smaller and more environmentally friendly packages contributes to reducing solid waste. Development of new combination products (such as detergent/bleach combinations) also decreases solid waste. As society has become more energy conscious, laundry washing temperatures have been decreased, which has required detergent reformulation. Introduction of new materials into the home often requires household product changes. Making major product improvements often requires more than simple modifications of already existing formulations. The new products may contain new chemicals, or combinations of chemicals that have never been used before. Often the safety of these materials has not been assessed. The only way to assure that such products are safe is through execution of an appropriate battery of safety tests.

Safety testing—What are the most commonly performed safety tests? A range of procedures may be conducted for a typical consumer or cosmetic product or its ingredients. Testing may include an assessment of eye and skin irritancy potential, acute oral and dermal toxicity, subchronic dermal and oral toxicity, contact sensitization, photoallergy, developmental and reproductive toxicity, respiratory toxicity, neurotoxicity, genotoxicity, and ecological safety. Each product undergoes a tailor-made safety evaluation that involves testing only in those assays that are necessary. When a material passes these tests, it usually is ready for the market from a safety point of view.

Introduction of alternative methods—Today, all of the tests listed require the use of animals. However, through the development and introduction of alternative methods, it has been possible to make some changes in animal safety testing. Alternative methods are any procedures that refine a particular test procedure, reduce the number of animals required, or replace a particular animal-based test. Reductions are procedures that decrease the overall number of animals required to make a safety assessment. Refinements are any changes in procedure that decrease the stress experienced by test animals. Replacements are any assays that can be used to eliminate the need for animals in a given testing procedure.

Important progress has been made in the development of alternative tests. For example, several alternative methods are being or soon will be incorporated into safety assessment program at our company and at other consumer product and cosmetic companies. In the area of ocular irritation testing, companies and some regulatory authorities are reducing the numbers of animals required per test from as many as 12 animals per substance to just 3. There also have been refinements of the Draize eye irritation test procedure, which decrease the stress of the test animals and produce results more predictive of human response. In vitro tests have also been introduced into the ocular safety assessment process. Since the introduction of these procedures, it has been possible in several instances to replace the need for ocular irritation tests without compromising the safety assessment.

Important work also has been done to develop alternatives for the acute oral toxicity test. Acute oral toxicity tests provide a value called the LD50, which gives toxicologists an indication of the relative toxicity of a test substance. Alternatives evaluated include the up/down acute toxicity test, the limit test, and the fixed-dose procedure. These alternatives are now being used to make acute oral toxicity assessments while substantially decreasing numbers of animals required to obtain the necessary information.

The local lymph node assay has been introduced as an alternative method for assessing contact sensitization. This procedure requires fewer animals than the standard test, takes less time to perform, and costs less than the currently accepted method. Another area of continuing research is directed toward developing in vitro models to assess dermal irritation. Enough progress has been made in this area to allow limited introduction of these methods into the safety assessment process. Progress is also being made in developing alternative methods for respiratory toxicology and developmental toxicology.

Finally, extensive work has been done developing in vitro genotoxicity tests. These methods have been in use for many years to help assess the ability of materials to cause DNA damage and provide information regarding the carcinogenic potential of a material.

Developing alternative methods—Despite the progress that has been made in the effort to develop alternative procedures, much more research needs to be done before it will be possible to replace animals in the safety assessment process. What is the most efficient way to develop these new procedures? Over the years, we have gained a considerable base of experience in the alternatives development process. From this experience we have developed a model (Fig 1) that has facilitated the efficient development of alternative methods.

The first step in developing viable alternative procedures is making the decision to establish a scientifically sound research program designed to specifically develop and evaluate procedures that will serve as an alternative for a particular safety test. At the initiation of the project, a wide range of sources for ideas and available methodology is consulted. This search will involve examining data bases containing information from many sources.

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including, but not restricted to, academic research laboratories, government research laboratories, and industrial laboratories. The researchers may then design studies and assemble sets of test substances to conduct a preliminary evaluation of the available technology.

When promising leads are identified through the preliminary work, the evaluation is continued in the next step of the process called parallel testing. Parallel testing involves evaluating materials in the standard safety test and the new alternative methods at the same time. This allows researchers to confirm or refute the findings of the preliminary evaluation. It also gives toxicologists performing safety assessments an opportunity to work with the new alternative method. They can compare the results of the new alternative assay with the standard tests with which they are familiar, and it helps them become comfortable with the reliability as well as the limits of the new system. It also provides data that can be shared with relevant regulatory agencies because they also must become familiar with the utility of the new tests.

Once experience has been gained with the assays suggesting that they will provide useful data, the new procedures are incorporated into the safety assessment process through a program called tier testing. Tier testing is a step-wise procedure that allows a toxicologist to assemble all of the available toxicity data that are useful for making a safety assessment. When in vitro alternatives are used in this process, they become just one part of the information used to support a risk assessment. Thus, the implementation of tier testing represents a key end point in the development of an alternative method, that is, it is the point where a new procedure is introduced thereby allowing decreased animal use.

The developmental process does not end with the implementation of tier testing. The correctness of the assessments made by use of the alternative procedures is continually followed by monitoring consumer experience in the marketplace. Research is also continued in an effort to identify procedures that improve upon the methodology in use. As new procedures are developed, the same process is used to prove their utility.

Ocular irritation alternatives—An example of the development of alternative methods is the in vitro ocular toxicology program that has been instituted at my company. When the ocular irritation alternatives program was initiated, the initial data search indicated there were over 30 methods that had been proposed as viable alternatives for ocular irritation testing. It was unclear however, whether any of the tests were truly useful. On the basis of the information obtained from the initial search, we established a preliminary evaluation of 7 currently available in vitro assay systems. A set of approximately 20 test materials of well-characterized ocular irritancy potential was assembled and evaluated in each in vitro assay. From that evaluation, 3 promising tests were identified. Those tests are the silicon microphysiometer, the microtox test, and the neutral red uptake assay. The silicon microphysiometer is a sensitive new cytometric device that measures the effects of test substances on cellular energy metabolism. The microtox test uses a luminescent strain of bacteria as an indicator of test substance toxicity. The neutral red uptake assay is a cytotoxicity test that uses the vital dye, neutral red, as an indicator of cell viability after treatment with test substance.

A parallel testing program was then initiated on the basis of the data from the preliminary evaluation. Toxicologists responsible for making safety assessments were asked to place all test substances being evaluated in the in vivo eye irritation test in the in vitro test battery as well. Through this process, it was possible to confirm where the tests worked well and where more work needed to be done to find procedures that more fully met the toxicologists' needs.

After approximately 8 months of parallel testing, it was possible to introduce in vitro tests into
the safety assessment process through the tier testing program (Fig 2). The tiered process begins with an evaluation of historical eye irritation data and physical/chemical data available for the test substance. If it is possible to make a safety assessment on the basis of this information alone, it is made without any additional testing. If this initial check does not allow a proper safety assessment, then the materials are evaluated in the in vitro test battery. Once the data from the in vitro battery are collected, the data are evaluated along with all other information available for the test material. If the in vitro data provide enough information to complement previously existing historical, physical, chemical, and other relevant data, it is possible to make a safety assessment without performing any in vivo testing. On the other hand, if there are reasons that the in vitro data and other information are not sufficient to make an assessment, it is necessary to determine the irritancy with limited in vivo testing. The in vitro data are used as a guide to determine the type of testing done and the numbers of animals used. For materials that appear innocuous on the basis of the in vitro data, confirmatory in vivo assays are conducted, using limited numbers of animals with strong assurance that minimal discomfort results. If the in vitro predictions indicate greater potential irritancy for a test material, judgement on whether testing needs to be done is made. Since the introduction of tier testing, our toxicologists are beginning to use the in vitro tests in their ocular risk assessments. Through execution of the tier assessment process, it has been possible to reduce the number of animals that otherwise would have been used in ocular irritation testing.

Although the in vitro tests are useful for many products, there are still a substantial number of test substances that are difficult to evaluate with currently available technology. Furthermore, little is known about the mechanisms underlying test substance-induced ocular injury, so additional research will be required to provide the fundamental understanding needed to develop scientifically strong tests.

Where do we go from here?—There are considerable challenges we face as we continue developing alternative methods that will enhance our ability to assess the safety of chemicals and product formulations while we decrease the need for animals. The veterinary profession can be a key player in finding solutions to the problems that need to be resolved. The following are areas where contributions from the veterinary profession may be fruitful.

There is great need for more research. Research needs can be divided into 2 key areas: basic mechanistic research and applied research. Basic mechanistic research includes activities directed toward learning how toxic materials cause their adverse effects. With a basic understanding of mechanism, it is far easier to develop predictive alternative methods. We need to gain greater understanding of the molecular and cellular mechanisms of toxicity in order to provide a basis for developing better, more easily interpretable tests. We also need to do a better job predicting whether test substances come in contact with target cells in target tissues. Research in physiologically based pharmacokinetic modeling and metabolism will help provide answers in this area. Currently, physical/chemical factors such as pH and reserve alkalinity are areas of considerable interest, but little is known about how these factors influence test substance toxicity. Greater understanding of the mechanisms underlying target organ specificity is also important. Such information would be useful in helping to predict potential adverse effects of test substances using in vitro methods.

Applied research includes activities directed toward removing blocks preventing use of available technology today. In this area we need solutions for the problems encountered as we attempt to test the wide variety of materials that need evaluation in in vitro tests. Examples of these materials include aqueous incompatible test substances and solids. There is also a need to explore the usefulness of many newly available technologies that have not yet been evaluated for use as alternative procedures. Examples include flow cytometry, image analysis, molecular biology, and morphometric procedures. Considerable work also needs to be done to validate currently available technology for use as screens in the safety assessment process.

Veterinary professionals working outside the laboratory have important contributions to make as well. Veterinarians working in industry must clearly communicate where new testing procedures are needed and must provide ideas that may be useful in the development process. Additionally, industry should provide resources such as test substances, in vivo data, and financial support for alternatives development.

Veterinary professionals in the regulatory community also have important contributions to make. Most importantly, there is a need for openness to innovation. Much work is being done in the alternatives area, but that effort will not come to fruition if we continue clinging to the status quo. Individuals working in the regulatory area must become active players supporting the development and validation process. Regulators ultimately are among the major customers for safety data. As suppliers of this information, developers must know what data are required to meet regulatory needs. Government support of research in the alternatives area (both in-house laboratories and through grants to academic or other institutions) will also foster the development process.

Conclusion—Safety testing of household prod-
ucts and cosmetics is necessary. Because consumer products and cosmetics are found everywhere, product manufacturers must be certain people, animals, and the environment are not harmed through their use. The safety of products has been assured for many years through the conduct of appropriate safety tests that require the use of animals. Although animal testing has been useful, it is important to begin developing alternative procedures that provide even better safety information and decrease our need for animals. Major progress has been made in the development of alternative methods. Many new procedures are now in use. Their introduction has helped improve our risk assessment capabilities and helped decrease the number of animals required to make certain safety assessments. The veterinary profession is in an excellent position to contribute substantially toward the continued development of alternative safety testing methods. It is an area of research in which contributions can be made both in the scientific and animal welfare area. We as veterinary professionals should be the leaders in this area.

References


