Title
The Draize Eye Irritancy Test

Permalink
https://escholarship.org/uc/item/5ds7v9tj

Author
Buyukmihci, NC

Publication Date
2017-12-20

Peer reviewed
The Draize Eye Irritancy Test

Nedim C. Buyukmihci, V.M.D.

Summary

This paper discusses the harmful and fatal use of non-human animals, particularly rabbits, in the Draize eye irritancy test. It argues and demonstrates that such use is not scientifically valid if the results are applied to human beings. Moreover, not only can this test result in extreme suffering (and death at the end) for non-human beings, dependence on this has the potential for harm to people. Alternatives to such use are discussed in the context of being more defensible ethnically and scientifically.

Keywords: alternative, animal welfare, compassion, Draize eye irritancy test, Draize skin irritancy test, ethics, kindness, morality, non-human animal, ocular irritancy test, rabbit, scientific method, species differences, toxicity testing

In the Draize eye irritancy test, any compound which might intentionally or accidentally gain access to the eye is tested by being placed onto the eyes of conscious, restrained rabbits. The animals are observed over a period of several days to see if there is an adverse reaction to the substance. There may be no reaction or there may be irritation ranging from minor to severe. In the worst situation, the cornea may ulcerate and perforate. Because the cornea is one of the most sensitive tissues in the body, rich in nerve endings, irritation or ulceration produces considerable pain. The rabbits usually are restrained in stocks which hold the animals by the neck and prevent them from rubbing their eyes. Therefore, they cannot in any way mitigate the discomfort or pain produced by the material placed in their eyes.

As an ophthalmologist and scientist, it is my professional opinion that the Draize test (19) has little, if any, relevance to human safety. It is fraught with technical and biological problems which make extrapolation of results to the human situation not only tenuous, but also dangerous.

The rabbit is the primary animal used in the Draize eye test (11). The rabbit’s eye and reaction to topical irritants often is considerably different from that of a human being’s (11, 14, 23, 28, 38, 41). A compound found to be safe in the rabbit may actually cause great harm to a human being. On the other hand, a compound found to be toxic to the rabbit may actually cause no problems for a person while providing great benefits.

From a practical standpoint, therefore, the tremendous suffering that some of the animals go through in these tests is absolutely unnecessary. Their misery in no way guarantees the safety of human beings. Similar criticism can be made against the Draize skin test (11, 20, 41, 45).

There are numerous alternative methods to obtain data to predict whether a particular material will be safe for human use. These include those for ocular irritancy (2, 3, 4, 10, 12, 13, 17, 18, 22, 23, 24, 27, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44) and for the Draize skin test (5, 6, 7, 8, 9, 16, 25, 26, 30). These methods are more reliable and more humane than the Draize test. In some cases the methods only represent a refinement in the test or a reduction in the numbers of animals used. In other cases, however, there is evidence that a total replacement, using a number of in vitro tests, is possible.

It often is stated that a proposed alternative to the Draize test must first be ‘validated.’ This means that the proposed alternative must be reasonably close in predicting what would be the result using the standard Draize test. There are at least two systematic errors with this approach. One, as mentioned, is that the data indicate the Draize test is not a reliable indicator of human reactivity. Another is that, to my knowledge, there has been no validation of the Draize test itself. It has been accepted as the standard with no rigorous attempt at verifying its reliability. Therefore,
although it is true that new methods of determining irritancy should be ‘validated,’ the standard should be against known reactions to various categories of substances by the human eye.

Tradition alone appears to be the major momentum behind the continued reliance on such an archaic and unreliable method as the Draize test. There are no regulations which require the use of this test for determining the safety of material which may be used by human beings (1). The data derived from testing on other animals may not even be admissible in court when a person brings action against a company due to injury from a particular product (21).

References:\n

\(^{3}\) In this paper, I have cited only a few references to document various points because the literature on this subject is substantial.


27. Kiopman, Gilles; Petchelintsev, Dmitri; Frierson, Manton; Pennisi, Stephen; Renskers, Kevin and Dickens, Michael. 1993. "Multiple computer automated structure evaluation methodology as an alternative to in vivo eye irritation testing." *Alternatives to Laboratory Animals* 21(1):14-27.


30. Neves, Bruno Miguel; Rosa, Susana Carvalho; Martins, João Demétrio; Silva, Ana; Gonçalo, Margarida; Lopes, Maria Celeste and Cruz, Maria Teresa. 2013. "Development of an in vitro dendritic cell-based test for skin sensitizer identification." *Chemical Research in Toxicology* 26(3):368-378.


The Consumer Product Safety Commission wrote a letter outlining the acceptable use of several alternatives to biological testing which included human experience with hazardous substances, previous test data as reported in the literature and testing for skin irritation. “If the test shows the substance to be a skin irritant, a prudent manufacturer might also...” The letter was published in the 11 February 1982 Congressional Record, vol. 128.

Discusses some of the alternatives such as the choioallantoic membrane (CAM) and agarose diffusion method (ADM) tests.

Uses in vitro precipitation of protein as basis. Very good correlation with standard Draize test results. “It was found that different types of chemical irritants produced aggregation of the Protein Matrix Reagent in direct proportion to their potential for ocular irritation.”

CAM (vascular assay one), corneal plasminogen activator assay, SIRC cytotoxicity test, cell protein accumulation assay, neutral red uptake assay and Tetrahymena motility test are actively being pursued because they will provide refinement and reduction.

Testskin combines Living Dermal Equivalent (LDE) and Living Skin Equivalent (LSE); LSE behaves like normal skin after grafting onto animals. Has good potential for estimating human dermal irritancy.

Showed the neutral red test, using human cells, to be “...reproducible, sensitive, rapid, and economical and can be readily incorporated into the testing protocols of industrial and pharmaceutical laboratories.”

“The data presented in this report indicate that mammalian cells propagated in vitro can be successfully used for preliminary screening and ranging of toxic concentrations of potentially irritant compounds. These assays are economical, easy to execute and can reduce the number of animals required for in vivo testing.”

“Moreover discrepant discrepancies in the response of the rabbit and human eye impede the extrapolation from animal data to man.”

“Anatomical, physiological and biochemical differences between animals and humans prevent direct prediction of the expected irritancy in man from data on experimental animals.”

“...the information that is necessary to prevent a possible hazard to man is the identification of an irritant or a corrosive substance. If this straightforward goal is kept in mind, in vitro techniques will soon provide reliable data for the estimation of the irritancy potential in man.”

“From the results of this preliminary study, it appears that the method could provide an alternative to animal studies on the ocular tolerance of cosmetics and toiletries.”

“...our data suggest the feasibility of using multilayered human tissues for automated multiple endpoint analysis.”

“These results suggest that the EYTEX [TM] test could be a reliable in vitro prescreening system for determining the eye irritation potential of cosmetic formulations, and that it should be accepted as a component of such a battery of alternative methods.”

“The ideal in vitro test must utilize human corneal tissue in order to obviate the need to extrapolate laboratory animal eye irritation to human situation. Unquestionably, the Draize alternative is sensitive to toxicities which result from a broad range of doses of test substances. The observed ED50 [effective dose 50%] of the most toxic material tested differs from the ED50 of the least toxic material by a factor of more than one million...Within this broad range of doses, the toxicities of many substances have been determined with sufficient precision that a statistically sound conclusion can be drawn...With the Draize test, this degree of precision has never been achieved. The preliminary studies support the contention that a simple, well designed, cell culture alternative can duplicate the findings of in vivo Draize experiments and extend these by obeying the quantitative, practical, and humanitarian problems associated with Draize testing.”

From Ciba-Geigy, Switzerland. Review of the skin test, mentioning shortcomings.

Evaluates the standard Draize test against the low-volume eye irritation test; demonstrates that the latter is a more reliable predictor of human response; as far as Draize test, problems include: “Common sense as well as years of empirical data tell us that the Draize test does not accurately predict human response to soaps and detergents...does not predict human ocular response to moderate irritants...the animal tests show a high proportion of longer clearing times than the human exposures, i.e., they overestimate the human experience.” Although “...safety assessments and labeling decisions...” are made using the Draize test, “...the reliability of the Draize test was never established for this purpose.”

Summary of some of the in vitro tests available: uridine uptake, colony inhibition assay, and macrophage chemotaxis.

The system, using human cells, is capable of detecting extremely minute quantities of the chemicals used as indicators; the test has good potential.

“The Multi-CASE program successfully predicted a priori the eye irritation potential of: 1) 21 individual chemicals; 2) chemical mixtures such as coconut oil, hydrogenated castor oil, rice bran, eucalyptus oils and potassium alginate; and 3) polymers, including polyvinyl alcohols, polyvinyl butyral, polyvinyl pyrrolidone, corn starch and nylon.”

“The Draize test is a poor scientific model and has little redeeming application to the development of preparations intended for use in the human eye, because animal results cannot be predictably extrapolated to humans. For the same reason, it is equally inappropriate to use the results of Draize testing for household products or for cosmetics which may reach the eye accidentally.”

“100% sensitivity and 85% predictability were described by the data, indicating the efficiency of EYTEX in identifying known irritants. The EYTEX system protocols, when used appropriately, can provide a conservative means of assessing the irritant potential of most cosmetic formulations and their ingredients.”

“The results demonstrate that the SIRC cell line is a suitable model system for screening shampoos of differing surfactant compositions without the need to use live animals.”

Showed that the kit is easy to use and there was adequate interlaboratory correlation.

Six surfactants were studied using a bovine hemolysis assay. The results were compared with data from other in vitro tests and with ocular irritation responses in vivo. The data generated using the hemolysis test was [sic] similar to those obtained with the other systems.

“The aim of this study was to evaluate a multi-endpoint cytotoxicity screening method using V79 cells based on four different endpoints of cytotoxicity: trypan blue exclusion, reduction of XTT, neutral red uptake and total protein content. In addition, cell morphology was routinely observed after each treatment. Test compounds were studied, which can be divided into five classes: protein synthesis inhibitors (cycloheximide, actinomycin D); inhibitors of cell division (bleomycin, vincristine); membrane-active compounds (Triton X-100); lysosomotropic agents (ammonium chloride); and general toxicants (sodium chloride). We obtained a variety of different toxicity profiles, which may be useful in defining the mechanisms of toxic action of these compounds. The multi-endpoint screening system proved to be readily applicable, robust and rapid, and gave reliable toxicity results over a wide range of chemical concentrations.”

“The results demonstrated that this easily performed method leads to few false negatives (6%) and thus merits its inclusion in a battery of screening tests for predicting eye irritancy without the use of animals.”

Good review of the alternatives mentioned in the title.

Some differences between rabbit and human are: maximum volume of fluid in conjunctival cul-de-sac is about 0.08 ml in rabbit, 0.01 ml in human; blink rate in the rabbit is a few per hour, about 12 times a minute in the human; although immediate reaction to irritant is sensitized (watery) tears in rabbits, sensation of eyes becoming aversively dry and stinging due to Harderian gland secretion (no Harderian gland in human); corneal surface area relative to that of whole globe is 25% in rabbit, 7% in human. The article reviews the Draize test and the scientific shortcomings with suggestions for refinement.
This study confirms the findings of an earlier report by De Sousa et al. (1984), and indicates that a high level of accuracy can be obtained with reduced numbers of rabbits per test.

Madin-Darby canine kidney cell line used; fluorescein usually will not pass, but will when the cells or their junctions are damaged. These results correspond well with the known Draize test results of these Tween compounds. It is suggested that this in-vitro test system represent [sic] a suitable alternative for the Draize Rabbit Irritancy Test.

This report...is concerned with circumstances in which essentially negative findings were obtained in laboratory screening tests although relatively severe visual disturbances and discomfort occurred soon after the product was launched in a test-market. In the course of laboratory investigations, there was only the mildest response in the rabbit eye. This was the case whether the product [a hair dressing] was instilled undiluted or diluted with water, and whether it was left in situ or rinsed out with water soon after instillation...Neither the neat product, nor a 25% aqueous dilution, produced any sign of irritation in the eyes of squirrel monkeys. Much greater irritancy could be shown on animal skin than in the eye. The original product in concentrated form, or diluted up to 1:4 with water, was distinctly irritable to intact and abraded rabbit skin and to the ears of female CF/1 mice, after four daily applications...On the other hand, there was no irritant effect on human skin either in prophetic patch-testing or in normal use.

"...the Draize score is a multidimensional measure of cell lysis, organelle dysfunction, breakdown of tight junctions and inability of cells to divide so as to repair the test lesion. To this end we have developed techniques which share in common the use of vital fluorescent stains, each of which is specific for one of these parameters."

Collection of exfoliated cells from the eyes of albino rabbits was performed in conjunction with the Draize ocular irritancy test. The number of cells which were retrieved, correlated well with Draize test scores. This non-invasive modification of the Draize test was well tolerated by rabbits and provides an objective assessment of ocular irritancy. It is hoped that this refinement of the Draize test will reduce the numbers of animals used and provide more objective data upon which validation procedures for alternative methods can be based.

This test has been scientifically validated and is well-established as an in vitro alternative test to screen the toxicity of plastics in medical devices...the agarose diffusion model may be slightly more sensitive than the Draize eye method. These results indicate a high degree of correlation between an in vitro screening test as an alternative to the Draize eye irritancy test..."