2-21-1988

Inventing the Skin You Love to Test

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Wanting nothing more than to darken her eyelashes, "Mrs. Brown went blind in the spring of 1933. She suffered constant pain for three months until her corneas peeled away, all for using an eyelash dye that promised to "radiate personality." Her experience earned her a place in a chamber-of-horrors exhibit presented to Congress by the fledgling Food and Drug Administration as part of a successful campaign to pass the Food, Drug and Cosmetic Act of 1938.

Countless such tragedies have no doubt been averted since the FDA gained, through that act, the authority to prohibit the sale of harmful cosmetics. But the legacy of "Mrs. Brown" (as she is referred to in FDA archives) remains a painful one for more than 100,000 rabbits each year that are subjected to similar fates under the act's provisions requiring toxicology tests on animals.

Here is what happens, for example, in the classic Draize test for ocular irritancy. The rabbits are removed from their cages and held firmly while their eyelids are pulled back and a measured dose of a suspected eye irritant is squirted onto the eye. The rabbits' eyes are then observed after 24, 48 and 72 hours for redness, blistering, bleeding or blindness.

In recent years, however, there has emerged a movement aimed at replacing such animal tests with *in vitro*, or test-tube, alternatives. More than 100 in vitro toxicology tests, most using cell or tissue cultures, are under development by scientists, including leading researchers in some of the largest household chemical and cosmetic companies.

Replacing animal tests used to be considered a flakey, humane idea," says Henry Spira, a leading spokesman for the movement against animal testing. But today, he says, "in vitro has moved into the mainstream.

The problem now, some researchers and activists say, is that federal regulatory agencies are failing to provide the leadership and incentives needed to nurture this toxicological transition. Federal agencies have committees looking into the value of *in vitro* methods, but Spira says people "get bored, with committees after a while."

Spira knows how to break up such boredom. In one of the turning points of the animal-rights movement, he organized a highly successful 1980 protest against Revlon, Inc., the cosmetics industry "flagship." Individuals from more than 400 groups dressed in rabbit costumes and marched outside Revlon's corporate offices in opposition to the company's use of the Draize test. Spira ran full-page newspaper ads depicting bandaged white rabbits asking, "How many rabbits does Revlon blind for beauty's sake?"

Six months later Revlon initiated a research program at Rockefeller University in New York City to look for alternatives to the Draize test. Within months, other cosmetic companies contributed hundreds of thousands of dollars to similar programs, and the search for *in vitro* alternatives got seriously under way.

Cheaper and Better

Today cosmetic companies widely publicize their commitment to reduce the number of animals used in testing their products. Such commitments are more than token gestures; companies are finding that *in vitro* tests can have a number of advantages over traditional animal tests. According to the congressional

Office of Technology Assessment, *in vitro* tests cost an average of $50,000 per product as opposed to $500,000 when animals are used. Moreover, *in vitro* tests can be more precise than many animal tests, making it possible to learn more about the molecular mechanisms that underlie inflammation, membrane damage and tissue toxicity. Such findings may lead to new strategies for the prevention or treatment of tissue injury.

Membrane damage is one of the early signs of tissue toxicity, and evidence of it is sought in a number of *in vitro* tests. The so-called Neutral Red Uptake test, for example, uses a biological stain to look for evidence of membrane damage in culture-grown human skin cells. Similarly, a test being developed at the Medical College of Pennsylvania in Philadelphia exposes cultured dog kidney cells to a suspected toxin, then looks for increased permeability to a fluoroscein dye due to membrane damage. In a related test under investigation by researchers at the Colgate-Palmolive Co. and others, a small piece of egg shell is removed from a chick egg, leaving the heavily vascularized, underlying membrane intact. A few drops of a suspected toxin, dissolved in saline, are placed on the exposed membrane and the amount of blood vessel breakdown is taken as a measure of toxicity.

Other *in vitro* tests are more specific. Researchers at Ohio State University are using sensitive, enzyme-based antibody tests to detect the production of a substance called C-reactive protein in white blood cells grown in culture with liver cells. C-reactive protein is an early indicator of tissue damage and a key element in the inflammatory response. And recent progress in molecular biology is allowing researchers to measure miniscule amounts of messenger RNA (mRNA), indicative of the production of telltale proteins in damaged cells.

"With the complexities of this [protein synthesis] system, many of us who were interested in the bio-medicine end have almost by necessity become molecular biologists," says Gerald Lazarus, of the University of Pennsylvania. Although it is a complicated area of investigation, he says, "We've gotten into this area because it gives us very crisp, critical and specific information, eliminating many of the problems inherent in biochemical studies."

There are disadvantages, however, to such specific measures of toxicity. Indeed, a fundamental problem with *in vitro* models is that they fail to mimic the complexity of the whole, living organism. Thus the premier caveat among *in vitro* toxicologists: Never settle for the results of a single test.

Any risk measurement of human ocular irritation or any other type of toxicity is going to have to be based on a spectrum of data generated from a battery of tests," says John Frazier, a professor of environmental health at Johns Hopkins University and associate director of the university's Center for Alternatives to Animal Testing (CAAT).

Table-Sized Tissues

In a broader approach to the problem, some researchers are seeking to combine the various components of skin into a living laboratory specimen that can be tested as a unit. Eugene Bell, professor emeritus at the Massachusetts Institute of Technology and chairman of Organogenesis, Inc., a biotechnology company in Cambridge, Mass, recently unveiled a "living skin equivalent" especially adapted for toxicology testing and expects to make it commercially available within 12 months. It is, in essence, a glob of living skin that can be painlessly exposed to various irritants.

Developed by Organogenesis as a skin graft for the treatment of burns, the "test" skin," Bell says, can be grown in sheets the size of conference tables. It has a synthetic skeleton and circulatory system, but is covered with a living outer layer and "feels like a piece of skin," he says. Pigment containing melanocytes

can be added so that the skin can tan after exposure to sunlight, Bell adds, add "it even repairs itself when wounded."

Bell makes the test skin in a two stage process that he calls "a bottle-filling operation." First, he mixes cultured dermal fibroblast cells with appropriate nutrients and other biological molecules, and pours them all into a mold. Over a period of a few days, this gel condenses into a "dermal equivalent," or a mass of cells similar to the deeper layers of skin.

Later, human keritinocytes—the type of cells that form the outer layer of skin—are cultured onto the dermal equivalent. They multiply and spontaneously organize themselves into a multilayered, differentiated epidermis within about four days. After three to four weeks, a complete basal lamina—a layer of specially arranged cells—is formed between the epidermis and dermis. The basal lamina, present in living akin but never before created *in vitro*, is believed to be an important region governing toxin penetration and the inflammatory response.

Not everyone is sold on Bell's new product. Paul Wegener, of San Diego-based Clonetics Corp., a maker of cultured epithelial cells, says there may be advantages to testing different cell types separately. "And one thing you can tell about Bell's product," he adds: "It's not going to be cheap."

But the successful modeling of a tissue system even hinting of the complexity of human skin bodes well for the future of *in vitro* testing. "I think that this is just the very beginning of a method to create hybrid organisms that will be useful for testing," Bell says. "We are really primitives at this point."

If the goal is to become less primitive, and a decline in the use of animal tests is one measure of that goal, the progress is being made, according to Animal Rights International, a coalition of animal-rights groups in New York City. Independent toxicology labs, cosmetic company trade associations and government agencies all are exploring *in vitro* alternatives, and no fewer than five scientific journals devoted to *in vitro* methods me been born in the past two years.

The Regulatory Bottleneck

So far, however, regulatory agencies—the key link between basic research and commercial use—have given few clues about which, if any, *in vitro* tests may be accepted as alternatives to current tests. And although committees abound, says Kailash Gupta, of the health-sciences division of the Consumer Product Safety Commission. "None of the agencies, as far as I know, has seriously sat down and said that these are the criteria that alternative tests should meet to be accepted."

Indeed, says Spira in a recent letter to federal regulators, although "regulatory agencies have publicly stated their support for alternatives … most of the regulatory agencies' actions have sent quite a different message to the industry: that, for regulatory purposes, it seems impossible even to begin replacing traditional methods with alternatives."

Spira notes that the Environmental Protection Agency still refuses to accept a more humane version of the Draize test, the Low Volume Eye Irritation (LVEI) test, that be says has been studied for more than a decade and has been designated a standard method by the American Society for Testing and Materials. The EPA has also been criticized for continuing to require the so-called LD50 test as a measure of mammalian toxicity—a test described by internationally renowned toxicologist Gerhardt Zbinden as "a ritual mass execution of animals."

Theodore M. Farber, director of the EPA's toxicology branch, confirms that the LD50 is still required but says EPA has "formed a committee that will be looking for some acceptable alternatives" to it. EPA also is

“actively looking at the Low Volume Eye Irritation test” and may validate alternatives for at least limited use as early as this spring or summer.

Bureaucratic sluggishness is not the only factor slowing acceptance of *in vitro* alternatives. Difficult scientific hurdles remain, and uppermost among them is the need to design objective validation criteria for new test methods. For example, to test the validity of two new *in vitro* tests that make use of different tissue cultures each culture might be exposed to standardized selection of common irritants—from the most innocuous to the most corrosive—and the results compared to traditional animal-test results. But because each cell culture will measure a slightly different variable, comparisons can be difficult to make.

Recommended Citation:

Weiss, R. (1988, February 21). Inventing the skin you love to test. *The Washington Post.*