HAZARD EVALUATION DIVISION

STANDARD EVALUATION PROCEDURE

GUIDANCE FOR EVALUATION OF EYE IRRITATION TESTING

Prepared by

Frank J. Vocci
and
Van M. Seabaugh

Standard Evaluation Procedures Project Manager
Orville E. Paynter, Ph.D., D.A.B.T.
Hazard Evaluation Division
Office of Pesticide Programs

United States Environmental Protection Agency
Office Of Pesticide Programs
Washington, D.C. 20460

302.2-101

REPORT DOCUMENTATION PAGE	1. REPORT NO. EPA-540/09-88-105	2.	3. PB89-124572
4. Title and Subtitle: HAZARD EVALUATION DIVISION: Standard Evaluation Procedure Eye Irritation Studies			5. Report Date: August 1988 6.
7. Author(s): Van M. Seabaugh and Frank J. Vocci			8. Performing Organization Rept. No. EPA 540/09-88-105
9. Performing Organization Name and Address: U.S. Environmental Protection Agency/OPP/HED/TS-769c 401 M Street SW Washington, DC 20460			10. Project/Task/Work Unit No. 11. Contract(C) or Grant(G) No. (C) (G)
12. Sponsoring Organization Name and Address: Same as #9			13. Type of Report & Period Covered 14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

The Standard Evaluation Procedure for Eye Irritation Studies states the purpose and requirements, and the test is further defined in the Pesticide Assessment Guidelines [Subdivision F, Hazard Evaluation: Human and Domestic Animals, (1982, revised 1984)]. Background information is given on the development of the test, animal models, scoring system, labeling, and factors affecting the results. There are discussions on objective and alternative technologies, evalation based on weight-of-evidence, epidemiological data, and low dose and dose response studies. A tier system is presented incorporating present methods and possible future alternatives. Data Reporting Guidelines (Subdivision F, Series 81-4, Eye Irritation) are available [National Technical Information Service (NTIS), accession no. PB88-161179; EPA document no. 540/09-88-023)].

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Unclassified and freely available	Unclassified	36
	20. Security Class (This Page) Unclassified	22. Price

STANDARD EVALUATION PROCEDURE

PREAMBLE

This Standard Evaluation Procedure (SEP) is one of a set of guidance documents which explain the procedures used to evaluate environmental and human health effects data submitted to the Office of Pesticide Programs. The SEPs are designed to ensure comprehensive and consistent treatment of major scientific topics in these reviews and to provide interpretive policy guidance where appropriate. The Standard Evaluation Procedures will be used in conjunction with the appropriate Pesticide Assessment Guidelines and other Agency Guidelines. While the documents were developed to explain specifically the principles of scientific evaluation within the Office of Pesticide Programs, they may also be used by other offices in the Agency in the evaluation of studies and scientific data. The Standard Evaluation Procedures will also serve as valuable internal reference documents and will inform the public and regulated community of important considerations in the evaluation of test data for determining chemical hazards. I believe the SEPs will improve both the quality of science within EPA and, in conjunction with the Pesticide Assessment Guidelines, will lead to more effective use of both public and private resources.

Anne L. Barton, Acting Director
Hazard Evaluation Division

Table of Contents

Preface

An application for registration of a pesticide requires toxicity data from which short and long term risks may be ascertained. Within the body of short term studies, the potential for a pesticide to produce eye irritation is an uppermost consideration in the assessment process. These concerns culminated in November 1982 when the EPA's Office of Pesticide Programs issued testing guidelines (Subdivision F: Pesticide Assessment Guidelines, Hazard Evaluation - Human and Domestic Animals) and Series 81-4 of these guidelines provides the basis for eye irritation testing.

Data on eye irritation are required by the Code Of Federal Regulations (40 CFR Part 158) to support the registration of each manufacturing-use product and each end-use product. The "Pesticide Assessment Guidelines" (EPA, 1982) further discusses these requirements. Refer specifically to 40 CFR 158.50 and 158.135 to determine if these data must be submitted. The section of 40 CFR 158.50 entitled "Formulators Exemption" requires a registrant of a manufacturing-use product to submit (or cite) any data pertaining to the safety of an active ingredient if the same data are required to support the registration of an end-use product that could legally be produced from the registrant's manufacturing-use product.

The purpose of this Standard Evaluation Procedure (SEP) is to provide supplementary information to §81-4. This document further assumes that human experience or epidemiological evidence is wanting, and judgments of classifications are wholly dependent upon animal tests. Therefore, the basis for classification may be superseded when valid, epidemiological evidence outweighs the surrogate test which previously had demonstrated either false negative or false positive results. Without the epidemiological evidence, it is anticipated that sufficient guidance for classifications is provided with this test.

Eye Irritation Studies

A. Historical Background

Ophthalmic toxicity reviews are available (EPA, 1981; McDonald et al., 1983; NAS, 1975). Mann and Pullinger (1942) described the use of rabbits to predict ocular toxicity of test substances in humans. These latter authors advocated the use of pigmented eyes rather than non-pigmented ones (albino rabbits), and relied on description of individual animal responses to address the irritant properties of test substances. Friedenwald et al. (1944) reported an albino rabbit method of assessing ocular toxicity that provided a scoring system based on the description of individual animal responses. Draize et al. (1944, 1955, 1959) modified Friedenwald's procedure, and published an eye irritancy grading system to further assist the evaluation of ocular toxicity of test substances. With the passage of the Federal Hazardous Substances Act (FHSA) in 1958, a modified "Draize" procedure became the required test and illustrated eye guides have been published as further aids in training (FDA, 1965; CPSC, 1976; EPA, 1981).

B. Anatomical Considerations (Bloom and Fawcett, 1975); (Figure 1)

1. Conjunctiva

The conjunctiva is the nonkeratinized squamous epithelium containing mucous secreting cells that covers the anterior sclera (bulbar conjunctiva) and inner surface of the eyelids (palpebral conjunctiva). It is continuous with the epidermis at the lids and the cornea at the limbus. The palpebral conjunctiva is more stratified and contains more goblet cells than the bulbar conjunctiva. Glycoproteins from the conjunctiva contribute to the stability of the tear film (Holly and Lemp, 1971).

2. Cornea

The cornea is an avascular transparent tissue which is inserted to the sclera at the limbus. From anterior to posterior the cornea has five distinct cell layers; the epithelium, Bowman's membrane, stroma, Descemet's membrane, and the endothelium. The epithelium, a nonkeratining stratified squamous type, is composed of five to six layers. The basal layer is attached to the basement membrane by numerous hemidesomosomes and is composed of cuboidal cells which are closely packed. The cells of the middle layers are polygonal in shape and bordered by many interdigitations attached by desmosomes. The most superficial layer of cells is squamoid in shape and covered with numerous microvilli (Phister, 1973).

Bowman's membrane, a modified portion of the anterior stroma, is a clear acellular layer whose thickness varies among species. This structure at a thickness of 2 um in rabbits is essentially unrecognizable but is prominent in humans and other primates. The thickness of this structure is humans has been reported as 8 to 12 um (Prince et al., 1960).

The stroma accounts for 90 percent of the thickness of the cornea. It is composed of parallel lamellae of collagen fibrils which run from limbal border to limbal border. Numerous keratocytes are scattered throughout the collagen fibrils. The diameter of collagen fibrils increases at the limbus where the fibers insert into the scleral stroma and begin to assume an interdigitated and twisted appearance.

The next layer, Descemet's membrane, is a clear elastic membrane of the final layer of the cornea, the endothelium. Descemet's membrane shows little interspecies variation in thickness of structure.

The endothelium is composed of a single layer of squamous cells; the apical surfaces are tightly attached by Zonula Accludentes. The cells form a hexagonal array when viewed via slit lamp, and are 4 to 5 um thick in humans and slightly thinner in other species. Their function is extremely important in maintaining the relative state of dehydration of the corneal stroma. For this reason, damage to the endothelium is more serious than damage to the epithelium.

Comparatively, the corneas of animals differ from those of humans in several ways. The rabbit cornea is thinner than the human cornea with most of the difference accounted for by the thickness of the stroma (Table 1). The collagen fibrils are more loosely packed in rabbits than in humans. As previously stated, Bowman's membrane is thinner in other animals than humans.

Examination of the cornea for irritant effects includes evaluation of both stromal lesions and epithelial lesions. Large epithelial defects are readily identifiable by slit lamp biomicroscopy whereas smaller defects are easily visualized by the addition of fluorescein sodium drops and the use of a cobalt blue filter on the slit lamp. Epithelial lesions may occur independently of stromal lesions provided the basal cell layer remains intact. Stromal lesions are detected as opacities which represent edema/infiltration of the cornea by inflammatory cells. Increasingly severe stromal lesions are indicated by an increase in opacity area of corneal involvement.

Prolonged corneal edema induces proliferation of endothelial cells at the limbus resulting in the formation of new vessels extending toward the center of the cornea.

Neovascularization of the cornea (pannus), occurs readily in rabbits and represents a permanent change secondary to ocular insult by irritants. Permanent corneal opacities may also result from severe epithelial and stromal lesions.

The penetration of chemical compounds into the cornea is biphasic. Lipid soluble substances can readily pass through the corneal epithelium and hydrophilic substances can pass through the stroma. A test compound must have a lipid soluble and water soluble phase to penetrate the cornea effectively.

Studies by Cogan and Hirsch (1944) and Cogan et al. (1944) have shown that the absence or presence of charge alone could not account for differences in permeability and that molecular size of a test compound correlated only approximately with corneal penetration. They further concluded that the corneal penetration of weak electrolytes was facilitated when the dissociation constant of the compound is small so that the test compound is present in an undissociated state at the pH of the eye and when the undissolved form of the test substance possesses lipophilic characteristics.

Krueger (1959) verified the findings of Friedenwald et al. (1944, 1946) that the corneal epithelium represents a barrier to the penetration of acids. Alkalies penetrated into the anterior chamber more quickly than acids in isolated pig eyes with intact corneal epithelium, but the rates of penetration of acids and alkalies were approximately the same when the corneal epithelium was removed. For this reason, the early assessment of ocular damage by acid burns is a measure of long-term damage to be expected (Potts and Gonasun 1975) whereas alkali burns result in progressive lesions so that initially mild lesions frequently develop into severe lesions as a late complication (Hughes 1946).

3. Iris

The iris is the anterior extension of the ciliary body. Thusly, ocular irritants applied topically to the cornea may produce pathologic changes in the iris.

Although not required by EPA, the slit lamp may be used to further ascertain eye injury (Baldwin et al., 1973). Injury to the iris in irritation studies is monitored by observing injection in the vessels, the thickness of the iris stroma, and aqueous flare. The vessels of the iris become hyperemic following irritation. Leakage of fluid from the unfenestrated capillary endothelium in the stroma results in edema recognizable in the slit lamp as a swelling of the iris. The Tyndall phenomenon,

commonly called aqueous flare, results from the release of proteinaceous material or cells into the aqueous humor. In the normal aqueous chamber, the light beam is not discernible. However, the proteinaceous material released during iritis changes the refractive index of light so that the light beam from a slit lamp becomes visible as it passes through the aqueous humor. Aqueous flare is, therefore, presumptive evidence of a breakdown in the blood aqueous barrier. The order of changes in iris irritation in increasing severity is hyperemia of vessels, stromal edema, and aqueous flare. The presence of inflammatory cells and aqueous flare are diagnostic of frank iritis.

C. Experimental Models

1. Albino Rabbit

An important criterion in selecting an animal or test model to evaluate potentially hazardous compounds is the ability of the animal test to predict the human response. Ideally, this animal would exhibit similar sensitivities to a wide range of chemical compounds, and would be docile, inexpensive, and readily available. Although several species of animals including dogs and rhesus monkeys have been used in ocular testing, the albino rabbit has several advantages and has been used most frequently. The albino rabbit is easy to handle and maintain and is relatively inexpensive. The corneal surface and conjunctiva are large and easily seen. The unpigmented iris is easy to evaluate in terms of congestion of iris vessels. A large data base exists for rabbits.

A review of the literature of ocular testing in albino rabbits reveals that this animal exhibits similar or more sensitive responses for many test compounds, less sensitive responses for a few test compounds, and totally failed to predict an irritant response for several compounds (Carter, 1906; Lewin and Guillery, 1913; Leopold, 1945; Carpenter and Smyth, 1946· Hogiwara and Sugiwia, 1953; Grant, 1974; Van Abbe, 1973; Marsh and Maurice, 1971).

In contrast to the above reports where the albino rabbit accurately predicted the human response, there are several reports where the sensitivity of the albino rabbit to test compounds was either greatly diminished or nonexistent (Estable, 1948; Gartner, 1944; Lewin and Guillery, 1913; Marsh and Maurice, 1971; Grant, 1974· Van Abbe, 1973· Beckley et al. 1969).

2. Other Species Used for Testing

The anatomical differences between the human and rabbit eye and the failure of the rabbit to predict the human response in several instances of ocular testing have led to criticism of the use of rabbits as the experimental model in

ocular toxicity testing. The use of primates has been suggested on the basis of anatomic similarities. However, it must be remembered that primates are expensive, difficult to obtain, costly to maintain, and not always easy to restrain. In general, studies of the comparison of responses of rabbits and primates to ocular irritants have shown that primates more closely predict the human response, and the rabbit is usually the most sensitive species (Beuhler and Newmann, 1964; Beckley, 1965; Beckley et al., 1969; Green et al., 1978; Hirst et al., 1981).

From the literature cited, it is concluded that the rabbit is a more sensitive species, but important exceptions exist to the general rule (Table 2). For this reason, one may consider the albino rabbit to be a very good, but not a failsafe test model. Data derived from rabbit tests may not be directly extrapolated to man in terms of anatomical location and/or severity of irritancy. The use of other species for testing purposes may be justified in some instances. Furthermore, epidemiological data as to potential exposure in humans must be considered (e.g., compounds dispersed as aerosols in the environment should be tested as aerosols).

3. Evaluation of Ocular Testing

a. Scoring Systems

The grades obtained from ocular testing are used for labeling purposes (CPSC 1976, EPA 1982).

b. Factors Affecting Results

Marzulli and Ruggles (1973) conducted a collaborative study using 10 laboratories. Each laboratory tested seven materials instilled directly on to the cornea and rated the ocular responses in terms of corneal opacity, chemosis, conjunctival injection, and iritis at 1, 2, 3, and 7 days. All laboratories were capable of distinguishing irritants when all four criteria were employed but varied widely in separating irritants from nonirritants when a single criterion was used.

McDonald and Shadduck (1977) reported the results of a statistically based experimental study designed to determine variability among a group of investigators and within a single investigator. Analyst uniformity among three investigators was considered very good for all ocular parameters except congestion and discharge (e.g., correlation coefficients for corneal opacity and corneal area ranged from 0.90 to 0.97). Analyst precision for each of three investigators ranged from 0.73 to 0.88. The authors reported that the scores obtained were acceptable and stressed that reliability was achieved by trained investigators.

Seabaugh et al. (1976) evaluated the length of exposure and the degree of chemically induced eye irritation in albino, New Zealand Rabbits. This study evaluated the utility of ocular irrigation to determine if such a procedure improves the predictive capability of the rabbit eye irritation test. All of the 32 chemicals tested were compared for effects of length of exposure and a 2 minute irrigation. In comparing 30 second vs. 24 hour exposure groups for irritancy changes, 20 percent of the chemicals decreased, 5 percent increased, and 75 percent showed no changes. With 2 minute vs. 24 hour exposures, 15 percent decreased, 10 percent increased, and 75 percent had no change. For 5 minute vs. 24 hour exposures, 13 percent decreased, 10 percent increased, and 77 percent did not change. For 6 chemicals tested, there were predominantly no apparent benefits from washing rabbit eyes with either 2 or 5 minute irritation at any of the chemical exposure times as compared to 24 hour exposures. With the exception of 3 powders among all of the other irritant chemicals tested, rabbit ocular responses did not change to the category of non-irritant following shorter exposures. This data indicated that irrigation of rabbit eyes does not contribute to the outcome of irritant/nonirritant eye classification categories for 16 CFR 1500.42 (Federal Hazardous Substance Act Eye Tests).

An additional factor affecting subjective scoring is the manner in which data are reported. Ballantyne and Swanston (1972) maintained that summed ocular scores are uninformative about individual tissue responses because identical mean scores could be obtained for two entirely different reactions. Shuster and Kaufman (1974) stated that the addition of scores assigned to different tissues is statistically improper because some lesions are more important than others (e.g., mild but persistent corneal lesions are more threatening than severe transient conjunctival injection. Ocular scores must be reported separately for each ocular tissue, as indicated in the illustrated guide (FDA, 1965).

D. Completion of Evaluation

1. Objective and Alternative Technologies

The previously described subjective tests have long been criticized for a lack of standardization in methods and assessment. The lack of uniformity in testing methods has provided the impetus to develop objective methods of *in vitro* assessment of ocular toxicity. One such method is the method of corneal thickness by use of a pachometer (Mishima and Hedbys 1968). Although the technique adds to the time and expense of ocular testing, it has the advantages of being both highly reproducible and noninvasive (Burton, 1972; Conquet et al., 1977).

Another line of investigation in the objective measurement of ocular toxicity is the measurement of intraocular pressure (IOP). Increases in IOP have been reported by several investigators following application of known eye irritants (Walton and Heywood, 1978; Ballantyne et al., 1972; Maul and Sears, 1976).

Histopathological analysis, while not truly an objective technique, is a sensitive and reliable technique for evaluation of ocular pathology (Weltman et al., 1965; Green et al., 1978).

Several additional techniques have been successfully employed to evaluate objective changes following the application of known irritants to the rabbit eye. Maul and Sears (1976) measured pupil diameter and the concentration of protein in the aqueous humor of rabbit eyes following exposure to nitrogen mustard. Jampol et al. (1975) had previously shown that the response to nitrogen mustard depends on sensory innervation and is not mediated by prostaglandins to any significant degree.

Maurice (1968) described a modification of slit-lamp biomicroscopy that he termed specular microscopy. The addition of this apparatus allows one to view and photograph endothelial cells of the cornea. Sugar (1979) has reviewed the use of the specular microscope in ophthalmological investigations. The utility of this instrument is in providing information and a photographic record of the effects of potential eye irritants on the corneal endothelium.

The use of scanning and transmission electron microscopy may prove useful in evaluating toxic effects of potential irritants and topical ocular products (McDonald and Shadduck 1977). However, Burstein (1980) pointed out that the major drawback of electron microscopy is the requirement of chemically fixed tissue in the dehydrated state. Electron microscopy is also expensive, time consuming, subject to sampling error, and requires highly skilled personnel. It probably finds its best application in the investigation of the process of ocular irritation and not the determination of irritancy for a specific test compound.

Both the subjective and objective testing parameters currently employed in ocular irritation rely on the use of intact animals for the endpoint determinations. There has been a large investigative effort to move toward the use of cell and organ cultures to determine the irritant potential of test compounds. The aim is to develop *in vitro* testing methods that would greatly reduce or eliminate entirely the need for animal testing. Such bioassay methods would provide objective data that is rapidly obtained, cost effective, and subject to standardization. This

line of investigation has diverged along two tracks, biochemical assays and morphologic assays. The biochemical assays will be summarized first, and followed by a summary of the morphologic methods.

Gasset et al. (1974) employed enzyme histochemistry to determine the effect of ophthalmic preservatives on rabbit eyes. Staining of NADH-2 oxidoreductase was employed to determine the viability of the corneal endothelium.

Borenfreund et al. (1983) described an in vitro assay based on the reduction of 3H-uridine uptake in HEP-G2, an established human hepatoma cell, and Balb/c 3T3, a mouse fibroblast cell line. A similar method, detailing a technique for determining irritant potential by ATP measurements was described by Kemp et al. (1985). Sciafe (1985) described a fluorometric and membrane bound enzyme released assay for determining in vitro the cytotoxic potential of surfactants. Other biochemical assays were investigated (Goldberg 1985). The University of Washington studied the release of plasminogen activator by corneal cells after exposure to irritants. The preliminary data suggested that corneal cell cultures release plasminogen activator in response to exposure to eye irritants. A similar line of investigation was pursued at Johns Hopkins University. Using Chinese hamster ovary cells, the potential of irritants to release hydrolytic enzymes from the fibroblasts was studied (Goldberg 1985).

Since irritants produce nonspecific cytotoxic effects, the damage produced may be simulated by in vitro studies which use a variety of endpoints. These endpoints for toxicity include cell viability as determined by trypan blue and fluorescence (Kemp 1985), morphological changes (Shopsis 1985), cell detachment, cloning efficiency (Reinhardt et al. 1985), and cell membrane integrity (Sciafe 1985).

Other morphological techniques applied to the problem of in vitro detection of irritants would include the following: Cytologic and colony inhibition assays employing Balb/c 3T3 cells. Semiconfluent cell cultures are exposed to the potential irritant for 24 hours then examined by phase microscopy for evidence of toxicity such as vacuolization and blebs. The results of these assays have correlated well with data obtained from the Draize test (Shopsis et al. 1985). Reinhardt et al. (1985) reported on a comparative study of 3 different cell culture lines exposed to 57 potential irritants. Their endpoints were cell detachment as determined by an automated cytometer, cloning efficiency, and growth inhibition. The results showed that all three cell lines and three endpoints were equally effective in determining the rank order of irritants. Muir et al. (1983) investigated the

hemolytic potency and ability to block spontaneous contractions in isolated mouse and rabbit ileum of eight surfactants. The results showed that hemolytic potency failed to correlate with *in vivo* ocular testing and the isolated rabbit ileum preparation gave the best correlation with *in vivo* findings in the rabbit eyes. Additional morphologic-based tests are in various stages of development (Goldberg 1985). Human corneal cell lines were evaluated at the Eye Research Institute in Boston for their ability to predict irritant potential. Cytotoxicity is evaluated by phase microscopy, trypan blue exclusion, and long-term survival as determined by repeated cell counts.

Although not strictly an *in vitro* test, the chick chorioallantoic membrane (CAM) has been proposed as a model for irritancy testing (Leighton et al. 1985: Luepke, 1985). However, Parish (1985) maintains that several problems exist in the CAM in the ability to predict for pure chemicals. Known irritants did not produce a dose response whereas several nonirritants killed most embryos and produced necrosis in the CAM test.

The problem of validity of *in vitro* tests as screening tools for eye irritancy is not confined to the CAM test, but is a problem for all *in vitro* tests at present. The ability of these tests to be used as large-scale screening mechanisms that would reduce the need for animal testing awaits the conformation that will come only from large scale testing and refinement in the techniques. The problem of validity was addressed by the FRAME (Fund for Replacement of Animals in Medical Experiments) laboratory in Nottingham, England (Balls and Horner 1985). This laboratory devised a scheme for validation of *in vitro* alternative methods that includes a set of coded chemicals to be used in blind trials of *in vitro* tests while summaries of the *in vivo* toxicity of the test compounds are being produced. Data submitted from blind testing will be evaluated for interlaboratory variation and its correspondence to *in vivo* testing data by an independent assessment team. Bosshard (1985) stated that only a small number of compounds have been tested to date by the newly developed *in vitro* methods; for this reason, there is a current drought of information on the interlaboratory variation in testing and the significance of the test data for predicting untoward effects in man. The systematic comparison of *in vitro* and *in vivo* data will be required to answer the issue of validity. Epidemiological data gained from accidental exposure to chemicals will be required for fine tuning of the estimation of the human response to irritants derived from *in vitro* data. While it is doubtful that a single *in vitro* test will fulfill the requirement for all test compounds, it is entirely possible that a battery of *in vitro* tests as proposed by Shopsis et al. (1985) will reduce animal testing to a minimum.

2. Evaluation Based on the Weight of Evidence

Toxicity in ocular tissues is synonymous with corrosive/irritant effects. These effects are qualitative, and not subject to the decision making process on the basis of numbers. It is for this reason that one must use their professional judgment to accept or reject data and to articulate the rationale for doing so. When data are ambiguous and nonconclusive, that is, one of six animals shows a positive response of significant duration, the reviewer should base his/her decision on the nature and duration of the response rather than the number of animals responding. The reviewer must then provide the toxicity category of the pesticide which can be interpreted from Table 4. In cases where both corneal involvement and irritation are observed, the test material should be placed in the highest category based on the duration of the response. The labeling of that test material is clearly then based on both the effects produced and their duration, and not on the number of animals responding. For this reason the EPA has adopted the NAS recommendation that the observation period in eye testing be extended to 21 days (NAS 1977; Campt, 1981). Considering the dose of 100 uL per eye, data which are not indicative of an all-or-none response are suspect, and one must carefully evaluate the effects in the positively responding animals for proper classification.

For a valid eye irritation test, at least 6 rabbits must survive the test for each substance. A trial test on three rabbits is suggested. No further testing is necessary if the test substance produces corrosion, severe irritation, or no irritation. If the data for a test compound shows only a mild transient conjunctivitis (2 or 3 days) in one or more animals with no delayed type reaction, then no further testing is required. However, if equivocal responses occur, testing on at least 3 additional animals should be performed. If the test substance is intended for use around the eye, then testing on at least 6 animals should be performed.

3. Epidemiological Data

Data derived from small animal testing is presumably predictive, but not necessarily indicative of the human response. Epidemiological data acquired from accidental exposure should be considered retrospectively and classification of compounds adjusted accordingly. Chlorpromazine, a phenothiazine tranquilizer that is still currently used clinically, was extensively tested in both animals and humans before its approval by the FDA in 1953 and found to be noninjurious to the eye (Marzulli, 1968). Clinical experience has shown that chlorpromazine induced deposits in both the cornea and lens of patients. Similar experience with a related compound, thorazine, has shown that it can produce cataracts and an increase in retinal pigmentation. The importance of

these clinical observations underscores the fact that animal testing is only presumed to be predictive and the true definitive answer usually unfolds only with extensive human experience.

McLaughlin (1946) detailed 602 cases of eye burns resulting from accidental exposure and reported that the most serious injuries were caused by highly acidic and basic materials. Among these patients, 7 injuries involved loss of vision whereas 458 were mild and cleared within 48 hours; the remainder required up to 10 days to heal. Clinical studies of this nature would greatly facilitate the expansion of the data base were they to include information on the type of compound, its concentration, and the exposure conditions. Evidence as to the irritancy potential of a compound derived from epidemiological studies and clinical experience should be considered prima faciae evidence that supersedes data derived from animal testing. Epidemiological data should, therefore, receive the highest consideration in the reclassification of a test compound.

4. Low Dose Testing and Dose Response Studies

Williams (1985) conducted a study of seven known irritants applied directly to the cornea of albino rabbits in volumes of 10 uL. The results showed that the severity of the reaction was reduced, but the rank order and ability to predict irritancy were not altered. The results were in agreement with a prior study of seven different compounds reported by Williams et al. (1982). A dose-response relationship was clearly established in terms of both duration of effect and maximal score for test compounds used at volumes of 10 ul and 100 ul. Again, test sensitivity was not compromised and the rank of severity remained unchanged for the 10 ul dose results. Griffith et al. (1980) performed a dose response study of eye irritation employing 21 test compounds at 4 different doses applied to the eyes of albino rabbits and scored for a 21-day observation period. By comparison of the maximal score and duration of effect with the best available data from human experiments, this group concluded that 10 ul represents a more realistic dose for hazard testing than the 100 ul currently employed by the Draize protocol. This reduced volume recommendation was considered at a recent meeting of the OECD in April 1986. The United States, the United Kingdom, and Switzerland supported the recommendation whereas opposition was expressed by France, Germany, Belgium, Spain, Japan, and The Netherlands. The use of the low-volume test as a screening test was discussed but not adopted as an official recommendation by the working group (Murphy 1986). It is EPA's (FIFRA) policy to insist on a dosage of 0.1 mL as stated in the rabbit eye irritation testing guideline until such a time that there is sufficient scientific data supporting the reduced volume. When this data becomes available, a recommendation will be made to EPA to change the guideline.

E. Toxic Classification of Compounds Based on the Weight of Evidence

Several definitions for the classification of test compounds have been proposed. According to 40 CFR Part 158, the difference between an eye irritant and a corrosive compound is that the corrosive produces irreversible tissue damage to the eye whereas the eye irritant produces changes which are reversible. It should be realized both irritants and corrosives produce cytotoxic effects that result in inflammation which in the case of corrosives yield either rupture of the globe, long-term erosions, or healing with scar formation. EPA uses a classification of irritants and corrosives based on both effect and duration of effect on ocular tissues (Table 4).

Beckley et al. (1969) proposed an eye irritant classification system based on fluorescein staining and slit lamp biomicroscopy. Although it is not part of EPA guidelines, it is instructive in that it details guidelines for acceptance of irritants based on minimal effects of short duration and more ominous effects that should alert oneself to the necessity for labeling a compound as hazardous (Table 3).

F. Tier System - Future Testing

Current EPA guidelines mandate that compounds to be registered be tested as manufacturing end-use products (40 CFR Part 158). These guidelines state strongly acidic or basic compounds with a pH of 2 or less or 11.5 or greater need not be tested owing to their predictive corrosive properties. Also, compounds that have been demonstrated as corrosives and irritants by dermal testing need not be further tested for eye irritation. It may be presumed that these substances could produce similarly severe effects in the eye. Williams (1984) found that 45 of 60 dermal irritants produced severe or moderate irritation in ocular testing. The remaining 15 skin irritants produced mild effects which cleared within 3 days. Perhaps the greatest change in the future testing will be the introduction of *in vitro* tests which have been covered, in part, in this document. These *in vitro* tests will require further research and more extensive testing for validation and to determine their overall utility in the risk assessment process. A prospective scheme for future testing might be as shown in Figure 2.

Table 1: Corneal Thickness of Several Species of Animals.

Species	Thickness (mm)	References
Cat	0.62	Marzulli and Simon (1971)
Dog	0.55	Marzulli and Simon (1971)
Rhesus Monkey	0.52	Marzulli and Simon (1971)
Rabbit	0.37	Marzulli and Simon (1971)
Mouse	0.10	Davson (1962)
Human	0.51	Maurice and Giardini (1951)

Table 2: Comparison of Response of the Rabbit and Monkey Eye to Irritants.

Test Agent	More Sensitive Species	Reference
Surfactant formulations	Rabbit (a)	Buehler and Newmann (1964)
1% Sodium hydroxide	Rabbit	Buehler and Newmann (1964)
Cytarabine hydrochloride	Similar (b)	Elliot and Schut (1965)
Liquid detergent	Rabbit	Beckley (1965)
Various materials	Rabbit	Carter and Griffith (1965)
5% Soap solution	Monkey	Beckley et al. (1969)
Detergent	Rabbit	Beckley et al. (1969)
Chloroacetophenone	Rabbit (c)	MacLeod (1969)
Iodine solution	Rabbit	Hood et al. (1971)
Surfactant formulations	Rabbit	Benke et al. (1977)
Commercial shampoos, cationic detergents	Rabbit	Gershbein and McDonald (1977)
Variety of substances	Rabbit	Green et al. (1978)
5% Sulfuric acid	Monkey	Green et al. (1978)

From Green et al. (1978)

(a) Response of the rabbit to test agent instilled with a corneal applicator more closely approximated the monkey response.

(b) Peak effects were observed in rabbits at 7 days and in monkeys at 8 to 12 days.

(c) In some cases, the two species showed similar effects.

Table 3. Eye Scoring Scheme Based on the Use of a Slit Lamp Biomicroscope and Fluorescein.

Site	"Accept"	"Accept with Caution"	"Probably Injurious to Human Eyes"
Conjunctiva	Hyperemia without chemosis	Chemosis, less than 1 mm at the limbus	Chemosis, greater than 1 mm at the limbus
Cornea	Staining, corneal stippling (a) without confluence at 24 Hours	Confluence (b) of staining at 24 to 48 hours	Staining with infiltration or edema
Anterior Chamber	0	0	Flare (c) (visibility of slit beam). Rubeosis of iris

(a) Corneal stippling: multiple discrete punctate irregularities in the corneal epithelial layer which retain fluorescein.
(b) Confluence: uniform zones for fluorescein retention larger than 1 mm in diameter.
(c) Flare: Tyndall effect in a beam traversing the aqueous humor.

The EPA guidelines for labeling are listed (Table 4, Campt 1981, Federal Register, 49, #188, 1984).

Table 4: Label Statements Regarding Eye Irritation Hazards Due to Pesticides.

Toxicity Category	Signal Word	Skull and Crossbones & "Poison" Required	Precautionary Statement	Practical Treatment
I Corrosive; (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Danger	No	Corrosive.* Causes irreversible eye damage. Harmful if swallowed. Do not get in eyes or on clothing. Wear (goggles, face shield, or safety glasses).** Wash thoroughly with soap and water after handling. Remove contaminated clothing & wash before reuse.	If in eyes: Flush with plenty of water. Get medical attention. If swallowed: drink promptly a large quantity of milk, egg whites, gelatin solution, or, if these are not available, drink large quantities of water. Avoid alcohol. NOTE TO PHYSICIAN: Probable mucosal damage may contraindicate the use of gastric lavage.

* The term "corrosive" may be omitted if the product is not actually corrosive.

** Choose appropriate form of eye protection. Recommendation for goggles or face shield is more appropriate for industrial, commercial, or nondomestic uses. Safety glasses may be recommended for domestic or residential use.

Table 4: (cont'd)

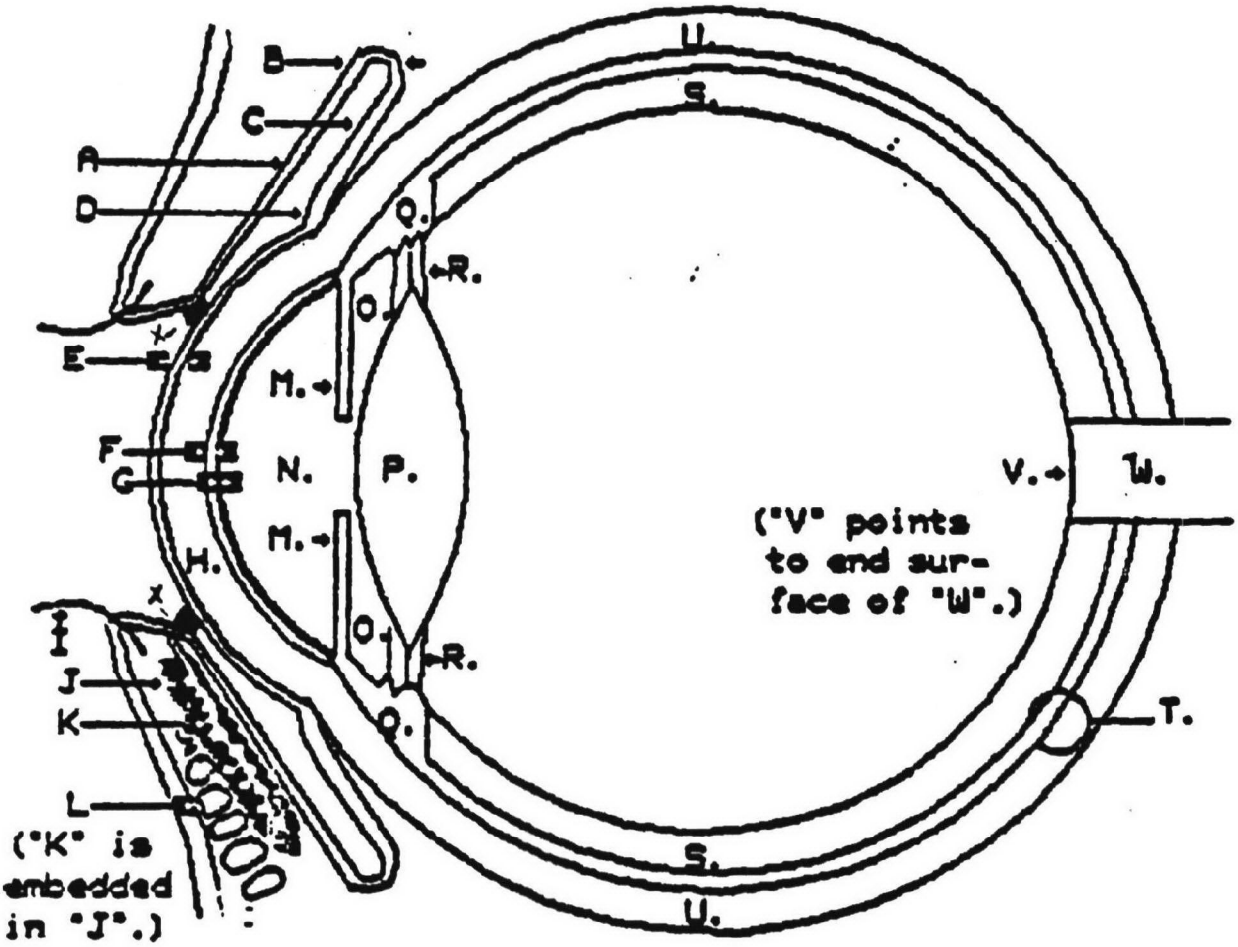
Toxicity Category	Signal Word	Skull and Crossbones & "Poison" Required	Precautionary Statement	Practical Treatment
II				
Corneal involvement or irritation clearing in 21 days or less	Warning	No	Causes substantial but temporary eye injury. Do not get into eyes or on clothing. Wear (goggles, face shield, or safety glasses.)** Harmful if swallowed. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.	Same as above; omit NOTE TO PHYSICIAN statement.

** Choose appropriate form of eye protection. Recommendation for goggles or face shield is more appropriate for industrial, commercial, or nondomestic uses. Safety glasses may be recommended for domestic or residential use.

Table 4: (cont'd)

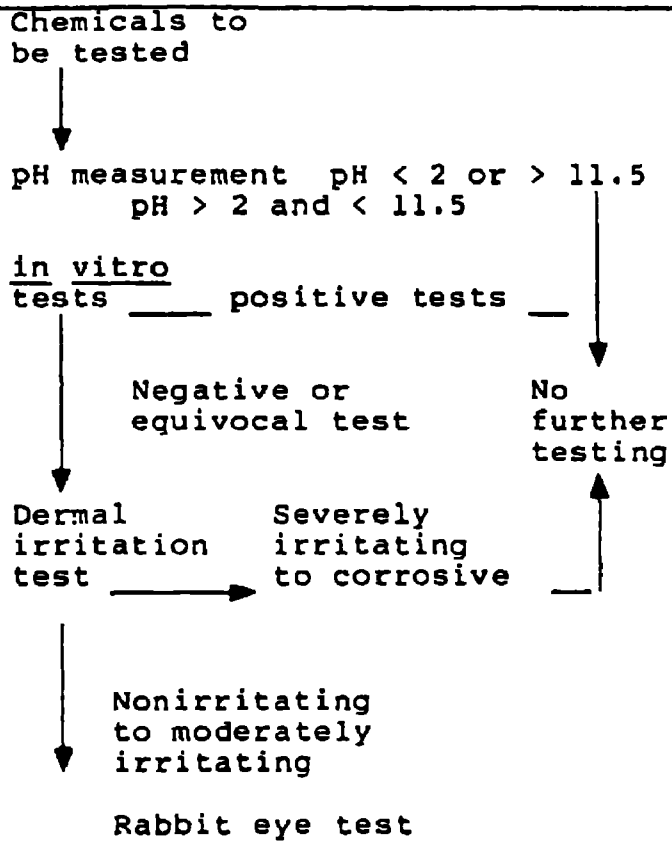
Toxicity Category	Signal Word	Skull and Crossbones & "Poison" Required	Precautionary Statement	Practical Treatment
III Corneal involvement or irritation clearing in 7 days or less	Caution	No	Causes (moderate) eye injury (irritation). Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling.	If in eyes: Flush with plenty of water. Get medical attention if irritation persists.
IV Minimal effects clearing in less than 24 hours	Caution	No	None required.	None required.

Figure 1: Eye Anatomy



A.	Palpebral Conjunctiva	K.	Meibomian Gland	U.	Sclera
B.	Fornix	L.	Orbicularis Membrane	V.	Optic papilla.
C.	Bulbar Conjunctiva	M.	Iris	W.	Optic nerve.
D.	Limbus	N.	Anterior Chamber	X.	Nicitating membrane.
E.	Corneal Epithelium	O.	Posterior Chamber		
F.	Descemet's Membrane	P.	Lens		
G.	Endothelium	Q.	Ciliary Bodies		
H.	Corneal Stroma	R.	Zonules		
I.	Cilia	S.	Retina		
J.	Tarsal Plate	T.	Choroid		

Figure 2: Proposed Testing Scheme.



Definitions For Eye Irritation Testing

Irritation: The production of reversible changes in the ocular structures following the application of a test substance to the anterior structure of the eye.

Corrosion: The production of irreversible changes in the ocular structures following the application of a test substance to the anterior structure of the eye.

Bulbar conjunctiva:

The mucous membrane loosely attached to the orbital septum, underlying anterior sclera, and Tenan's capsule except at the limbus, where the conjunctiva fuses with Tenan's capsule for 3 mm.

Palpebral conjunctiva:

Lines the posterior surface of the eyelid, firmly attached to the underlying tarsus, and attaches to the sclera to become the bulbar conjunctiva.

Chemosis: Conjunctival swelling.

Blepharitis: The inflammation of the eyelids.

Blepharospasm: Twitching of the eyelids.

Anterior chamber: The space filled with aqueous humor bounded anteriorly by the cornea and posteriorly by the iris.

Injection: Congestion of blood vessels, a term synonymous with hyperemia.

Hyphema: A term for blood in the anterior chamber.

Limbus: Junction of the cornea with the sclera.

Pannus: The infiltration of the cornea by blood vessels.

Sclera: The external white layer of the eye composed of fibrous tissue.

Uveal tract:	Composed of the iris, ciliary body, and choroid. The ciliary body is composed of the ciliary processes which make the aqueous humor and the ciliary muscle which functions in accommodation. The iris and ciliary body are called the anterior area and the terms anterior uveitis, iritis, and iridocylitis are synonymous.
Choroid:	The posterior portion of the uveal tract and the middle tissue of the eye between the sclera and retina. The terms posterior uveitis and choroiditis are synonymous.
Uveitis:	A general term for inflammatory disorders of the uveal tract and may involve one or all three portions simultaneously.
Pachometer:	An instrument used to measure corneal thickness.
Tonometer:	An instrument for measuring intraocular pressure.

Bibliography

Baldwin, H.A., McDonald, T.O., Beasley, C.H. (1973). Slit Examination Of Experimental Animal Eyes. II. Grading Scales And Photographic Evaluation Of Induced Apthological Conditions. J. Soc. Cosmet. Chem. 24:181-195.

Ballantyne, B., Swanston, D.W. (1972). Ocular Irritation Tests (1972). Br. J. Pharmacol. 46: 577-578.

Balls, M. and Horner, S.A. (1985). The FRAME Interlaboratory Program In Vitro Cytotoxicity. Fd. Chem. Toxic. 23: 209-213.

Bosshard, E. (1985). Review On Skin And Mucous Membrane Irritation Tests And Their Application. Fd. Chem. Toxic., 23: 149-154.

Beckley, J.H. (1965). Comparative Eye Testing; Man Vs. Animal. Toxicol. Appl. Pharmacol. 7: 93-101.

Beckley, J.H., Russell, T.J., Rubin, L.F. (1969). Use Of The Rhesus Monkey For Predicting Human Response To Eye Irritants. Toxicol. Appl. Pharmacol. 15: 1-9.

Benke, G.M., Brown, N.M., Walsh, M.J., Drothman, R.B. (1977). Safety Testing Of Alkyl Polyethoxylate Nonionic Surfactants. I. Acute Effects. Fd. Cosmet. Toxicol., 15 (5): 309-318.

Bloom, W., Fawcet, D.W. (1975). In: Textbook Of Histology. W.B. Saunders Co., Philadelphia, Pa., pp. 917-963.

Borenfreund, E., Shopsis, C., Barrero, O., Sathe, S. (1983). In Vitro Alternative Irritancy Assays: Comparison Of Cytotoxic and Membane Transport Effect Of Alcohols. Ann. N.Y. Acad. Sci., 407: 416-419.

Buehler, E.V., Newman, E.A. (1964). A Comparison Of Eye Irritation In Monkeys And Rabbits. Toxicol. Appl. Pharmacol. 6: 701-710.

Burstein, N.L. (1980). Corneal Cytotoxicity Of Topically Applied Drugs, Vehicles And Preservatives. Supp. Ophthalmol. 25 (1): 15-30.

Burton, A.B.G. (1972). A Method For The Objective Assessment Of Eye Irritation. Fd. Cosmet. Toxicol. 10: 209-217.

Campt, D.D. (1981). Letter, Label Improvement Methods: Change In Test Methods For And Categorization Of Eye Irritation. EPA. PR 81-3.

DUE TO CIRCUMSTANCES BEYOND OUR CONTROL,
PAGE 24 OF THIS REPORT IS MISSING.
ALL EFFORTS TO OBTAIN THE MISSING PAGE
HAS BEEN UNSUCCESSFUL, WE ARE MAKING THE
REMAINER OF THIS REPORT AVAILABLE TO THE
PUBLIC

Environmental Protection Agency (1981). Eye Irritation Testing. An Assessment Of Methods And Guidelines For Testing Materials For Eye Irritancy. EPA-560/11-82-001, Washington, D.C.

Estable, J.L. (1948). The Ocular Effect Of Several Irritant Drugs Applied Directly To The Conjunctiva. Am. J. Ophthalmol. 31: 837-844.

Food & Drug Administration, Illustrated Guide For Grading Eye Irritation By Hazardous Substances. Washington, D.C., Government Printing Office (1965).

Federal Register, Interagency Regulatory Liaison Group, 46 (14): 7075-7077 (1981).

Friedenwald, J.S., Hughes, W.F., Herrmann, H. (1944). Acid-base Tolerance Of The Cornea. Arch. Ophthalmol. 31 (4): 279-283.

Friedenwald, J.W., Hughes, W.F., Herrman, H. (1946). Acid Burns Of The Eye. Arch. Ophthalmol. 35: 98-108.

Gartner, S. (1944). Blood Vessels Of The Conjunctiva. Arch. Ophthalmol. 36: 464-471.

Gasset, A.R., Ishil, Y., Kaufman, H.E., Miller, T. (1974). Cytotoxicity Of Ophthalmic Preservatives. Am. J. Ophthalmol. 78 (1): 98-105.

Gershbein, L.L., McDonald, J.E. (1977). Evaluation Of The Corneal Irritancy On Test Shampoos And Detergents In Various Animal Species. Fd. Cosmet. Toxicol. 15: 131-134.

Goldberg, A.M. (1985). An Approach To The Development Of In Vitro Toxicology Methods. Fd. Chem. Toxic. 23: 205-208.

Grant, M.W. (1974). Toxicology Of The Eye, 2nd edition, Springfield, IL, Thomas.

Green, W.R., Sullivan, J.B., Hehir, R.M., Scharpf, L.F., Dickinson, A.W. (1978). A Systematic Comparison Of Chemically Induced Eye Injury In The Albino Rabbit and Rhesus Monkey. The Soap And Detergent Association, New York.

Griffith, J.A., Nizon, G.A., Bruce, R.D., Reer, P.J., Banaan, E.A. (1980). Dose-response Studies With Chemical Irritants In The Albino Rabbit Eye As A Basis For Selecting Optimum Testing Conditions For Predicting Hazard To The Human Eye, Toxicol. & Appl. Pharmac. 55: 501-513.

Hagiwara, H., and Sugiwia, S. (1953). The Use Of Caster-oil And Tween 80 As An Ophthalmic Base. Acta Soc. Ophthalmol. (Jap) 57: 1-5.

Hirst, L.A., Kenyon, K.R., Fogle, J.A., Hanninen, L., Stark, W.J. (1981). Comparative Studies Of Corneal Surface Injury In The Monkey And Rabbit. Arch. Ophthal. 99:1066-1073.

Holly, F.J., Lemp, M.A. (1971). Wettability And Wetting Of Corneal Epithelium. Exp. Eye Res. 11: 239-250.

Hood, C.I., Gasset, A.R., Ellison, E.D., Kaufman, H.E. (1971). The Corneal Reaction To Selected Chemical Agents In The Rabbit And Squirrel Monkey. Amer. J. Ophthalmol. 71: 1009-1017.

Hughes, W.F. (1946). Alakli Burns Of The Eye. 1. Review Of the Literature And Summary of Present Knowledge. Arch. Ophthalmol. 34: 423-449.

Jampol, L.M., Newfeld, A.H., Sears, M.L. (1975). Pathways For The Response Of The Eye To Injury. Invest. Ophthalmol. 14: 184-189.

Kemp, R.B., Meridith, R.W., Gamble, S.H. (1985). Toxicity Of Commercial Products On Cells In Suspension Culture: A Possible Screen For The Draize Eye Irritation Test. Fd. Chem. Toxic. 23: 267-270.

Krueger, R. (1959). Experimentelle Und Klinische Beobachtungen Zur Behandlund Der Alkaliveratzung Mit Ascorbinsaure. Bericht D. Phthalm. Ges. 62: 255-258.

Leighton, J., Nassauer, J. and Tchao, R. (1985). The Chick Embryo In Toxicology: An Alternative To The Rabbit Eye: Fd. Chem. Toxic. 23: 293-298.

Leopold, I.H. (1945). Local Effects Of Detergents On Ocular Structures. Arch. Ophthalmol. 34: 99-102.

Lewin, L. and Guillery, H. (1913). Die Wirkungen Von Arzneimitteln Und Giften Auf Das Age, 2nd ed. Berlin: Hirschwald.

Luepke, N.P. (1985), Hen's Chorioallantoic Membrane Test For Irritation Potential. Fd. Chem. Toxic. 23: 287-291.

MacLeod, I.F. (1969). Chemical Mace; Ocular Effects In Rabbits And Monkeys. J. Forensic Sci. 14: (1) 34-47.

Mann, I., Pullinger, B.D. (1942). A Study Of Mustard Gas Lesions Of The Eyes Of Rabbits And Men. Proc. R. Soc. Med. 35: 29-244.

Marsh, R.J., Maurice, D.M. (1971). The Influence Of Non-ionic Detergents And Other Surfactants On Human Corneal Permeability. Exp. Eye Res., 11:43-48.

Marzulli, F.N. (1968). Ocular Side Effects Of Drugs. Fd. Chem. Toxic., 6: 221-234.

Marzulli, F.N., Simon, M.E. (1971). Eye Irritation From Topically Applied Drugs And Cosmetics: Preclinical Studies. Am. J. Optom. 48: 61-79.

Maul, E., Sears, M.L. (1976). Objective Evaluation Of Experimental Ocular Irritation. Invest. Ophthalmol. 15 (4).

Maurice, D.M. and Giardini, A.A. (1951). A Simple Optical Apparatus For Measuring The Corneal Thickness, And The Average Thickness Of The,Human Cornea. Brit. J. Ophth. 35: 169-177.

Maurice, D.M. (1968). Cellular Membrane Activity In The Corneal Endothelium Of The Intact Eye. Experimentia. 24: 1094-1095.

McDonald, T.O., Baldwin, H.A., Beasely, C.H. (1973). Slit Lamp Examination Of Experimental Eyes. I. Technique Of Illumination And The Normal Animal Eye. J. Soc. Cosmet. Chem. 24: 163-180.

McDonald, T.O., Shadduck, J.A. (1977). Eye Irritation. In: Advances In Modern Toxicology, Marzulli, F.N. and Maiback, H.I. (eds)., Vol. 4 Hemisphere, Washington and London.

McDonald, T.O., Seabaugh, V.M., Shadduck, J.A., Edelhauser, H.F. (1983). Eye Irritation. In: Dermato-toxicology, 2nd edition, Marzulli, F.N., and Maiback, H.I. (eds). Hemisphere, Washington.

McLaughlin, R.S. (1946). Chemical Burns Of The Human Cornea. Amer. J. Ophthalmol. 29 (11): 1355-1362.

Mishima, S., Hedbys, B.O. (1968). Measurements Of Corneal Thickness With The Haig-Streit Pachometer, Arch. Ophthalmol. 80: 710.

Muir, C.K., Flower, C., Van Abbe, N.J. (1983). A Novel Approach To The Search For In Vitro Alternatives To In Vivo Eye Irritancy Testing. Tox. Letters 18, 1-5.

Murphy, J.J. (1986). Memorandum, Trip Report For OECD Ad Hoc Meeting On Acute Toxicity Testing. Paris, France, U.S. EPA.

National Academy Of Sciences - National Research Council (1975). In: Principles For Evaluating Chemicals In The Environment. NAS Publication For The Environmental Protection Agency: Eye Irritation, 104-105.

National Academy Of Sciences - National Research Council, (1977). In: Principles And Procedures For Evaluating The Toxicity Of Household Substances, Prepared By Committee For The Revision Of NAS Publication 1138 for the Consumer Product Safety Commission. Eye Irritation, 41-54.

Organization For Economic Cooperation And Development (OECD) (1986). Acute Eye Irritation/Corrosion, OECD Publications And Information Center, Washington, D.C.

Parish, W.E. (1985). Ability Of In Vitro Tests To Represent Histopathological Features Of Acute Eye Inflammation. Fd. Chem. Toxic. 23: 215-227.

Phister, R.R. (1973). The Normal Surface Of Corneal Epithelium: A Scanning Electron Microscopic Study. Invest. Ophthalmol. 12: 654-668.

Potts, A.M., Gonasun, L.M. (1975). Toxicology Of The Eye. In: Toxicology - The Basic Science Of Poisons. Casarett, L.J. and Doull, J. (eds). Macmillan Publishing Co., Inc., N.Y., N.Y., 275-309.

Prince, J.H., Diesem, C.D., Eglitis, I., Ruskell, G.L. (1960) Anatomy And Histology Of The Eye And Orbit In Domestic Animals. Springfield, IL: Thomas.

Reinhardt, C.A., Pelli, D.A., Eglitis, I., Ruskell, G.L. (1985). Interpretation Of Cell Toxicity Data For The Estimation Of Potential Irritation. Fd. Chem. Toxic. 23: 247-252.

Seabaugh, V.M., Osterberg, R., Hoheisel, C.H., Murphy, J., Bierbower, G. (1976). A Comparative Study Of Rabbit Ocular Reaction To Various Exposure Times To Chemicals, Toxicology And Appl. Pharm. Vol. 37, No. 1.

Sciafe, M.C. (1985). An In Vitro Cytotoxicity Test To Predict The Ocular Irritation Potential Of Detergents And Detergent Products. Fd. Chem. Toxic., 23: 253-258.

Shopsis, C., Borenfreund, E., Walberg, J., Stark, D.M. (1985). A Battery Of Potential Alternatives To The Draize Test. Uridine Uptake Inhibition, Morphological Cytotoxicity Macrophage Chemotoxic, And Exfoliate Cytology. Fd. Chem. Toxic., 23: 259-266.

Shuster, J., Kaufman, H.E. (1974) Letter, Invest Ophthalmol., 13: 892-893.

Sugar, A. (1979). Clinical Specular Microscopy. Surv. Ophthalmol. 24 (1): 21-32.

Swanston, D.W. (1985). Clinical Specular Microscopy. Surv. Ophthalmol. 23: 169-173.

Van Abbe, N.Y. (1973). Eye Irritation: Studies Relating To Responses In Man And Laboratory Animals. J. Soc. Cosmet. Chem. 24: 685-692.

Walton, R.M., Heywood, R. (1978). Applanation Tonometry In Assessment Of Eye Irritation. J. Soc. Cosmet. Chem. 29: (6): 365-368.

Weil, C.S., Scala, R.A. (1971). Study Of Intra- And Interlaboratory Variability In The Results Of Rabbit Eye And Skin Irritation Tests. Toxicol. Appl. Pharmacol. 19: 276-319.

Weltman, A.S., Sparber, S.B., Jurtshuk, T. (1965). Comparative Evaluation And The Influence Of Various Factors On Eye Irritation Scores. Toxicol. Appl. Pharmacol. 7: 308-319.

Williams, S.J., Graysel, G.J., Kennedy, G.L. (1982). Evaluation of Ocular Irritancy Potential: Intralaboratory Variability And Effect Of Dosage Volume. Toxicol. Lett. 12, 235.

Williams, S.J. (1985). Changing Concepts Of Ocular Irritation Evaluation: Pitfalls And Progress. Fd. Chem. Toxic. 23: 186-193.