European Union Revamps Toxicology Testing in Attempt to Limit Animal Use, Improve Prediction

The U.K. clinical trial disaster earlier this year underscored at least one thing: Animal models poorly mimic human physiology.

Prior studies of the TeGenero leukemia drug, a monoclonal antibody, showed no sign of the catastrophic reaction that hospitalized six people. As companies increasingly develop protein-based therapies that specifically target human cells, animal models are likely to prove even worse at predicting how humans will respond.

“These types of drugs make up more than 50% of new-drug applications,” said Thomas Hartung, Ph.D., head of the European Centre for the Validation of Alternative Methods (EVCAM) of the European Union Joint Research Centre. “We can’t use toxicology that is 60 years old.”

Now new tests are in the works. In March the center approved six new toxicology screens that promise to speed up research on some drugs while making others safer. These are the latest of several tests to spring out of a European initiative to minimize animal testing. Since 1991, the center has focused on reducing, replacing, and refining existing animal-based toxicology screens for drug, chemical, and consumer product regulation. Once the center validates a test, companies within the EU are required to use it.

The United States, with its relatively tame animal rights movement, has approached the issue more lightly. No single agency is responsible for creating new tests and, once developed, they are not mandated. In recent years, however, the search for alternatives has grown beyond what some consider soppy sentiment. As the gap between powerful, targeted drugs and the technology used to screen them for toxic side effects widens, alternative testing can also improve safety.

Of the new European tests, for example, five detect pyrogenic contaminants in drugs—bacteria that infiltrate injectable or intravenous drugs during manufacturing and cause lethal immune reactions. The tests rely on cultured human white blood cells and might replace two existing, more expensive methods—the Limulus assay and testing on rabbits.

The new techniques improve on both methods. The Limulus assay picks up only gram-negative bacteria, whereas the new cell culture methods detect both gram-negative and gram-positive bacteria, as well as viruses and fungi, Hartung said. Drugs not suitable for the Limulus test, such as monoclonal antibodies and other biological compounds, typically use rabbits instead, but they only test whether there is contamination—not how much. What’s more, a drug that elicits an immune reaction in rabbits may not in humans. In addition to improving safety, Hartung estimates the new pyrogen tests will eliminate the need for 200,000 rabbits a year that were used to test biological compounds.

The sixth test uses cultures of human cord blood and mouse bone marrow cells to detect low white blood cell counts—a common side effect of cancer drugs. The test will speed up phase I clinical trials of cancer drugs because it assesses whether a specific dose will elicit a response without causing toxic effects before the drug is given to clinical trial participants, Hartung explained. There is no test to find the appropriate initial human dose of an experimental cancer drug so researchers typically extrapolate from animal studies. Consequently, much time is wasted simply establishing the safest effective dose.

“We’re optimistic about [the tests] having some utility,” said Abigail C. Jacobs, Ph.D., from the Center for Drug Evaluation and Research at the U.S. Food and Drug Administration. Jacobs represents the FDA on the U.S. Intergovernmental Coordinating Committee on the Validation of Alternative Methods, a group comprising scientists from several U.S. government agencies who evaluate alternative testing methods. ECVM has submitted its new tests to the committee for examination.

Success Story

The European Commission established ECVM in 1991 in response to protests against the use of animals in research and product testing. The center’s mission is to reduce unnecessary animal suffering and create alternative tests by identifying and investigating new techniques, comparing them to existing methods, and establishing standards. The European Union has since spent $300 million on the search for alternatives to animal testing. In addition to the six tests already approved, another nine in vitro tests are in the final stages of peer review and 25 more are in various research phases.

ECVM has grown since 2003, when a policy change mandated that animals be phased out of cosmetics testing over the next 10 years; it now has a staff of 55. Another directive, which is expected to pass into law next year, would require testing of more than 30,000 existing chemicals whose toxic effects have never been recorded. Faced with spending billions of euros on testing and the
ire of Europe’s staunch animal rights activists, industries are keen on alternatives too. Several companies are developing new assays for toxic effects in collaboration with regulators and the center. By law, researchers will be required to use it. The effect is that in Europe, validation—evidence that a test is required to use it, is systematic. The center has validated more than a dozen in vitro screens over the past several years, and Hartung estimates that these have reduced the need for hundreds of thousands of animals each year. A combination of in vitro assays to test a chemical’s acute toxic effects, for example, cut the number of animals required from 45 per substance to only eight. That figure works out to about 250,000 fewer animals used per year for this purpose alone. If the center were to validate all tests presently under review, studies suggest they could cut by 70% the number of animals required under proposed legislation for testing the toxic effects of existing chemicals.

In the United States, no single government agency is responsible for developing tests for regulatory purposes. This contrast reflects a cultural difference, said Alan Goldberg, Ph.D., head of the Center for Alternatives to Animal Testing at Johns Hopkins University. “U.S toxicologists are trained to look at basic biology,” he said.

That means grant money primarily goes toward studying mechanisms of toxic effects rather than detection methods or specific assays. In the private sector, pharmaceutical, chemical, or consumer product companies develop their own assays for toxic effects and submit validation data to regulatory agencies. “So far we’ve been very successful,” Jacobs said. Although human-specific proteins such as monoclonal antibodies do present a challenge, often it is simply a matter of finding the appropriate species to test, she said. The agency’s primary concern is safety, and drugs based on monoclonal antibodies have already hit the market without a hitch.

With no official directive to replace animals in testing toxic effects, the United States has been passive about reducing the number of animals required in research. In 1997, the National Institute of Environmental Health Sciences established the interagency commission to evaluate alternative testing methods. The group is made up of 15 federal agencies including the FDA, but recommendations for alternatives can come from any source. Once the committee decides an alternative test is sound, it will recommend the test to its member agencies. It does not carry out research or provide funding. And once recommended, companies are not required to use the alternative method. Such passivity, however, is not neglect, said Alan Poland, the National Cancer Institute’s representative to the interagency body.

“Everybody would love to have cell culture techniques,” he said. “It just hasn’t worked out.”

For example, the National Institute for Environmental Health Sciences funded projects several years ago to investigate alternatives to the standard 2-year chronic bioassay that tests compounds in animals to look for carcinogenic effects. At best, the other tests predicted carcinogenicity only 85% of the time, Poland said. “That’s not good enough for cancer.”

Moreover, although in vitro tests can certainly help some aspects of toxicology research, they cannot replace animal testing, said Samuel Cohen, Ph.D., chair of the department of microbiology at the University of Nebraska Medical Center in Omaha. “Cells are not as metabolically active in culture as they are in vivo,” he explained, and cells cannot reflect overall metabolism. For example, the liver often detoxifies drugs and chemicals and alters their potency. At the least, animal models enable toxicologists to look at mechanisms of toxicity and assess how they might apply to humans.

**Toxicology Methods**

Still, new methods are needed, Hartung said. “Toxicology testing has developed like an onion,” he explained. Over the years, researchers have added layer upon layer of tests, usually in response to a crisis. A notable case is thalidomide, which, when given to pregnant women in the late 1950s to combat morning sickness, caused birth defects. In response, the government mandated that companies develop animal-based tests to predict the foreseeable toxic effects of drugs or chemicals. Since then, most tests were developed in a similar way.

Of primary importance, said Hartung, are in vitro assays that predict how a drug might affect the heart and liver. Up to 30% of experimental drugs fail during clinical trials, most because of toxic effects that were not detected during preclinical studies. Drugs toxic to the livers of animals, for instance, predict human liver toxic effects only 50% of the time. Toxicologists do use human cells, like immortal cells that divide indefinitely, or cells from cadavers, but both sources have shortcomings: immortal cells proliferate abnormally, and cadaver cells quickly deteriorate in culture.

Several companies are developing assays of liver cells or heart muscle cells cultured from human stem cells. Gerón in Menlo Park, Calif., plans to sell human liver cells for toxicology research later this year. Cellular Dynamics International in Madison, Wis., cofounded by stem cell pioneer James Thomson, is developing human heart and liver cells expressly for toxicology research. And Cellartis, based in Göteborg, Sweden, has already developed human heart and liver stem cell assays and is collaborating with ECVAM on validation studies.

The increasing interest in the field of alternatives is not necessarily to replace animal experiments per se, Hartung concluded, but to have screens that are proven to more accurately predict toxic effects in humans.

“We’re asking whether a method is actually doing its job and if not, coming up with alternatives.”

—Gunjan Sinha

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