Hunting for Animal Alternatives

Scientists in product testing and research are trying to reduce animal experiments, but face roadblocks ranging from regulatory sluggishness to the scientific value of a whole-animal response

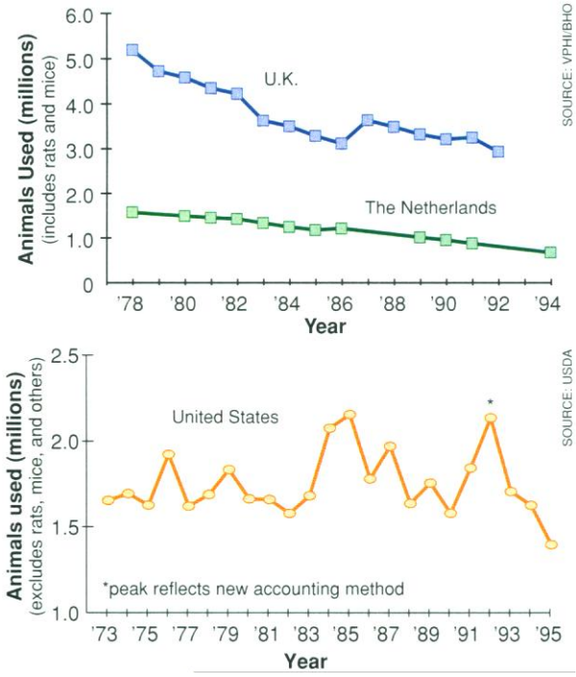
For Callaway Chemicals of Columbus, Georgia, determining which of its dozens of new chemical products each year should be classified as "corrosive" was once an unsavory affair. In tests required by the U.S. Department of Transportation (DOT), the company paid a commercial laboratory $400 to $1200 per test to shave the hair off a rabbit's back, then drizzle on a sample of Callaway's textile or water-treatment chemicals. The depth of the rabbit's wounds minutes to days later indicated the sample's corrosivity. "We weren't keen on using animals for this kind of testing," says Dale Bauer, the company's environmental, health, and safety manager. "We often just chose to mark things as 'corrosive' rather than go through it."

Last year, the company found a way out of its dilemma in the form of Corrositex, an in vitro test approved by DOT in 1993 as a substitute for the traditional rabbit skin test. Developed by California-based In Vitro International (IVI), the test gauges corrosivity according to the time required for a chemical sample to break through a skinlike protein membrane. The method yields results within a few hours and for as little as $100 per test, according to IVI. And that, says Bauer, makes it a plus "from the time and money aspects as well as the humane aspect."

Corrositex, which may soon become the first in vitro testing method to win multiagency regulatory approval from the U.S. government, is a much-needed success story in the search for ways to apply "the Three R's"—reduce, refine, and replace—to the use of animals in testing, research, and education. Concern about expense, a slow shift in attitudes among scientists, and political pressure from animal activists—who sometimes use extreme tactics—have made alternatives to animal testing a growth industry. The Three R's are the rallying cry of a burgeoning international movement that has already begun to lower the number of animals used in experiments and that will mount its second World Congress—sponsored by consumer-products firms—in Utrecht, the Netherlands, in 2 weeks.*

Despite such progress, there are clearly roadblocks ahead for those seeking to move away from animal experiments. For starters, most scientists affirm that in many areas of basic research, there is no substitute for measuring the response of a living organism. "There are adjuncts—not replacements—that may reduce the number of animals used," says Gerald Van Hoosier, a veterinarian at the University of Washington, Seattle. "But that doesn't eliminate the need to use animals for some types of research," such as vaccine development or AIDS research, he says. And the climate of intimidation created by violent groups such as the Animal Liberation Front has caused a "hardening of attitudes" that slows progress toward concrete solutions, says pathologist Ian Silver of Bristol University in the United Kingdom, who has been threatened by animal-rights groups for his work on the effects of stroke and shock on the primate brain.

Willingness to use alternative methods is increasing in industry, but many companies won't be able to switch until the new methods have been rubber-stamped by regulatory bodies, which still exhibit "a huge conservatism," says Michael Balls, director of the European Center for the Validation of Alternative Methods (ECVAM) in Ispra, Italy, and co-chair of the Utrecht conference. Indeed, in the face of regulatory delays and market sluggishness, California-based Advanced Tissue Sciences, one of IVI's two U.S. competitors, dumped its line of alternative testing products 3 weeks ago.



Animal abatement. The number of animals used in research has fallen, especially in Europe, where figures include mice.

Moreover, even in the relatively straightforward world of toxicity testing, animals can sometimes provide a much simpler guide than alternative test systems. That point was brought home last year in a surprise finding by 37 laboratories participating in a joint European study. Nine of the most promising in vitro alternatives to the notorious Draize rabbit eye-irritation test got failing marks. As a result, many observers predict that Europe's scheduled 1998 ban on the sale of cosmetics containing ingredients tested with the Draize—a major impetus behind the development of alternative methods there—will slip to 2000 or beyond.

Advocates of alternatives insist that the movement still has momentum—there is no going back to the days when the number of animals used was simply not an issue. And in contrast to the vilification of science by extreme animal-rights groups, a growing number of scientists are quietly working with moderate animal-welfare organizations to slowly reduce the number of animals used. "We've defined the Three R's, and we've persuaded people to accept that concept as being good for science, not just good for animals," says Balls. "But the third phase is implementing the Three R's, and this is the most difficult of all."

Attitude adjustment

Scientists and industrial toxicologists weren't always willing to spend their efforts researching alternative methods. When British researchers William Russell and Rex Burch first formulated the Three R's in a 1959 book, alternatives were taken much less seriously, recalls Silver.

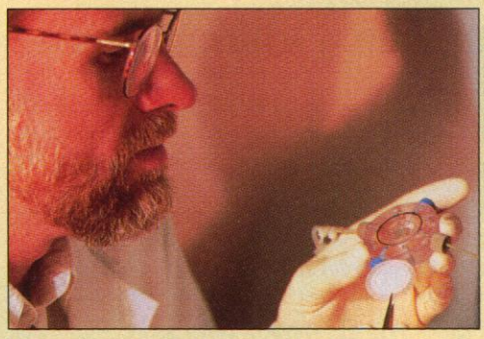
But over the decades there has been a shift in thinking. An increasing number of

* 2nd World Congress on Alternatives and Animal Use in the Life Sciences, Utrecht, the Netherlands, 20–24 October.

Animal-Free Experiments

How can a scientist find out how a chemical affects biological tissue without trying it on a living creature? Research on that problem is fast becoming a discipline unto itself, complete with journals and conferences such as the upcoming Utrecht Congress (see main text), where proponents ranging from lone academics to major corporations document the potential of a range of creative in vitro methods.

For example, rather than testing toxic compounds such as lead and mercury on pregnant mice, developmental biologist Ellen Silbergeld of the University of Maryland School of Medicine exposes cultured mouse embryos to toxicants at the two-cell stage, when the embryo's new genome begins to express itself. That allows her to quantify the direct genetic damage the compounds cause, and "no animal is exposed in vivo," Silbergeld says.



D. HENTGES/ NORTHWESTERN UNIV.

Rabbitless results. Miller's test could partially replace the Draize rabbit eye-irritation test.

Other researchers are adapting old techniques to fill the new demand for alternatives. For example, William Miller, a biochemical engineer at Northwestern University in Evanston, Illinois, has adapted a traditional cell-monitoring method to function as an in vitro system for eye irritancy and corrosivity testing. Researchers have long used the ease with which charged particles can pass between adjacent cells as a measure of their health, because normal cells set up a resistance barrier to such passage. This wall, called transepithelial electrical resistance (TER), lowers if the cells are disrupted, say by a toxicant. With help from a 3-year, $150,000 grant from Procter and Gamble (P&G), Miller and graduate student Andrew Pasternak set up a more realistic measuring apparatus that continuously bathes cells in liquid. Each chemical applied to the system decreases TER in a particular way over time, producing a unique, sloping "time signature" that roughly predicts ocular irritancy and could partially replace the Draize rabbit eye test. Commercialization "is not imminent," Miller says, "but I don't think it's decades away either."

For firms such as consumer products giant P&G, using fewer animals means cutting the amount of time and money poured into government-mandated rodent carcinogenicity assays, and so company scientists have tried several approaches. For example, they have coaxed cells from Syrian hamster embryos (SHE) to divide uncontrollably—"to recapitulate the cancer process in a petri dish," says Robert LeBoeuf, an associate director of the company's Human Safety Department in Ross, Ohio. In recent tests of over 300 chemicals, the SHE assay correctly predicted the outcome of rodent assays 80% to 90% of the time, says LeBoeuf. Another major initiative is predicting a substance's toxic properties from its molecular structure. Using a proprietary database of over 12,000 chemicals, P&G investigators can identify materials so similar to known toxicants that they don't need to be tested separately. Those trying to reduce the number of animal tests may be cheered by P&G's example: With these and other methods, the company has expanded drug research eightfold since 1984 without using more animals—and has reduced nondrug use of animals by 90%. **–W.R.**

researchers believe they have a responsibility to minimize the pain and distress they cause in animals, says veterinarian Andrew Rowan, director of the Center for Animals and Public Policy at Tufts University in Medford, Massachusetts. That shift is clear in the growing emphasis on animal "well-being" seen in successive editions of the U.S. National Research Council's *Guide for the Care and Use of Laboratory Animals*; the most recent revision gives institutional animal-care committees more flexibility to achieve animal welfare and downplays rigid requirements on cage construction (*Science*, 22 March, p. 1664). There is also a spreading conviction that in some cases, experiments that cause less pain and distress—or that bypass animals altogether—can produce purer results, says Rowan. For example, to explore the detailed mechanistic effects of drugs on tissue, in vitro tests offer "more opportunities to control the variables of your experiment," says pharmacologist Silvio Garratini, director of the Mario Negri Institute for Pharmacological Research in Milan, Italy.

The shift is also a reflection of social and legal pressures from outside science. Prodded by animal-welfare groups, both the United States and Europe have built the Three R's into law: In 1985, amendments were added to the U.S. Animal Welfare Act that require federally funded investigators to consider alternatives to animal use, and an even stronger 1986 European Council directive permits animal experimentation only when no other satisfactory method is available.

It's with the first two R's, refinement and reduction, that basic researchers—who use about 40% of all laboratory animals—have made the most progress. New statistical methods have allowed researchers to slash the numbers of animals used in some experiments without lowering precision, notes Rowan. And transgenic techniques that make mice more susceptible to human diseases such as cancer or polio are making it possible to screen potential carcinogens more quickly and to reduce the number of primates used in vaccine research, says William Stokes, a veterinarian at the U.S. National Institute of Environmental Health Sciences.

But for many basic researchers, the third R, replacement, is problematic. Alternative methods such as tissue-culture systems simply can't approximate the complexity of whole animals, especially in areas such as neuroscience and behavior, notes Van Hoosier: "Toxicology and pharmaceutical development are much more amenable to alternatives than basic research, where you need to study interactions in organic systems." Many scientists avoid even the word "alternative," thinking that it implies an unrealistic policy of total replacement. "You cannot say in vitro methods are an alternative to in vivo—they are complementary," says Garratini. And others point out that without any animals at all, much vital research simply couldn't be done. "You can't study learning and memory, or the effects of emotion or stress, except in a fully functioning animal," says neuroscientist Steven Rose of the Open University in London, noting that such data are central to developing treatments for human neurological conditions. "We applaud the Three R's, and we think science has already come a long way with the first two," says Frankie

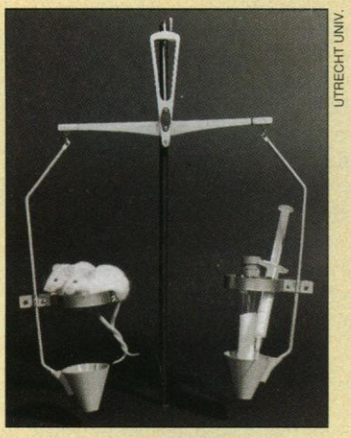
Trull, president of the National Association for Biomedical Research in Washington, D.C. However, replacing animals completely, she says, "is certainly a pipe dream during our lifetimes."

Even so, the total number of animals used in research is falling. According to the U.S. Department of Agriculture (USDA), animal use in U.S. research, including company testing but excluding all rats and mice, dropped about 35% from 1985 to 1995, to some 1.4 million. In the United Kingdom, where rats and mice are included in tallies, animal use dropped from 5.6 million in 1974 to 2.9 million in 1992, and the Netherlands has made even deeper cuts, from 1.6 million in 1978 to only 673,000 animals in 1994 (see charts).

Going Dutch: Mainstreaming Alternatives

In 1977, the Netherlands adopted a radical law, requiring that biomedical researchers use nonanimal methods whenever possible. Since then, the country has done more than any other to promote alternatives, reducing the number of animals used in research and testing by some 60% since 1978. "They're far, far ahead of everybody else," says Joanne Zurlo, associate director of Johns Hopkins University's Center for Alternatives to Animal Testing.

Even back in the 1970s, when public discussion of moral obligations toward animals became more intense, the debate in the Netherlands wasn't violent, says medical biologist Jan van der Valk, director of the Netherlands Center for Alternatives to Animal Use (NCA), a government-funded research and advocacy center in Utrecht. "Laboratory-animal scientists opened their doors to people from animal-welfare organizations," he says. And "they in turn appreciated that there are scientists who have feeling for laboratory animals."



UTRECHT UNIV.

Weighing the cost. Dutch scientists lead the way in alternatives to animal experiments.

Since 1985, many of those scientists have taken a government-mandated course on laboratory-animal science taught by geneticist Bert van Zutphen of Utrecht University. The 3-week course hangs instruction on biology, husbandry, and experimental design around the principle that no experiment should be undertaken "without considering whether all or part of the problem can be solved without the use of animals," as van Zutphen puts it. Students also learn the "Three R's"—reduction, refinement, and replacement methods. "We've now educated more than 3000 young scientists in the Three R's," he says. And although some researchers complain about the extra bureaucracy, others say it doesn't impede science unduly. Says geneticist Richard Bootsma of Erasmus University in Rotterdam: "People are much more strict in planning their experiments, but I don't think animal work suffers from that."

In 1988 the Dutch parliament followed up its mandates with money, creating the Alternatives to Animal Experiments Platform as a clearinghouse for grants to explore alternative methods. The platform will give away about $1.5 million this year, keeping some for the 2-year-old NCA, which organizes symposia and advises Dutch schools and corporations. Just 1 month ago, parliament strengthened this commitment with a new law declaring that animals are sentient beings with "intrinsic value" and moral status, and requiring that all animal experiments be vetted by ethical review committees.

Of course there's still a big gap on these issues between researchers and radical animal-rights groups such as People for the Ethical Treatment of Animals (PETA), which counts some 17,000 members in the Netherlands. Indeed, another recent Dutch law would forbid genetic modification of animals except with permission from an ethical review committee—which could take up to 6 months. In that form, the law could end genetic research in the Netherlands, says Bootsma—but as a result of talks among scientists, the government, and animal advocates, a compromise will likely be reached and the law will not be implemented in its present form. "It's probably an example of the way things should work," says Bootsma. Explains Netherlands PETA manager Geoffrey Deckers: "In Holland we have a tradition of compromising." –W.R.

Razing the Draize

The biggest potential for further reduction is in product testing, drug development, and quality control, which together account for nearly half of all animals used in the life sciences. However, companies face a big barrier to alternatives to animals: validation. In product testing, government regulations usually require a specific method such as the Draize test. When a new method comes along, the rules have to be modified. Validation, as Balls explains, is the effort to establish both the reliability of a new method and its relevance to the biological effect being tested, from eye or skin irritancy to carcinogenicity. Marrying the two sounds easy enough, but "I couldn't have imagined some of the difficulties we have had" putting validation into practice, Balls says.

The stalled European effort to validate a substitute for the Draize test is a case in point. The 52-year-old test rates eye irritancy according to the level of inflammation a substance causes when applied to rabbits' eyes, and animal-welfare groups, cosmetics firms, and many scientists have long hoped to make it obsolete. As director of ECVAM—created by the European Council 4 years ago to help implement its 1986 directive—Balls led a large international validation study of nine in vitro alternatives to the Draize test.

The findings, reported last December in *Toxicology in Vitro*, were dismaying: None of the new methods predicted the eye irritation potential of 59 test substances as reliably as the Draize test itself. As a result, it is likely that the planned 1998 European Union ban on cosmetics containing ingredients tested with the Draize will be postponed for at least 2 years, observers predict.

The disappointing results also revealed a deeper reality: It is unlikely that any single new method will be able to act as a total replacement for the Draize test. The study "sent a real note of caution across the alternatives community," says senior scientist Rosemarie Osborne of Procter & Gamble. As Alan Goldberg, director of Johns Hopkins University's Center for Alternatives to Animal Testing, says, "What you get from the Draize test is a very complex information set that we should not try to model" using any single in vitro test. Rather, groups, or "tiers," of tests may be required, each designed to test a specific biological effect or class of chemicals.

Balls agrees, saying that alternative methods are best used in combination and that regulators may have to adopt a more flexible approach if whole-animal tests are to be reduced. "We've been seduced by trying to replace the Draize test," he says. That test is simply the historic standard, not necessarily the best predictor of true eye irritancy in humans, say Osborne and others. The dif-

ficulty in proving equivalence to an accepted method goes beyond the Draize test, says Fred Schramm, vice president of Advanced Tissue Sciences in La Jolla, California. His company championed its Skin^2 tissue-culture system as a substitute for corrosivity testing, but the market was evolving slowly and regulatory approval in Europe was "still a ways off"—so last month the company abandoned the test.

Regulators admit that there have been bottlenecks but say they are gearing up to fix them. The 2-year-old U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), an ad hoc group of validation advocates from USDA, DOT, and other agencies, hopes to secure funding to become a permanent advisory body next year, and has already informed IVI that it will do an "interagency peer review" of Corrositex as a shakedown run. "Regulatory acceptance will remain the purview of the agencies, but we'll facilitate the review of new methods," explains co-chair Stokes.

ICCVAM and its counterparts in Europe have also begun to "harmonize" the international validation process, producing comparable validation criteria at recent workshops in the United States and Sweden. "We've been through a long learning phase, but now we're feeling more confident about the future," says Balls, who expects that the Utrecht meeting will spur the field of in vitro testing—one new research area that benefits animals as well as people.

–Wade Roush

SCIENCE IN FRANCE

New Research Strategy Draws Criticism

PARIS—To judge by two recent events, French scientists would seem to have plenty to cheer about. Late last month, research escaped relatively unscathed from brutal government cuts designed to make France's 1997 budget conform to the requirements of European monetary union (*Science*, 27 September, p. 1790). And last week, the government announced that it will create a major new gene sequencing center, marking France's long-awaited entry into the international effort to sequence the human genome. But just as researchers were breathing a sigh of relief at their reprieve from the ax, they learned that it comes with strings attached.

On 3 October, an interministerial committee chaired by Prime Minister Alain Juppé unveiled a sweeping panoply of measures designed to harness French science to serve the needs of France's ailing economy. The new strategy, which will take effect with the 1997 budget, will shift research funds into priority areas and push scientists to reorient their own priorities—for example, by including patent records as part of the evaluation of publicly funded researchers. Juppé's office declared in a statement that the pressures of international competition mean that France's "grand national research ambitions ... must be translated into economic development and the creation of new jobs." But the strategy could backfire: By laying such a heavy hand on the reins of French research, the government has provoked anxiety that basic research will be compromised. "The programs are very short-sighted," comments Harry Bernas, a physicist at the Orsay campus of the University of Paris.

The 23-member committee, which included the heads of all government ministries involved in or affected by research, set down seven "priority themes" for French science: electronics and information technology, transportation, industrial chemistry, food and agriculture, product innovation, medical research, and environmental technology. The committee also earmarked funds for specific projects in biotechnology, industrial chemistry, infectious diseases, and gene sequencing.

To make the new strategy stick, the government has mandated that France's public research agencies set aside a portion of their budgets for these priority programs. For example, in 1997, the Centre National de la Recherche Scientifique and the biomedical agency INSERM will be required to set aside 7% of their laboratory budgets (excluding salaries) for this purpose, a figure that will rise to 20% over the next several years. Moreover, publicly employed scientists will find that the number of patents they file will be taken into account along with their publication record when promotions are considered. And, to provide an extra incentive—and a sweetener—Juppé signed a decree on 2 October that allows individual researchers to reap 25% of the profits from any patentable discoveries they make.

FRENCH EMBASSY PRESS & INFORMATION DIVISION

Taking the reins. Prime Minister Alain Juppé.

Even with this inducement, the plan drew mixed reactions from French researchers contacted by *Science*. Many expressed concern that it might upset the proper balance between basic and applied research. "We all want science to be useful," says Pierre Chambon, director of the Institute of Genetics and Molecular and Cellular Biology near Strasbourg. "But to tie it so tightly to industry is a mistake." For example, says Orsay's Bernas, "the interface between biology and physics, which is growing exponentially, is completely out of the scope of the programs being designed."

A more favorable but cautious view of the policy is expressed by André Capron, director of the Pasteur Institute of Lille in northern France, a largely self-supporting institute that has seen a 15% drop in its total income in recent years. "It is clear we will not survive if there isn't a strong approach toward applied research and industrial applications," Capron says, although he adds that researchers must "maintain their vigilance about the proper balance between basic research and private activities."

Capron also applauds the decision to allow researchers to share directly in the profits by patenting their own discoveries. In contrast, Chambon argues that profits should go to the laboratories rather than individual researchers. "This is not just," Chambon says. "You are going to favor people working in fields that are immediately applicable at the expense of people doing basic research." And the ultimate result, he adds, might turn out to be the opposite of that intended by the government's new strategy. "What is bad for basic research is bad for applied research," says Chambon.

While researchers were anything but unanimous in their response to the government's tilt toward economically important research, they did give widespread approval to another big announcement last week—the decision to create a human gene sequencing center with a research staff of 120 to 140 people. The center, which will be located in the Paris suburb of Evry, will be directed by Jean Weissenbach, an internationally known geneticist and currently head of the Généthon gene research center, also in Evry. It will have the capacity to sequence 20 million to 30 million DNA bases per year with an error rate of only one in 10,000 bases. The government, which is committed to supporting the project for 8 to 10 years, has allotted $11.5 million to the center for 1997 and up to $19.3 million for each subsequent year, placing it among a handful of such top centers in the world. The announcement provided at least some salve for the anxiety created by the government's new grand strategy for French research.

–Michael Balter