

IN VITRO ALTERNATIVES TO ANIMAL TESTING: BREAKTHROUGHS AND CHALLENGES

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Over the last 15 years, there has been considerable concern by members of the animal protection community and the general public about the use of animals for testing cosmetics and personal care products. The desire to end animal testing, however, does not obviate the need to protect consumers by obtaining safety information on products and ingredients. How then do we balance the ethical need to abolish animal testing with the scientific (and ethical) need for safety data? The most promising answer lies with non-whole-animal testing methods commonly referred to as *in vitro* alternatives. The last 15 years have been dedicated to the development of these methods for use by industry, government and academia. Although many promising methods have been identified, we have yet to completely replace the infamous Draize Rabbit Eye Test or implement many alternative methods within the regulatory community. Why has the pace of replacing animals in testing been so slow? As this article will explain, the path to replacement is much more complicated than first anticipated.

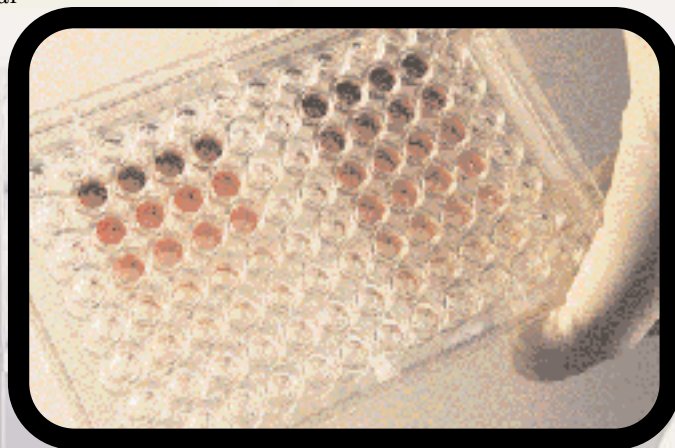
Why Companies Use Animals

It is fairly well known that many industries, such as those producing pharmaceuticals or pesticides, are required to submit results from safety tests to a regulatory agency — the Food and Drug Administration (FDA) or the Environmental Protection Agency (EPA) (for US companies) —

before a new drug or chemical can be marketed. In most cases, the regulatory agency requires that these safety tests be conducted on animals. The cosmetic and personal care industries, however, are not so tightly regulated and are not required to submit safety data before marketing a new product. However, both the FDA and the Consumer Product Safety Commission (CPSC) require these

in the event of consumer complaints.

In some cases, companies use the animal model because not all the necessary *in vitro* methods exist. In the drive to stay competitive, many companies turn to novel ingredients which have never been tested. These compounds may be newly synthesized or may be harvested from natural sources. In either case, they are considered chemicals which will be



Neutral Red Assay – 96 well plate containing cells (usually human) stained with Neutral Red Dye. Viable cells are stained red. A decrease in color indicates a decrease in viability. (Dead cells do not take up red dye).

companies to insure the safety of their products — the means by which they substantiate this safety is left up to their discretion. Why then do some of these companies continue to test their products on animals? For many it is an issue of liability. Although neither the CPSC nor the FDA require the Draize Rabbit Eye Test, it is suggested in the guidelines as an appropriate method of evaluating the safety of new products. Since there is no recognition of alternative methods in the guidelines, many companies elect to test in animals to show they have used the best method available (i.e. the one recognized by the agencies)

used by thousands and perhaps millions of consumers and their safety must be evaluated. Since there is no existing safety data, companies must generate their own. Although alternative methods exist for many areas, there simply are not methods available to completely evaluate the safety of a novel compound. Therefore, companies are forced to either test in animals or abandon the use of these innovative materials. There is an ever increasing number of companies, especially cosmetic companies, that face this situation. Many companies which have committed to No Animal Testing claims must invest in the process of identifying alternative methods so that they will be available for future testing. If all companies worked together to help develop sound alternative methods, all companies would benefit in the future.

In other cases, companies use animals because they are not aware of alternative methods or do not have the expertise within their company to use them. Scientific and educational organizations such as the Institute for *In Vitro* Sciences dedicate themselves to

assisting these companies with the use of alternative methods. Making *in vitro* testing services available to broad sectors of industry and government turns the idea of eliminating animals from testing into a reality.

What Alternative Methods Are Available

The advantage of alternative methods is the ability to isolate a tissue or organ of interest (e.g. the eye, skin or liver) and ask questions in a very controlled environment. The disadvantage of the alternative methods is that many important components and pathways (e.g. circulatory and immune systems) are absent. Therefore, in some cases, it may be impossible to model exactly what might happen in a whole animal or human.

The most promising methods rely on biologically (cell) based systems which relate directly to the human. Detecting the damage or death (cytotoxicity) of human cells after exposure to ingredients and products can assist product development chemists in designing safer or less irritating products. Sometimes, human cells of different types are cultured together to form simple tissues representative of the skin or eye. These types of human-based cell culture system are used throughout the world to develop new products and determine their safety.

Some types of human cells are impossible to grow in the laboratory. While research is continuing to make more types of human cells available, cells derived from animals are sometimes used. In most cases, these cells have been immortalized so that those taken from a single animal can be propagated and used over many years in thousands of experiments. In other situations, slaughterhouse by-products are used (e.g. cow or chicken eyes).

Although some animal protection organizations do not support the use of animals as food, many have recognized that using these by-products in the short-term will prevent many additional animals from being sacrificed in testing. The use of human eyes would be more relevant and thus preferred, however, the limited supply and high demand for eyes for transplantation make them generally unavailable for testing large numbers of products. Research is continuing to utilize cells from human eyes in order to re-construct some of the tissues that are of primary interest in this type of testing (e.g. the cornea). When these human-based constructs are avail-

animal methods that are available for use. With all of these methods, one may ask why they have not yet replaced the animal models altogether? Once again, there is a complicated answer. First, there are no perfect tests. Each is limited in its ability to test different types of products. Others are not generally available or are too costly to use. Still some of the methods require more research until they can be used on a routine basis. Second, once a promising method has been identified, it must be validated and accepted for use.

The Concept of Validation

Validation is the scientific process used to show that a test really “works,” and to define the areas within which the test can be used with confidence. Until the reliability and relevance of a new test is established, it will not gain corporate or regulatory acceptance. The process of validation can be extremely time consuming and very costly. Most formal validation projects involve four to six laboratories and span two to three years. It is, however, critical to the

process of identifying methods that will eventually replace the animal model. If we forego the process of validation, many “bad” (i.e. unreliable or irrelevant) tests may be used. The result could be serious human injury which would cause a setback for the alternatives movement. It is crucial that new methods undergo this rigorous process and prove themselves ready for implementation.

Once a promising method has wound its way through the validation process, it must be declared validated by an authorizing body and then considered for regulatory acceptance.



BCOP Assay – The cornea of a cow eye is held in the dosing chamber while a test material is applied. After incubation, the opacity of the cornea will be determined.

able, the need for animal by-products may be eliminated.

Other types of alternatives rely on computer-based systems which analyze the structure of a compound and predict toxicity. These types of systems are used predominantly in the chemical and pharmaceutical industries. However, computer-based systems are generally only sophisticated enough to predict the toxicity of a single compound used alone. They cannot predict the consequences of combining different chemicals together in a formulated product.

These are just a few cursory examples of the variety of non-whole

The Concept of Acceptance

Hand-in-hand with the use of alternative methods is acceptance of the results generated by these tests. There are two types of acceptance with which we must be concerned: corporate and regulatory acceptance. Once the management of a corporation has made a commitment to the use of alternative test methods and provided funding to implement a program, the reduction in the use of animals can begin. Since the liability to companies not testing on animals remains large, most will adopt a weight-of-evidence approach. In this case, all information known about a new product and its ingredients will be considered, including, but not limited to, data from one or several alternative tests. Other information might be gathered from the ingredient supplier or from market-use information of other similar products. If, after reviewing all the information available, a company cannot substantiate the safety of the product, it will elect to either stop the development of the product or will conduct animal testing.

Valid alternative methods which are accepted by regulatory agencies for specific uses (e.g. eye irritation) would allow a company to utilize that test with more confidence and abolish the need for confirmatory animal testing.

The Route from Validation to Regulatory Acceptance

The US and European Union offer two different approaches to the validation and eventual acceptance of alternative test methods. It is still early in the process to see which will provide the smoothest path to regulatory implementation.

The European Center for the Validation of Alternative Methods (ECVAM) was established in 1991 by the European Commission. ECVAM's prime objective is "to promote the scientific and regulatory acceptance of alternative methods which are of

importance to the biosciences and which reduce, refine or replace the use of laboratory animals" (*ATLA* 22:1, 7-11, 1994). Funded solely by the government, ECVAM has commissioned numerous research and validation programs in addition to workshops and publications which cover a variety of the life sciences. Once ECVAM's Scientific Advisory Committee has pronounced a test valid and ready for regulatory acceptance, it is the responsibility of member states of the European Union to implement it. Simultaneously, there is pending legis-

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lation prohibiting the use of animals in testing if a validated *in vitro* method exists. Therefore, pressure from the public and government sectors should speed the implementation of new tests.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established as a collaborative effort by 14 regulatory and research agencies in the US. ICCVAM "coordinates issues within the Federal government that relate to the development, validation, acceptance and national/international harmonization of toxicological test methods" (NIH Publication No: 99-4495). Unlike ECVAM, ICCVAM does not have the resources to fund research or validation projects. Instead, these efforts are funded mainly by industry and animal welfare organizations. ICCVAM organizes

experts to critically review submissions of new test methods and then distributes the recommendations to the 14 regulatory agencies. Similar to the member states of the European Union, each government agency has the responsibility of implementing the new test method. In this country, however, there is not the same level of public or government pressure to insure the implementation of new tests.

Pressure from animal protection advocates will assist in moving federal regulators and industry to accept alternative test methods. Support for the "ICCVAM Authorization Act," a mechanism for consideration of alternatives by US regulators, is a first step to providing pressure.

Moving Forward

As this article is being written, results from ICCVAM's first review of an *in vitro* method (Corrositex™) are being distributed to government agencies and the general public. Hopefully by the time this article is published, agencies will be drawing up guidelines for acceptance of these new results. The use of Corrositex™ to determine if materials are corrosive to the skin would save hundreds of animals from an extremely painful experiment. Moreover, the acceptance of data from this test would set a precedent for *in vitro* methods and hopefully pave the way for many more.

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The Institute for In Vitro Sciences is a non-profit organization dedicated to reducing the use of animals in research and testing by offering educational and non-animal laboratory alternative testing services to industry and government. The Institute's programs are supported by members, industry, animal welfare groups, and the general public. To learn more about our organization and how you can support these critical programs, please visit our website (www.iivs.org) or contact Erin Hill at (301) 947-6526.

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Similarly, last year ECVAM's Scientific Advisory Panel declared non-whole-animal tests for corrosivity and phototoxicity as valid and ready to be considered for regulatory acceptance. These tests are now being considered by the member countries of the European Union.

Non-whole-animal tests are our greatest tool in continuing the reduction of animals used in research and testing. Investment in their development, validation and use needs to continue to be supported at a high level to insure a steady decline in the use of animals. 🐾