



The End of Animal Testing?

THE HSUS WORKS WITH SCIENTISTS, GOVERNMENTS, AND CORPORATIONS TO PIONEER METHODS THAT REPLACE ANIMAL USE IN PRODUCT SAFETY EVALUATION

by ANGELA MOXLEY

A hidden cost lurks behind the sweetener in your morning coffee, the paint on your living room walls, perhaps even the soap you use to wash your hands.

At some point in their development, these products or their raw ingredients may have been applied to the eyes or skin of live animals, injected into their bodies, or pumped into their stomachs or airways. In tests of potential carcinogens, subjects are given a substance every day for two years; other tests involve killing pregnant animals and studying the fetuses.

Governments in most developed countries require a battery of experiments on materials including food additives, drugs and vaccines, pesticides, and many other chemicals. The results may be used to ban potential toxins, formulate standards to protect workers who handle them, warn certain classes of people such as pregnant women against taking a drug, or create packaging labels to let consumers know what to do if, for example, the product splashes into their eyes.

But the real-life applications for some tested substances are as trivial as an “improved” laundry detergent, a new eye shadow, or a copycat drug to replace a profitable pharmaceutical whose patent has expired.

No one knows exactly how many animals suffer each year for toxicity testing, but the annual toll is likely in the millions in the U.S. alone. Each test consumes dozens to thousands of animals apiece, says Troy Seidle, Humane Society International director for research and toxicology; registration of a single pesticide requires more than 50 experiments and the use of as many as 12,000 animals.

But recent years have brought a growing recognition among scientists and government officials that the welfare of animals in laboratories matters to the public, that animal tests often don't pre-

dict effects in people, and that they are simply too inefficient to meet the high demand for chemical testing. These changes have spurred a revolution that is moving the field toward tests performed in computer simulations and modern-day petri dishes—developments that could spell the end of animal use in toxicology within two decades.

In conducting conventional animal tests, says Martin Stephens, HSUS vice president of animal research issues, “you don't have to know a lot about animals' biology; you hope that it's similar to our own and you just do the test. Whereas now we know a lot more about human and animal biology, and we can model things in the test tube that we couldn't necessarily do 50 years ago.”

The old methods are “basically using animals as surrogate people,” he says. “We can do better.”

SUFFERING FOR SHAKY SCIENCE

In addition to the often severe suffering inflicted during safety tests, which are almost always conducted without pain relief, animals used in toxicity testing suffer mental and emotional stress from repeated, often rough handling and nearly constant confinement.

While rabbits, guinea pigs, dogs, birds, and fish are used in toxicity testing, rats and mice are the mammalian species most frequently experimented on—yet these rodents are exempted from even the modest provisions of the Animal Welfare Act, and facilities are not required to report how many they use.

Chosen for their small size, high reproduction rate, and relatively docile nature, rats and mice are too often perceived as content in a lab setting; many people mistakenly think the need for a natural environment has been bred out of them, says Stephens. But several years ago, a film documenting rats taken



Most rodents in labs live alone in shoebox-size cages with nothing to do; experiments may leave them in near constant pain with no relief.

from a lab and put in an enclosed natural area revealed something different: They immediately began digging burrows, building nests, and creating their own society.

“There’s a sense that these animals don’t quite experience the suffering that other animals can experience. Partly it has something to do with their small size, and it has something to do with their stoic demeanor,” says Stephens, noting that rodents have evolved behaviors to hide weaknesses from predators. “They’re not like dogs, who will let you know what’s going on. So it’s especially sad that these are the animals who are used the most in the lab—because they are the hardest to figure out when they’re in pain or distress.”

It’s too easy for a researcher to glance into a cage, see a quiet mouse huddled in the corner, and move on—especially during the day, when these nocturnal animals are resting, says Stephens. But to those willing to pay attention, the signs are there. Distressed rodents may be hunched over with a disheveled appearance, says Seidle. Or they may be highly agitated and hyperactive, exhibiting repetitive behaviors such as running in circles or climbing the walls of their shoebox-sized cages.

When the tests are over—after up to two years in some studies—rodents are decapitated by mini-guillotines or have their necks broken. Many are gassed to death with carbon dioxide, a method that’s

been shown to be distressful.

Animal testing has historically been a “health-crisis-driven enterprise,” Stephens says. In the 1930s, a toxin in a mascara product caused blindness in women and one death, leading to the development of eye irritation tests on rabbits. In the 1950s and 1960s, birth defects in babies born to pregnant women who took the drug thalidomide triggered new requirements for widespread reproductive toxicity testing in animals. And concern about pesticides resulted in the creation of a laundry list of required animal tests in the 1970s and ’80s.

As many as
12,000
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Fear of toxins in products and the environment, combined with the development of lifesaving substances such as penicillin and the polio vaccine, has led to entrenched support of animal testing among many scientists and government regulators.

But numerous prominent failures over the decades have underscored the weaknesses of animal tests. Results can vary from lab to lab and species to species. Seidle points out that birth defect studies in rats and rabbits failed to detect the developmentally toxic effects of PCBs (industrial compounds widely used in the U.S. until a 1979 ban on their manufacture), for example, while cancer tests have missed the hazards of substances such as asbestos, benzene, and cigarette smoke, delaying consumer and worker protection measures by decades in some cases.

Cancer study results are particularly problematic, says Stephens. Every day for two years, animals receive the largest dose possible that is not immediately sickening, a concentration far higher than what people would be exposed to, he says. “These chemicals could be no problem given in realistic doses, but when you overwhelm the body—the liver, the kidney—there can be a kind of indirect toxicity just from that,” he says.

Interpretation of the results also poses problems, Stephens notes. “Some animals are going to get cancer just because animals



Rats and mice have complex needs for burrowing and foraging that are denied to them in labs.

get cancer. So what levels should raise alarm? What you find is that at the end of the day, there is a gross characterization where unless results are at one end of the extreme or the other, you typically don't have high confidence that people are going to react the same way."

Out of 3,000 cancer tests in animals, 53 percent identified the tested substances—some as common as acetaminophen—as carcinogenic, says Thomas Hartung, director of the Center for Alternatives to Animal Testing at Johns Hopkins University. Hartung says this figure is likely 10 times higher than it would be in humans, probably because rats are much more prone to tumors.

Animal testing of pharmaceuticals has also provided dubious information. Ninety-two percent of drugs that pass the animal testing stage are ultimately abandoned, according to a 2004 Food and Drug Administration report. Of these, 60 percent caused adverse effects in human clinical trials that weren't predicted in animals; the other 40 percent were found to be ineffective in people, despite promising results in animals.

"That's 92 percent of drug candidates down the drain after many years of research, development, and investment," says Seidle. "It's a tremendously high attrition rate, which speaks very poorly of the methods that are currently available. So for many of the companies, animal testing is just bad for business. It's costing them way too much, and in this economic climate they can't afford it."

Hartung agrees. "I think that people have learned over the last few years that the 'gold standard' for what we have been doing is not that gold. The limitations are increasingly obvious," he says. "Some of the big companies are running into enormous problems. They see it as the toxicology was not always giving them the best advice."

FINDING A BETTER WAY

With nearly 300 brands used by 3 billion people every day, Procter & Gamble is well-positioned to help drive change in the field. The company has spent more than \$250 million over the past 22 years on alternatives, reducing its animal usage by 98 percent in that time, aiding in the development

Full-body restraints hold rabbits during the application of a test substance to their eyes—in some cases, for more than 24 hours following the procedure.

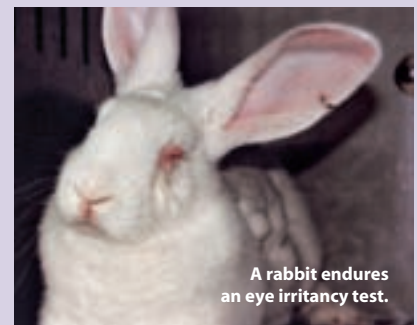


The Way Forward: Eye Irritation

Sixty-five years after it was devised by toxicologist John Draize, a rabbit test remains the standard animal study for measuring eye irritation caused by chemicals and products. The test substance is put in the rabbit's eye sac, says Martin Stephens, vice president of animal research issues for The HSUS, "and the eyelids are held shut so the substance can get on the cornea and all around the eye." The eyes are rated for irritancy as indicated by redness, ulceration, hemorrhaging, cloudiness, or blindness. The rabbits are held for up to 21 days and then killed.

A leading alternative method uses the corneas of cow eyes left over from the meat industry. The cow eye test and a similar test using chickens' eyes have been approved in the U.S. and Europe as alternative methods for assessing severe eye irritants or corrosive materials, meaning live animals need no longer be used to test for substances likely to cause the worst suffering. Negative test results using these methods, however, require further tests in rabbits, and no alternative methods have yet been approved for evaluating milder irritants.

But hope is on the horizon. In one recent victory, The HSUS helped ensure that germ-killing cleaning products no longer need to be tested on rabbits' eyes to determine safety labels in the U.S. "I think the Draize eye test is almost history," says Thomas Hartung, director of the Center for Alternatives to Animal Testing at Johns Hopkins University, which formed in 1981 largely in response to public outrage over the test. "And people are waiting for this—nobody likes this assay. We don't have an answer to all the possible applications, but ... give it a year or two."



A rabbit endures an eye irritancy test.

of 50 alternative methods, and completing more than 400 publications on the topic, says Len Sauers, vice president for product safety and regulatory affairs. P&G also funds jointly with The HSUS *alttox.org*, a website devoted to the advancement of nonanimal methods, and partners with The HSUS to give financial awards to encourage alternatives development.

The investment stems from a commitment to animal welfare and a recognition that alternatives are often more objective and efficient, Sauers says. For example, a previously standard version of an allergy test involved applying substances to the skin of guinea pigs, observing the effects over a month, and then subjectively grading the response. An alternative test conducted on mice uses fewer animals and causes less suffering; researchers measure the proliferation of a certain cell type, says Mark Lafranconi, a P&G toxicologist. “So we’re not waiting to see the expression of the disease; we’re seeing a subtle or biochemical change that occurs,” he says. “And it becomes much more objective, much more refined. It also can be conducted in about a third of the time of the original test.”

Through its work with P&G and other corporations, The HSUS is advancing alternative methods based on the “Three Rs” approach of replacing, reducing, and refining

The Way Forward: Skin Irritation and Corrosion

Testing for skin irritation and corrosion closely parallels the Draize eye test. The test substance is applied to a rabbit’s shaved skin, then removed after four hours. The skin is observed for up to 14 days.

Today this test can be fully replaced with what is known as a nonanimal tiered approach. First, a proven nonanimal test is carried out for skin corrosion, or irreversible tissue destruction. One such test consists of a glass vial capped by an artificial skin-like membrane; when a corrosive substance destroys the barrier, the fluid in the vial changes color or texture. This and other skin model tests have been accepted in the U.S. and Europe as alternatives to the rabbit test.

If a substance is shown to be noncorrosive, a second test for milder irritation is still necessary, for which several high-tech, three-dimensional human skin models have been developed. Thanks largely to the work of Humane Society International as part of its Hop to It, Europe! campaign to end animal use in skin irritation testing, the EU recently accepted three such models—a victory that “we project, given the testing requirements for chemicals alone, will probably save upwards of 30,000 rabbits annually,” says HSI research and toxicology director Troy Seidle. The HSUS anticipates global acceptance of these human skin models within the next year.



animal use. Some of these alternatives use human tissues or animal cell lines grown in a test tube or computer models that map cellular structures and processes. Other strategies involve conducting tests on animals only when negative results are ob-

tained in the nonanimal test, prioritizing the more toxic and common chemicals for testing, and eliminating redundant or needless testing requirements.

The HSUS is pressing government agencies and politicians on both sides of the Atlantic to become more accepting of animal-free alternatives in regulatory programs for drugs, pesticides, and other chemicals—a tough task in a sometimes hostile environment, says Seidle. “You have to convince government regulators that Door No. 2 is just as good, if not better than, Door No. 1 that they’ve been using for decades—in some cases for most of their careers—and often can’t be bothered changing.” Advocates must also persuade authorities to accept methods validated in other countries so that multinational companies don’t have to follow a maze of regulations.

In spite of the red tape, progress is being made, however incrementally. To date, nearly 50 alternative tests have been declared scientifically valid. Acceptance of alternative techniques is becoming mainstream, says Hartung, as evidenced by his recent invitation from a leading science



Toxicology at the National Institutes of Health Chemical Genomics Center revolves around assessing compounds using trays with tiny cell-filled wells. Robots can perform thousands of tests in an afternoon. Experiments are conducted at several dosage levels and examine multiple cell effects to ensure precision and accuracy, says Sunita Shukla, a post-doctoral fellow whose position is funded in part through an alternatives award from The HSUS and Procter & Gamble. “We’re not just here to pump out data,” she says. “We’re here to get data that’s meaningful for the toxicology world.”

journal to reflect on the future of toxicology.

Hartung acknowledges the success of animal advocates in challenging the status quo, and Stephens notes that after decades of being shut out of influential decision-making and advisory bodies, animal welfare organizations finally have a role in many of them. For one, The HSUS's international affiliate, HSI, has been a leader in advancing acceptance of alternatives through the global Organisation for Economic Co-operation and Development. "We're seen as legitimate players," Stephens says. "We're not just standing outside the castle, lobbing stuff over the parapet—we're engaging on science policy issues."

TOXICITY TESTING FOR THE 21ST CENTURY

In Europe, the push for alternatives has long been driven by cultural views on animal protection, as well as laws such as a 1986 requirement that nonanimal tests be used wherever available and a 2003 EU directive phasing in a ban on animal testing of cosmetics products and their raw ingredients. Even a large-scale EU chemical testing program has pushed corporations and governments to invest in nonanimal tests, says Stephens.



Workers at the Institute for In Vitro Sciences assess products for eye irritation using cow corneas left over from the meat industry and kept in cubelike chambers—a method preferable to using live animals. After a test substance is applied, the corneas are measured for opacity; the cloudier the cornea and the more the outermost tissues swell, the greater the irritation. A second test is performed using dye to measure the effects of irritation on the cornea's permeability. These precise assessments replace the subjective Draize test on rabbits, where researchers visually determine the level of irritation and assign a score.

The Way Forward: Acute Toxicity/Lethal Dose 50 Percent

One of the most notorious animal experiments, the Lethal Dose 50 Percent or "LD₅₀" test involves giving animals a substance through force-feeding, inhalation, and/or skin applications and measuring the amount that kills half the test subjects. Rats, mice, and rabbits are mostly used as surrogates for humans, while tests on fish and birds are carried out to examine potential hazards to wildlife populations.

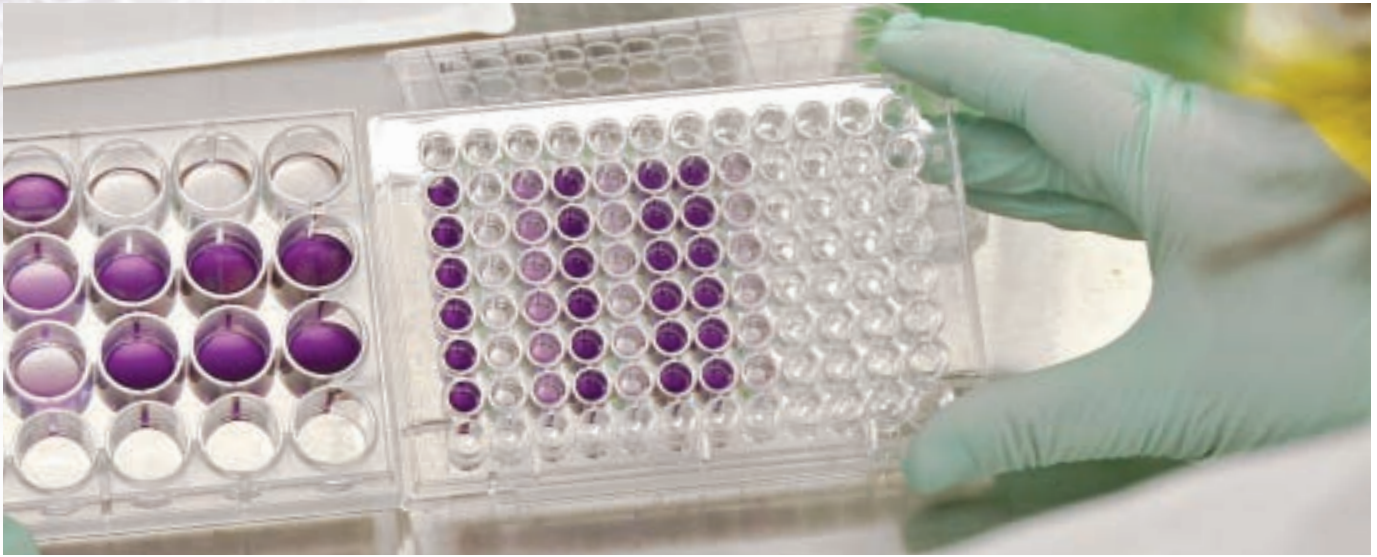
LD₅₀-type studies have evolved since their creation in 1927 and are today referred to more generally as "acute toxicity" studies. Thomas Hartung, director of the Center for Alternatives to Animal Testing at Johns Hopkins University, notes that the test "is dramatically losing importance." Test group sizes have been reduced from 150 in the 1970s to 40 or fewer today. In some parts of the world, such as the U.K., regulators now accept nonlethal signs of toxicity instead of death.

But this modest animal welfare refinement has been implemented only for force-feeding tests; for inhalation and skin studies,

lethal poisoning remains the objective. Furthermore, for pesticides and each of their raw ingredients, authorities in the U.S. and other countries require acute toxicity studies to be carried out by all three methods. Humane Society International research and toxicology director Troy Seidle is working with progressive corporate and government scientists in Europe to challenge such testing requirements "in the hopes of sparing tens of thousands of animals each year from one of the cruelest fates imaginable," he says.

Alternative methods are also being developed; one promising approach uses death of cells in a test tube as a starting point for predicting the dose that kills half the animals. Martin Stephens, HSUS vice president of animal research issues, hopes these alternatives will eventually be supplanted by wholly cell-based methods, as well as the use of emerging technology to examine what causes toxicity in humans and predict "what would be the lethal dose in a person in a much more sophisticated way."





In the nonanimal skin irritation test developed by Massachusetts-based MatTek Corporation, test substances are applied to tiny pieces of MatTek's EpiDerm™ human skin-like tissue for an hour. After the substances are removed, the tissues are incubated for 42 hours before being mixed with a special chemical. When the mixture's color intensity is measured in a spectrophotometer, dark purple indicates the test substance will not be irritating to human skin.

A government alternatives center was established in 1991 as the cornerstone of a well-funded strategic approach in Europe. By comparison, it wasn't until 1997 that the U.S. government—long considered underfunded and scattered in its Three Rs efforts—established an alternatives agency, which has since been criticized for sluggish movement on

approving nonanimal tests. And although California, New Jersey, and New York have passed laws requiring the use of available alternatives, no such federal law exists.

But recent developments have vaulted the U.S. to the forefront of the field. In 2004, the Chemical Genomics Center at the National Institutes of Health began applying a technology borrowed from the pharmaceutical industry to the testing of compounds.

Using high-speed automated robots and, instead of animals, cells and isolated molecular targets, the technique turns traditional toxicology on its head. Rather than observing effects in animals and trying to extrapolate the results to people, the technology attempts to uncover the processes in the human body that lead to toxicity and to pinpoint the chemicals that trigger them. The shift is expected to not only drastically reduce animal testing but produce results that will better serve public health and safety.

Center director Dr. Chris Austin says the new science will enable researchers to rapidly assess tens of thousands of compounds, an impossible outcome with animal testing. But the project is exploring uncharted territory, he notes, and scientists must prove that results obtained in cells are relevant to those that occur in the entire body.

A 2007 report commissioned by the Environmental Protection Agency formalized the new approach as the way of the future. *Toxicity Testing in the 21st Century*:

A Vision and a Strategy calls for limiting animal testing to situations where new alternatives are not yet ideal—and predicts that such targeted animal testing will eventually diminish. Early last year, three federal agencies signed an agreement to cooperate in the research, development, and validation of the new strategy.

Stephens, who served on the committee that produced the report, estimates that the effort will require an investment of \$2 billion over 10 years. For its part, The HSUS is lobbying for federal funding, encouraging conversation through forums such as *alttox.org*, and pushing scientists, regulators, and industry to work together. “Some people will say, ‘This is a generation away; talk to us when you’re finished,’” Stephens says. “But we’re trying to encourage the development of pilot programs that will demonstrate pieces of the approach, so that people won’t be so daunted by the prospect of the approach having to conquer the whole field at once.”

Austin notes that the Tox 21 project has enjoined groups that have not always seen eye-to-eye on the issue of animal use in toxicity testing: “We are working very closely together to meet a common goal in a very cooperative, respectful, collegial way,” he says. “Whenever you have groups of human beings who have seen themselves as adversaries ... working together for a common goal, for the common good, that ought to be celebrated. That’s what this represents.” ■

Cruelty-Free Shopping



Many companies conduct animal testing on cosmetics, personal care, and household products to satisfy safety requirements, though it is not specifically mandated for these materials in the U.S. The HSUS is a founding member of the Coalition for Consumer Information on Cosmetics, which maintains a list of products where no animal testing was used in any phase of development by the company or its laboratories or suppliers.

TO FIND OUT which products are on the list—identified on their packaging by the CCIC's leaping bunny logo—and to order a free pocket-sized shopping guide, visit leapingbunny.org.