More than a cosmetic change

Commercial and political pressures are pushing for a halt to the use of animals in toxicology tests in Europe. This change will also mean a move towards better science, says **Alison Abbott**.

Every time you reach for an eyedrop or reapply a lip salve, you do so confident that the chemicals they contain are safe to use. But the toxicology tests on which regulators rely to gather this information are stuck in a time warp, and are largely based on wasteful and often poorly predictive animal experiments. Efforts in Europe are about to change this, and the man charged with bringing toxicology into the twenty-first century is a plain-talking German: Thomas Hartung. Although Hartung acknowledges the immense challenges ahead, he sees this as an opportunity for toxicology "to turn itself at last into a respectable science".

Three years ago, when Hartung became director of the European Centre for the Validation of Alternative Methods (ECVAM) in Ispra, Italy, he didn't know that the job was about to shift gear dramatically. ECVAM was set up in 1993 to support European Union policy aimed at reducing the number of animals used in regulatory testing.

The centre, which nestles on the sleepy shores of Lake Maggiore in the Italian Alps, originally had ten members of staff and faced an uphill struggle to cut back the millions of animal tests carried out in Europe every year. Then in 2003, two major policy changes were announced from above, increasing the pressure on the centre's labs. ECVAM found itself facing an unexpectedly short deadline for delivering a slew of animal-free methods for testing chemical toxicity.

ALAMY

Skin deep: animal testing for all cosmetics is being phased out in Europe.

Rule change

The first change was to the European Union's Cosmetics Directive, which phases out over ten years the use of animals in cosmetics testing. A short while later, the European Commission proposed its controversial REACH legislation (Registration, Evaluation and Authorization of Chemicals). Europe produces some 30,000 chemicals for which toxicity data have never been registered. REACH aims to make registration mandatory for both future and existing chemicals — even those that have been on the market for decades.

If, as expected, the REACH directive is approved next year, it will come into effect in 2007. Animal-welfare groups fear that this will mean millions more animals will be used in tests to meet the regulatory requirements. And industry claims that the testing process could cost it billions of euros. Almost overnight, industry's interest in cheaper, animal-free testing skyrocketed.

Last month ECVAM was put in charge of developing, with industry and regulatory agencies, the testing strategies for REACH. Now commanding 50 staff, Hartung is rising to the challenge. The toxicity tests that have been used for decades are "simply bad science", he explains. "We now have an opportunity to start with a clean slate and develop evidence-based tests that have true predictive value."

Many of the animal tests used today were developed under crisis conditions. The notorious Draize test, which assesses the irritation or damage caused by chemicals simply by putting them into the eyes of rabbits, is a prime example. It was developed by the US Food and Drug Administration in 1944 after reports in the 1930s that some cosmetics were causing permanent eye injuries. One 38-year-old woman had gone blind after dyeing her lashes with Lash-Lure, a product that contained a derivative of coal tar.

Then came the calamity of thalidomide, which was given to pregnant women in the late 1950s to control morning sickness, but which caused horrific birth defects. By this time, governments were highly sensitive to public concerns and called on their authorities to develop animal-based tests that would predict all conceivable toxic effects of drugs and chemicals. The principles behind most of those tests remain more or less unchanged today.

"To test a chemical for its potential to cause cancer takes five years and involves 400 rats. More than 50% of the results are positive, of which 90% are false positives."

The battery of tests demanded by European authorities covers all eventualities, from acute effects that are seen shortly after exposure — such as eye and skin irritation — to concerns about whether in the longer term a compound might cause cancer, or brain or birth defects. In addition, tests must be carried out to define the risk a chemical might pose to the environment, such as toxicity to fish and other aquatic species.

Safety catch

Each chemical that goes through the multiple tests required for registration can use up to 5,000 animals — or 12,000 if the chemical is a pesticide. The cost of doing this for the 30,000 unregistered chemicals so that they comply with REACH has been estimated at between €5 billion (US$6 billion) and €10 billion.

In the decade since ECVAM was established, the number of animals used in toxicology testing has fallen slightly, although it still hovers at about one million per year. This reduction is a result of the refinement of existing tests, and the introduction of some alternative methods that rely on *in vitro* tests using cell cultures.

ECVAM is still pushing on both fronts. For

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example, the LD_{50} acute toxicity test, which involves feeding animals with a chemical to determine the lethal dose, still accounts for one-third of all animal tests worldwide. But the numbers involved have fallen from 150 animals per chemical in the 1970s to just eight animals in 2002.

ECVAM believes that it can halve the total number of animals used for regulatory testing within a decade. It has just completed its first large-scale validation study of an *in vitro* cytotoxicity test, which monitors death of cultured cells following short-term exposure to a chemical. Chemicals shown to be harmful in this test would be excluded from any LD_{50} animal tests. At least 70% of the chemicals registered in the past two decades fall into this category, says Hartung. And this is just the beginning.

"We've made a lot of progress with the low-hanging fruits, but many of the remaining animal tests — particularly the tests for toxicity in the long term — are much more challenging to replace," says Hartung.

CREDIT

Tests that put chemicals into the eyes of rabbits have changed little since the 1940s.

Poor prediction

This is despite the acknowledged poor quality of most animal tests, which have never undergone the rigours of validation that *in vitro* alternatives now face. Most animal tests over- or underestimate toxicity, or simply don't mirror toxicity in humans very well.

Take the embryotoxicity test in which chemicals are fed to pregnant animals and the fates of their embryos, and the progeny of two subsequent generations, are studied. "Animal embryotoxicity tests are not reliably predictive for humans," says Horst Spielmann, a toxicologist at the Federal Institute for Risk Assessment in Berlin. "When we find that cortisone is embryotoxic in all species tested except human, what are we supposed to make of them?"

The same goes for cancer. To test a single chemical for its potential to cause cancer takes five years and involves 400 rats, each of which is treated with the maximum tolerated dose. It is dramatically over-predictive: more than 50% of the results are positive, of which 90% are false positives[1]. Yet the number of compounds proved to be carcinogenic to humans is very low — the International Agency for Research on Cancer in Lyons, France, has identified just 95 proven and 66 probable human carcinogens.

As their experience grows, ECVAM staff are finding out just how complicated it is to develop persuasive alternative tests. A highly strategic approach is required, says Hartung. "In most cases it is not just a question of replacing one animal test with one *in vitro* test," he explains. Instead, a number of tests will be required, each of which has been shown to match data on toxicity in humans, assuming such information is available.

For example, several *in vitro* alternatives have been developed to replace the Draize test, each with its own advantages and limitations. Among these, some are better at predicting mild irritation than physical damage. Others are more suited to a particular chemical class, such as detergents.

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Life-savers: researchers at ECVAM are developing alternatives to animal toxicity tests.

Life or death

Scientists also cannot assume that *in vitro* alternatives are automatically better, says Spielmann. In 1971, a comparison of animal Draize tests in different labs revealed the test to be hopelessly non-reproducible[2]. But Spielmann's 1995 study of animal-free alternatives to the Draize test showed that they were equally unreliable[3]. Since then the *in vitro* tests have been standardized, and they are intrinsically more reproducible. "Although reproducibility and relevance are not the same thing," Spielmann cautions.

Relevance requires a good match between the test results and human data. At an ECVAM workshop in February, 30 industrial scientists met to develop the most effective strategy for using the alternative Draize tests, so that the false negatives and false positives of each test compensate for each other. This strategy is now going through the crucial validation procedure, in which human data, often from occupational health databases, will be used as points of reference (see 'The validation game', overleaf).

ECVAM has so far seen 17 alternative tests through validation — 11 use *in vitro* methods, another six involve refining *in vivo* tests to reduce the number of animals used. An additional 40 or so tests are under peer review, with more to come.

Most of the new tests assess acute toxicity,

The validation game

The number of toxicity tests required by regulatory authorities mushroomed in the 1960s and 1970s. But tests accepted by authorities in one country were rarely identical to those accepted elsewhere. This forced companies to repeat the same test with small variations in every country they wanted to market their product, wasting both money and animals.

So in the early 1980s, the Organisation for Economic Co-operation and Development (OECD) launched a major harmonization initiative. National authorities met regularly under OECD auspices to negotiate modifications to their test schedules that would make them compatible with those in other countries. Most of the differences have now been resolved.

But at the same time there was growing pressure from the public to replace or reduce animal testing, so the OECD expanded its role to oversee global acceptance of new animal-free, or animal-lite, tests. Validation — the proof that a test accurately predicts a specific effect in humans — is the biggest challenge for alternative methods.

At the European Centre for the Validation of Alternative Methods (ECVAM) in Ispra, Italy, validation typically requires testing a new protocol in three or four different external laboratories. The test chemicals are analysed by personnel who do not know the compounds' identities. If a test proves reproducible, it is sent for peer review by ECVAM's Scientific Advisory Committee, whose members include scientists, representatives from all member states of the European Union and relevant industrial and animal-welfare groups.

Once approved by the committee, the test is adopted by the European Chemicals Bureau, also based at Ispra, and then sent to the OECD for the all-important global validation. Most of the labs involved in ECVAM testing are European, but the centre has been careful to involve other regulatory authorities in early stages of the development process to speed OECD validation.

In 2000, the 3T3 Neutral Red Uptake Phototoxicity Test became the first replacement test to be validated by European authorities. The test identifies whether a chemical becomes toxic when exposed to light. Chemicals are added to a culture of skin cells, which is then irradiated with ultraviolet light. The rate at which the cells die before and after irradiation is monitored. The test was developed by Horst Spielmann from the Federal Institute for Risk Assessment in Berlin.

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Ideally, the predictive value of a new protocol is tested against human rather than animal data. Human toxicity data are, of course, hard to find, so Spielmann was gratified to be invited to join in a clinical trial assessing the potential phototoxicity of drugs to which physicians had raised concerns. He was able to compare his test's data for some standard chemicals with the irritation they caused on a patch of human skin. He found a perfect match. The test was accepted by the OECD last year.

One of the 40 or so tests now going through validation is the new cytotoxicity test to help replace the animal lethal-dose (LD_{50}) test (see main story). It was the first validation study to involve both US and European groups from the start. It is also the first to use data from the records at national poison centres. The predictions of the *in vitro* test provided a better match than the rat LD_{50} test when compared with the toxicity information on 42 chemicals listed as having poisoned people.

but animal use is highest when testing for the toxic effects of prolonged exposure to chemicals for long-term consequences such as cancer and reproductive toxicity. These costly procedures are harder to mimic *in vitro* and may never be completely replaced.

Sounds familial

This is why, apart from the €30 million it uses to support ECVAM annually, the European Commission is funding three multimillion-euro 'Integrated Projects'. Under these, dozens of labs will collaborate for five years to tackle more difficult issues, such as allergic reactions or widespread toxicity resulting from chemicals entering the bloodstream.

Scientists know that they are likely to find it hardest to convince regulators about alternative tests for highly emotive issues such as cancer and birth defects. More than half of all animals that will be needed to support REACH legislation are likely to be used in reproductive toxicology testing. The €9-million Integrated Project called ReProTect has 27 labs dedicated to developing alternatives to these tests. The ReProTect consortium has broken down the human reproductive cycle into smaller elements, from male and female fertility to implantation, to pre- and postnatal development, and is trying to develop a meaningful package of tests[4].

"Quite correctly everyone feels uneasy about taking risks where stakes are so high and issues so emotive," says Hartung. "We all want to be sure that there is real evidence that alternative tests are predictive of human toxicity."

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Rats are widely used to assess whether compounds can cause cancer.

P. GOETGHELUCK/SPL

For example, regulators know the weaknesses of the rat cancer test as well as scientists but, wanting to be safe rather than sorry, they accept it because it is believed to throw up few false negatives. They prefer to let industry prove the innocence of any compound that shows up positive. Any replacement tests will need to reassure both regulators and industry.

"We all want to be sure that there is real evidence that alternative tests are predictive of human toxicity."
— Thomas Hartung

With so much yet to be achieved, is Europe on target to deliver toxicity tests to meet the new regulations? The Cosmetics Directive phases out animal use in acute toxicity testing in 2009, and in testing for long-term effects in 2013. "We are on target for the first deadline, but the second deadline may be more difficult," says Hartung. Fortunately, the 2013 deadline can be renegotiated.

The REACH legislation is yet to be finalized, and alternatives to tests that present the highest financial and animal burden, such as reproductive toxicology and carcinogenicity, will not be in place when REACH first becomes law. But the longer-term picture should see a reduction in animal suffering going hand in hand with use of better science. ■

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