Innovations

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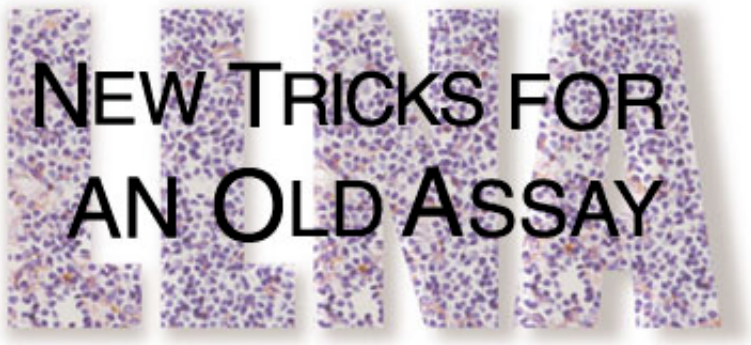


A worker accidentally spills an industrial chemical on her bare arm, a child splashes a household cleaning liquid on his legs; how will their skin react? If the product has the potential to induce an allergic response, one reaction could be the redness and swelling known as allergic contact dermatitis, a sometimes serious occupational and consumer health problem.

The chemicals in workplace and consumer products sold worldwide must be tested for their potential to cause such allergic reactions. For decades, several guinea pig tests have been used to identify human contact allergens--the most common have been the guinea pig maximization test (GPMT) and the standard Buehler test. Though accepted by the Organisation for Economic Co-operation and Development (OECD), which helps set industrial health and safety guidelines for its 29 member countries worldwide, both tests have limitations: they use a large number of guinea pigs, provide subjective rather than objective results, and are difficult to measure and interpret when colored chemicals are evaluated. Since the 1980s, investigators in the United States and the United Kingdom have sought an alternative test method that would reduce the number of animals required to test for dermal toxicity, and refine the test process in order to circumvent those limitations.

"The requirements for a proposed new test are that its performance has been adequately assessed and the reproducibility [of its results] demonstrated in different laboratories around the world," says William Stokes, associate director for animal and alternative resources of the NIEHS's Environmental Toxicology Program.

When a proposed alternative test method seems relevant to federal testing requirements, an independent scientific peer review panel is put together to discuss the proposed protocol and data, according to Stokes. In September 1998, a peer review panel brought together by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program's (NTP) Center for the Evaluation of Alternative Toxicological Methods began studying an alternative test method to identify human contact allergens known as the local lymph node assay (LLNA).

The LLNA has been used since the mid-1980s as a screening test for skin sensitization, but only positive

results could be used to make a regulatory decision. If the test produced negative results, researchers were required to perform a guinea pig test to validate the results. The LLNA's supporters hope it will now be accepted as a "stand-alone" assay for testing the effects of chemicals on the skin. They argue that the LLNA requires fewer animals than the other test methods. "It could also cut the price of testing in half. And . . . testing time [is reduced] from two months to one week," says Frank Gerberick, a principal scientist at Procter & Gamble, one of three major laboratories testing the LLNA (the other two are Zeneca Central Toxicology Laboratory and Unilever Research, both in the United Kingdom). Says Gerberick, "We're not asking to replace the current methods; we just want a little bit more of a choice."

How the LLNA Works

First conceived in 1984 by Ian Kimber, head of research at the Zeneca Central Toxicology Laboratory, the LLNA has been the subject of numerous intra- and interlaboratory collaborations and published papers. Whereas the guinea pig tests observe an animal's skin reaction to a chemical, the LLNA measures the response of the lymph nodes to a substance.

Allergic contact dermatitis is a disease mediated by lymphocytes, the central cell type in the immune system. When susceptible individuals are exposed to a chemical allergen, those lymphocytes that are able to recognize it as a foreign substance divide and increase in number. It is this increase in the number of chemical allergen-responsive lymphocytes that renders the individual sensitized; the stimulation of lymphocyte division is, therefore, a central event in contact allergy. Says Kimber, "The potential of chemicals to cause skin sensitization is measured as a function of their ability to stimulate lymphocyte proliferative responses in lymph nodes draining the site of exposure. So we reasoned that measurement of this proliferative activity might provide a sensitive endpoint for the identification of those chemicals that are able to cause sensitization."

The LLNA requires that a test chemical be applied to the backs of the ears of 4 or 5 young adult (6- to 16-week-old) female mice. Each mouse is treated for 3 consecutive days with varying concentrations of the material, then rested for 2 days. On the sixth day, the mice are euthanized and their lymph nodes are excised and examined. A test substance that causes a stimulation index of three or greater, meaning a three-fold proliferation of lymph node cells in the test mice, at one or more concentrations is considered to have skin sensitizing activity. "If the material is an allergen, it [will] stimulate the immune system," says Gerberick. "If you have a chemical that's an allergen, it stimulates the immune system so cells divide, and that's what we're looking at."

In the GPMT, on the other hand, the guinea pigs are injected with a chemical in the shoulder region. After 6-8 days, sensitization is boosted by wearing a patch that has been treated with the chemical over the injected site for 48 hours. Twelve to 14 days later, another occluded patch is attached to one flank for 24 hours. The two sites are then scored for redness and swelling after removal of the patches, at 48 and 24 hours, respectively.

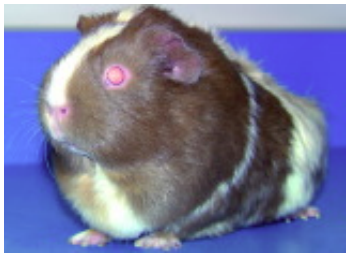
The standard Buehler test requires 20 animals in the test group plus another 20 for control groups. Three 6-hour patches are applied, one per week, to the same shaved site. After a 2-week rest period, the test animals and half of the control animals receive another 6-hour patch at another site. Then the test animals are tested again 7-15 days later. Reactions are graded according to a five-point scale.

Most studies have compared LLNA test results with those from the GPMT and the Buehler test on the same batches of chemicals. Comparison with human data began in 1991 and discussions with the Food and Drug Administration (FDA) regarding regulatory review were initiated in 1996. Results from some of the laboratories working with the FDA were published in 1998. "In contrast to the other procedures, the LLNA appears to offer increased efficiency, less cost, and less time," says Stokes. "It's a mechanistic test that looks at

the proliferation of lymphocytes in the regional lymph node. Counting the cells gives researchers a more objective measurement, rather than the subjective assessment of redness and swelling on the skin. And, from an animal welfare perspective, it requires fewer animals and [causes] less pain and distress."

Advantages and Limitations

As an alternative test method for allergic contact dermatitis, supporters claim the LLNA would also eliminate the need to boost the immune system response with an adjuvant in guinea pig tests in order for the response to be more easily measured. Injected at the chemical site, the adjuvant can cause local toxicity and unnecessary trauma to the guinea pig. The adjuvant, says Gerberick, "causes most of the discomfort in the guinea pigs, perhaps worse than a booster shot. The LLNA does not require an adjuvant."



No worries. Approval of the local lymph node assay as a stand-alone toxicity test for contact dermatitis means that guinea pigs are no longer required (as they were in standard allergenicity tests).

In traditional guinea pig tests, allergic activity is measured by observing the skin for redness. This subjective method may fail to adequately judge colored materials that can obscure reddening of the skin sites. Because the LLNA analyzes lymph node activity rather than a topical skin reaction, the color of a test material does not influence the test's performance.

The LLNA can also be used to evaluate chemical relative potency. Potency measurements are important when assessing the likely risk of sensitization in humans. Current guinea pig tests are by and large unsuitable for use in potency assessments, says Gerberick, because they are designed only for hazard identification, not to analyze the response at different concentrations, or potencies.

In the LLNA, it also is not necessary to clip or shave the fur from the mice, as must be done on several occasions during the standard guinea pig tests. Says David Basketter, a dermatotoxicologist at the Safety and Environmental Assurance Centre, part of Unilever Research, and an LLNA investigator, "[This is a] minor point, but it adds to the weight of the argument that the LLNA provides a considerable refinement in the way it minimizes the chances for distress [to the animal]."

But the LLNA does have limitations. Says Gerberick, "One question is whether it is sensitive enough. The GPMT is a very sensitive method because of its use of an adjuvant. But we feel the LLNA is sensitive enough to identify significant allergens."

The Next Step

As animal welfare advocates and the general public express their concern over the treatment of laboratory animals, researchers have begun to seek more efficient and less painful methods to test for the potential of allergic contact dermatitis in humans. After 14 years of international trials, the LLNA may satisfy this need.

The NTP center's peer review panel will draw conclusions on the usefulness of the assay from its review of the test method's protocol and data, says Stokes. This conclusion will then be forwarded through ICCVAM to

federal regulatory agencies, primarily the FDA, the EPA, and the Occupational Safety and Health Administration, which have the final say on whether the LLNA will be incorporated into their testing regulations and guidelines. The alternative test method must also undergo the OECD process for approval by its member countries in order to achieve widespread international acceptance.

Suggested Reading

Basketter DA, Gerberick GF, Kimber I, Loveless SE. The local lymph node assay: a viable alternative to currently accepted skin sensitization tests. Food Chem Toxicol 34:985-997 (1996).

Kimber I, Basketter DA. Contact sensitization: a new approach to risk assessment. Hum Ecol Risk Assess 3:385-395 (1997).

Kimber I, Hilton J, Dearman RJ, Gerberick GF, Ryan CA, Basketter DA, Lea L, House RV, Ladics GS, Loveless SE, Hastings KL. Assessment of the skin sensitization potential of topical medicaments using the local lymph node assay: an inter-laboratory exercise. J Toxicol Environ Health (in press).

Loveless SE, Ladics GS, Gerberick GF, Ryan CA, Basketter DA, Scholes EW, House RV, Hilton J, Dearman RJ, Kimber I. Further evaluation of the local lymph node assay in the final phase of an international collaborative trial. Toxicology 108:141-152 (1996).

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