

## skin corrosion

Corrosive agents are chemicals that cause irreversible damage and destruction of the skin, often burning through several layers of tissue. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and discoloration.

Corrosivity data are mainly collected by regulatory agencies concerned with the transportation of hazardous substances, in the event of a highway accident and chemical spill. In the U.S., the Department of Transportation requires the submission of skin corrosion data consistent with the standards of the United Nations Transport Authority. Corrosion is also an endpoint in skin irritation studies mandated by the Environmental Protection Agency in its assessments of pesticide formulations and ingredients. In this case, corrosivity represents the most extreme form of skin irritation, in which the skin is literally destroyed beyond the body's ability to heal.

### animal test

Rabbits are locked into full-body restraints and a test chemical is applied to the shaved skin on their back. The wound site is then covered with a gauze patch for the duration of the exposure period, normally four hours, after which the patch is removed and the degree of skin damage is read and scored at specified time intervals. Untreated skin areas serve as the control. A chemical is considered to be corrosive if, by the end of a 14-day observation period, the chemical has burned through the outer layer of the skin of one or more animals, leaving visibly dead tissue in its wake. No painkillers are provided.

Despite their years of use, animal-based skin corrosion studies have never been properly validated. In fact, evidence exists that animal studies are highly variable, of limited reliability, and generally poor predictors of human skin reactions.

**For example, a comparison of data from rabbit tests and four-hour human skin-patch tests for 65 substances found that 45 percent of classifications of chemical irritation potential based on animal tests were incorrect.** (MK Robinson et al., Food Chem Toxicol 40, 573-592, 2002)

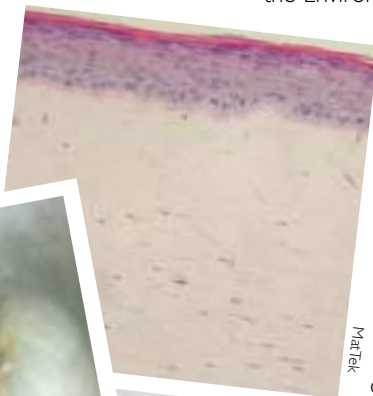


### non-animal test

Human skin equivalent tests such as EpiDerm™ and EpiSkin™ have been validated and accepted in Canada, the European Union, and virtually all other member countries of the Organization for Economic Cooperation and Development (OECD), as total replacements for animal-based skin corrosion studies. These methods consist of normal, human-derived skin cells, which have been cultured to form a multi-layered model of human skin. The reliability and relevance of human skin equivalent models has been established through rigorous, inter-laboratory validation studies overseen by the European Centre for the Validation of Alternative Methods (ECVAM), and these methods have been accepted as an official OECD test guideline. However, their acceptance as stand-alone replacements in the U.S. has been undermined by several members of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), most notably the Environmental Protection Agency, the Food and Drug

Administration, and the Consumer Product Safety Commission, which insists that "confirmatory" testing still be carried out using animals.

**Corrositex™ is another non-animal method of assessing skin corrosion. Using a protein membrane instead of skin, Corrositex™ can measure whether, and at what rate, a chemical is capable of penetrating the simulated skin barrier according to a color-change reaction.** Corrositex™ was pioneered in the U.S., assessed by ICCVAM to confirm its validity, and subsequently accepted by both the U.S. Department of Transportation and European Union as a partial replacement for animal-based skin corrosion studies.



## skin absorption

Skin absorption studies are carried out to determine the rate at which a chemical is able to penetrate the skin. A chemical's dermal delivery rate is mainly of interest to regulatory agencies concerned with chemical exposures in the workplace. U.S. federal agencies that require the submission of skin absorption data include the Environmental Protection Agency, the Food and Drug Administration, the Occupational Safety and Health Administration, and the Agency for Toxic Substances and Disease Registry.

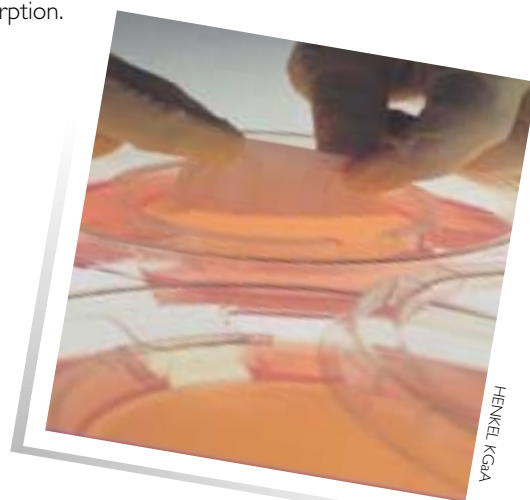
### animal test

Rats' backs are shaved and a chemical is smeared on them for an exposure period of up to 24 hours, after which the rats' skin is washed and the animals are housed individually in "metabolism cages" to permit the collection of their excrement for analysis. Animals are later killed and their skin, blood, and excrement are analyzed, after which the rate of skin absorption is calculated. Despite their years of use, animal-based studies of skin absorption rate have never been properly validated to establish their relevance to people. Other disadvantages not mentioned include the potential for biasing the results of the animal studies by the process of washing off the test chemical from the animals' skin, thus facilitating absorption of the test chemical.



### non-animal test

Various tissue culture methods have been rigorously evaluated and accepted in Europe as total replacements for animal-based skin absorption studies. These methods use skin from a variety of sources to measure the passage of a test chemical into and across skin to a fluid reservoir. Absorption of a test chemical is measured over time by analysis of the receptor fluid and the treated skin. The reliability and relevance of *in vitro* skin absorption studies have been thoroughly established through a number of international expert reviews, and these methods have been codified and accepted as an official test guideline of the Organization for Economic Cooperation and Development (OECD). The non-animal tests have a number of scientific advantages over the animal tests, including the ability to study a broader range of doses, including those at the actual level of exposure that occurs in the occupational or ambient environment. Despite these clear advantages, however, most U.S. agencies continue to rely on animal testing to measure skin absorption.



## skin irritation

Irritants are chemicals that cause skin damage that is reversible (unlike corrosion, which is irreversible). Clinical signs of irritation include the development of a rash, inflammation, swelling, scaling, and abnormal tissue growth in the affected area.

A number of U.S. federal agencies require the submission of skin irritation data, including the Consumer Product Safety Commission (cosmetics and household products), the Environmental Protection Agency (pesticide formulations and ingredients), and the Food and Drug Administration (pharmaceuticals).

### animal test

Rabbits are locked into full-body restraints and a test chemical is applied to the shaved skin on their back. The wound site is then covered with a gauze patch for the duration of the exposure period, normally four hours, after which the patch is removed and the degree of irritation is read and scored at specified time intervals. Untreated skin areas serve as the control. A chemical is considered to be an irritant if it causes reversible skin lesions, such as inflammation or other clinical signs, which heal partially or totally by the end of a 14-day observation period. No painkillers are provided.

Despite their years of use, animal-based skin irritation studies have never been properly validated. In fact, evidence exists that animal studies are highly variable, of limited reliability, and generally poor predictors of human skin reactions. For example, a comparison of data from rabbit tests and four-hour human skin-patch tests for 65 substances found that 45 percent of classifications of chemical irritation potential based on animal tests were incorrect. (MK Robinson et al., Food Chem Toxicol 40, 573-592, 2002)



PETA

### non-animal test

Government regulators in Canada accept the use of a skin-patch test in human volunteers as a valid replacement for animal-based skin irritation studies. Human patch tests offer the benefit of being directly relevant to people, thus obviating the questionable practice of extrapolating the results of rabbit tests to humans. However, before a chemical is considered for a human skin-patch test, scientists first confirm that a chemical is not corrosive (using a non-animal method described in the Skin Corrosion factsheet) and carry out computer modeling and various test-tube studies to be certain that a chemical does not possess other harmful properties. Only chemicals that appear to be non-irritating move on to a human skin-patch test to confirm their safety.



# phototoxicity

Phototoxicity, or photoirritation, is an inflammatory skin reaction caused by exposure to a chemical and subsequent exposure to sunlight or ultraviolet radiation. Phototoxicity typically appears as exaggerated sunburn, which is characterized by the presence of a rash, swelling, and inflammation.

This endpoint is mainly a concern for drugs and pharmaceuticals that are either ingested or applied directly to the skin in the form of a cream. The only regulatory agency in the U.S. that routinely requires phototoxicity studies is the Food and Drug Administration.

## animal test

Mice or guinea pigs are locked into restraints and different concentrations of a test chemical are applied to patches of shaved skin on their backs. Half the animals are then exposed to ultraviolet radiation for two or more hours, after which the chemical is removed. The animals are then kept restrained for several days while experimenters examine their skin. Swelling and sores are common. No painkillers are provided. Despite their years of use, animal-based phototoxicity studies have never been properly validated to establish their relevance to people or even codified into a standardized test guideline. In fact, the only internationally recognized guideline for phototoxicity studies is the non-animal, cell-based test described at right.



PETA

## non-animal test

The 3T3 Neutral Red Uptake (NRU) Phototoxicity Test was developed and validated in Europe and has since been accepted at the international level as a total replacement for animal-based phototoxicity studies. In this test, cells from the 3T3 cell line are exposed to a test chemical in the presence and absence of light. Photo-cytotoxicity is evaluated by the relative reduction in viability of cells exposed to the chemical in the presence versus absence of light, where cell viability is measured by degree to which they are able to absorb the dye, neutral red. Although the reliability and relevance of the 3T3 NRU Phototoxicity Test have been established through rigorous, inter-laboratory validation studies overseen by the European Centre for the Validation of Alternative Methods (ECVAM), and this method has been accepted as an official test guideline of the Organization for Economic Cooperation and Development (OECD), the Food and Drug Administration and the National Institute of Environmental Health Sciences continue to rely on animal testing to assess the phototoxic potential of new drugs and pharmaceuticals.



Institute for In Vitro Sciences

## pyrogenicity

Pyrogens are fever- and inflammation-causing agents that can pose a serious health hazard, especially in the case of intravenous drugs and pharmaceutical products. The only regulatory agency in the U.S. that routinely requires phototoxicity studies is the Food and Drug Administration.

### animal test

A rabbit pyrogen test has been in use since the 1940s. In this test, rabbits are locked in full-body restraints and a test substance is injected into their bloodstream while their body temperature is monitored. The animals can suffer effects ranging from fever to breathing problems, circulatory and organ failure, and even fatal shock. Despite its long history of use, the rabbit pyrogen test has never been formally validated to establish its reliability or relevance to humans. In fact, there are a number of well-documented drawbacks to this test, including marked species and strain differences in sensitivity. (T Hartung et al., ATLA 29, 99-123, 2001)



### non-animal test

An In Vitro Pyrogen Test has been developed and validated in Europe as a total replacement for animal-based pyrogenicity studies. As an immune reaction, pyrogenicity involves an interaction between a contaminant in a drug formulation and cells of the immune system. Using human blood donated by healthy volunteers as the test medium, this non-animal method is able to fully model the interaction between the immune system's white blood cells and the test drug, thereby confirming the presence or absence of pyrogen contamination. Additionally, this determination can be made *in vitro* with greater speed and sensitivity and at a lower cost than animal-based methods would allow. Despite these advantages, the Food and Drug Administration continues to rely on animal testing to assess the pyrogenic potential of new drugs and pharmaceuticals.

