ICCVAM Workshop Series on  
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January 19, 2011: Assessing the Potential for Chemically Induced Eye Injuries  
Poster Session  
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Porcine Cornea Confocal Assay (PorFocal), For Your Eyes Only!

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MB Research has developed a procedure for the culturing of excised porcine corneas for a period of up to 21 days. Using these corneas, we have developed a method to evaluate the effects of multiple doses of low-level irritants. In order to detect and quantify low-level damage to corneal tissue, the PorFocal assay was created to assess ocular irritation by measuring cell viability using a dead stain, ethidium homodimer (EtH), and fluorescence confocal microscopy. For the PorFocal assay, eight cultured corneas (four per test material) were treated with either phosphate buffered saline (PBS) or 0.01% benzalkonium chloride (BAK) for a total of 10 doses (2x/day on days 1, 2, and 6; 1x/day on days 0, 3, 7, and 8) at 50 µl/treatment. On day 8, these corneas were incubated for 30 minutes with 2 µM EtH dead cell stain and imaged using confocal microscopy. The EtH stained dead cell nuclei were imaged in six random 450 µm x 450 µm x 56 µm-deep tissue fields via confocal z-stacks composed of eight 8 µm-thick optical slices. A maximum projection of image z-stacks was created so that no nucleus was counted twice. All dead nuclei (cells) were counted for each tissue field, the counts were summed, and statistical analysis was performed using analysis of variance. PBS-treated corneas (n=4) exhibited 1659 dead cells and 0.01% BAK-treated corneas (n=4) exhibited 3591, a 216% increase in cell death, which was statistically significant (p<0.001). These data indicate that low-level damage can be detected by using confocal microscopy. Future directions for this project include increasing the amount of replicates to decrease variance. Also, the complimentary component of the staining kit is a live cell stain. This stain could be further developed and a ratio of live to dead cells in each group could yield higher-sensitivity corneal irritation measurements.
Development of the Replacement Ocular Battery – Tiered Testing Strategy of Alternative Toxicology Tests to Replace the Need for Rabbit Eye Tests

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Over 10,000 rabbits per year are sacrificed in the determination of acute ocular irritation potential. Using a series of non-animal assays, the replacement ocular battery (ROBatt) can accurately predict the range of ocular irritation from none to corrosive.

At present no single alternative assay has been accepted by regulatory agencies as a complete replacement for the use of live animals. Some assays, such as the bovine cornea opacity/permeability test, have been accepted as a screen for severe and corrosive materials. Noncorrosive materials still need to be evaluated in rabbits. The ROBatt tiered-testing approach uses a series of two to four non-animal assays to categorize materials through the range of potential irritation.

Societal pressure has already reduced the testing of laboratory animals for nonregulated cosmetics. MB Research has worked together with our cosmetic clients to validate and perfect non-animal assays to predict ocular irritation. ROBatt has been proposed as a combination of proven assays that can be used in a regulatory setting rather than solely for nonregulated industries.

Next steps in the development of ROBatt include collaborating with industry leaders and government officials on the validation of the tiered testing strategy using a broad range of ocular irritants and submission of analyzed data to regulatory agencies for consideration/acceptance as a testing alternative to the Draize rabbit eye test.
PorCORA Ocular Reversibility Assay Testing with Personal Care Products

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To ensure consumer safety, ocular irritation testing is routinely performed on personal care products. Two ocular assays, the chorioallantoic membrane vascular assay (CAMVA) and bovine corneal opacity and permeability assay (BCOP), are widely used in the cosmetic industry since they do not require the use of animals. These assays provide reliable data predicting ocular irritation and are inexpensive to conduct. To complement the CAMVA/BCOP assays, the porcine corneal opacity reversibility assay (PorCORA) was developed using an *ex vivo* model to predict reversibility of ocular irritants. In the current study, three commercially available consumer products (a shampoo, a hair color glaze, and a 12% hydrogen peroxide product) were tested in the PorCORA for ocular damage reversibility. The PorCORA indicates that under the exaggerated *in vitro* study conditions the surfactant-based shampoo may cause irreversible ocular damage: histological changes occurred in the squamous-cell layer of the corneas and mild to moderate changes in the basal-cell layer. However, published *in vivo* data does indicate that reversibility of ocular damage occurs following exposure to shampoo. Furthermore, the PorCORA predicts that under the same study conditions used for the shampoo, ocular damage caused by a hair color glaze and a 12% hydrogen peroxide product are fully reversible with histology reporting only minimal or mild microscopic effects to the superficial squamous-cell layer. Like the shampoo, literature also indicates that reversibility of ocular damage occur *in vivo* following exposure to hydrogen peroxide. The PorCORA assay, in conjunction with other ocular irritation assays can be used to predict the extent of ocular damage and reversibility that products may cause following consumer eye exposure.
Historical Data on Personal Care Products Over Fourteen Years Using the Chorioallantoic Vascular Membrane Assay and the Bovine Cornea Opacity/Permeability Assay

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The chorioallantoic membrane vascular assay (CAMVA) and bovine corneal opacity and permeability assay (BCOP) are two common assays used to determine ocular irritation for consumer-use products. These assays do not require the use of live animals, provide reliable predictive data, provide results similar to in vivo models and are rapid and inexpensive to conduct. Data from 321 studies performed from 1995 to 2009 (a total of 345 test materials assessed by CAMVA and/or BCOP) were compiled to determine the feasibility of predicting ocular irritation for various formulations. Review of the data from both assays found that hair shampoos, skin cleansers, and hair styling sprays (containing ethanol) were repeatedly predicted to be ocular irritants. In contrast, skin lotions/moisturizers were repeatedly predicted not to be ocular irritants. Based on the findings for these product types, future ocular irritation testing (i.e., CAMVA/BCOP) can be nearly eliminated as long as formulations are compared to those previously tested. For example, skin cleanser irritation appears to be solely dependent on surfactant species and level in these formulations. For other product types (e.g., deodorants, makeup removers, hair styling, body sprays) it was concluded that these products should continue to be tested in CAMVA/BCOP for ocular irritation potential because either significant variability exists in the historical data (nonspray hair stylers) or the historical sample size is too small to permit definitive conclusions (deodorants, makeup removers, massage oils, facial masks, body sprays, and hair styling products).

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5

Applicability of Validated and Adopted In Vitro Methods to Assess Detergents and Cleaning Products

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In 2008, the CLP Regulation (1) on classification and labelling, which implements the United Nations Globally Harmonised System of Classification and Labelling of Chemicals (2), was adopted into European Union legislation. It establishes, amongst others, a new calculation method for default classification of mixtures (from June 2015) which might result in the over-labelling of many cleaning products currently not requiring classification based on consistent animal, in vitro and human experience data. Such over-labelling could confuse end-users and lead to underestimation of real risk when this is merited due to trivialisation of labelling.

In order to ensure robust and appropriate product classification, the European Detergent Association (A.I.S.E.) initiated in 2010 a programme to investigate the applicability of validated and adopted in vitro eye and skin irritation/corrosion methods to classify detergent and cleaning product formulations. The programme addresses skin irritation, eye irritation/corrosion, and skin corrosion and eye effects for extreme pH products. Each area includes a review of existing literature and existing data shared by A.I.S.E. member companies, and the practical testing in selected in vitro test methods of representative formulations supported by existing animal and/or human data.

The knowledge gained through the program will be used to develop guidance on the use of in vitro methods for classification and labelling of detergent and cleaning products and/or recommendations for future research and/or studies. The latest developments and examples in the area of eye irritation will be presented.

References

Development of the EpiOcular Eye Irritation Test

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The recently implemented European Union (EU) Cosmetics Directive and Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) legislation has heightened the need for in vitro ocular test methods. In response, the European Centre for the Validation of Alternative Methods (ECVAM) eye irritation task force requested that submitters, model developers, and companies involved in EpiOcular™ prevalidation studies work toward expanding the test chemical applicability domain (AD) of the model. The EpiOcular model producer (MatTek Corp.) therefore undertook development of an expanded EpiOcular AD protocol. Based on results for 59 test materials, a prediction model (PM) was developed that uses a single exposure period and a single tissue viability cut-off (determined by the MTT assay) for classification: ≤60% viability = irritant (EU Classifications R36 and R41); >60% viability = non-classified (NC). In the current poster, we report results from evaluation of 35 additional materials (94 materials tested in total), including alcohols, hydrocarbons, amines, esters, and ketones. For the combined 94 test materials, the PM provided 100.0% sensitivity and 68.0% specificity for discriminating between ocular irritants and nonirritants. This PM was subsequently evaluated in a 2007-2008 multilaboratory study by the European Cosmetics Association (COLIPA). Twenty coded chemicals were tested in seven laboratories (four EU and three U.S.). Overall, 298 independent trials were performed, demonstrating 99.7% agreement in prediction (NC/I) across the laboratories. Coefficients of variation for the percent survival of tissues across laboratories were generally modest (<16%) except where tissue survival values were low. Using these data, a formal submission was sent to ECVAM in 2008, and the EpiOcular Eye Irritation Test is currently part of a formal validation study. The expanded AD, together with a long history of reproducibility and proven utility for ultra-mildness testing, makes EpiOcular an extremely useful model for addressing current legislation related to animal use in the testing of potential ocular irritants.
3D Organotypic EpiOcular Eye Irritation Test Results – Use of Alternative Tissue Culture Inserts

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Highly differentiated organotypic tissue models are being used increasingly in lieu of animals to meet regulatory testing requirements. The ongoing quality of these tissue models is of prime importance so that U.S. and European Union regulators and industry users can be assured that the toxicological system is reproducible both during the validation process and afterwards. The purpose of this study was to investigate the effects of various tissue culture inserts (TCI) on EpiOcular™ tissue morphology and assay reproducibility. Four types of TCI from three commercial manufacturers were obtained, and standardized culture conditions were used to produce the EpiOcular organotypic tissue model, which is comprised of normal human cells. The EpiOcular tissues were subjected to quality control tests, including histological evaluation and determination of the exposure time of a common surfactant (Triton X-100) that reduces the tissue viability to 50% (ET-50). In addition, the EpiOcular Eye Irritation Test (EIT) was run using 94 test articles (73 liquids, 21 solids). The histology of tissues produced on all four types of TCI was structurally equivalent to the control tissues. The average ET-50s produced for EpiOcular were: TCI-1: 23.8 +/- 6.5 min (n=8, p=0.4), TCI-2: 21.3 +/- 5.4 min (n=6, p=0.11), and TCI-3: 26.5 +/- 7.9 min (n=6, p=0.28). These values were not statistically different than that of tissues cultured on the control TCI-C (24.4 +/- 3.7 min). In addition, use of the alternative TCI in the EpiOcular EIT showed no differences in irritant/non-irritant predictions for a wide range of chemical categories and irritancies. For TCI-1, EIT sensitivity and specificity were 98.0% and 63.8%; for TCI-2: 96.9% and 67.2%, for TCI-3: 89.9% and 71.7%. Control cultures had specificity and sensitivity of 100.0% and 68.0%. In summary, the TCI is one of the crucial parameters in producing high-quality and reproducible organotypic tissue models; however, multiple commercially available TCI appear to have appropriate properties.
Eye care cosmetics (ECC) need to be nonirritating in order to be successful in the marketplace. In addition, in order to avoid complaints by customers with sensitive eyes, many ECCs are formulated to be ultra-mild. However, testing of and discrimination between ultra-mild formulations is difficult since traditional Draize rabbit eye testing is insensitive to the low levels of irritation caused by ECCs. Furthermore, animal testing is not possible due to animal rights concerns and due to current European legislation banning cosmetics that have been tested using animals. Human clinical testing can be performed, but because only a low level response is expected large numbers of subjects would be necessary and hence testing costs would be high if not prohibitive. Cells in monolayer culture could be used for testing; however, the test materials would need to dissolved in aqueous media which for many non-water-soluble cosmetics is not possible. The current study investigated use of the organotypic EpiOcular tissue model as a means of discriminating between ultra-mild formulations.

Ten commercially available mascara products were purchased and tested using EpiOcular with an extended time exposure protocol. Because the tissue model is cultured at the air-liquid interface (apical tissue surface left dry), both water-soluble and water-insoluble test materials could be applied neat to the apical tissue surface. Exposure times between 8 and 24 hours were used after which the tissue viability was determined using the MTT assay. Dose response curves were constructed and the exposure time that reduced tissue viability to 50% (ET-50) was determined by mathematical interpolation. For the 10 mascaras tested, a broad range of ET-50s was obtained from 8.7 hours to >24 hours. Other studies with low levels of surfactants known to be irritating at higher concentrations could also be discriminated by ET-50s. As such, the extended time exposure protocol appears to be a facile, cost-effective means to screen ultra-mild ECCs and other materials.

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The isolated chicken eye (ICE) test method is an *in vitro* model that provides short-term maintenance of normal physiological and biochemical function of the chicken eye. Potential eye damage is assessed by changes in corneal swelling, opacity, and fluorescein retention. ICCVAM recommended that ICE could be used to classify positive substances as ocular corrosives and severe irritants. While not a complete replacement for the rabbit eye test, the ICE test method can be used in a tiered-testing strategy for regulatory classification and labeling within a specific applicability domain. These recommendations were accepted by U.S. Federal agencies, and positive results from ICE may now be used in the U.S. instead of the rabbit eye test for certain regulatory hazard classification decisions. To have the greatest impact on reducing animal use, ICCVAM, with input from stakeholders in the U.S., EU, and Japan, drafted an Organisation for Economic Co-operation and Development (OECD) test guideline that was based on the ICCVAM-recommended ICE protocol. This protocol was developed following an international peer review evaluation with contributions from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Evaluation of Alternative Methods. OECD TG 438 has now been formally adopted by OECD and will be accepted by all 33 OECD member countries in accordance with OECD Mutual Acceptance of Data. The use of ICE will reduce the use of rabbits for eye safety testing and eliminate such testing in animals of most substances likely to cause severe pain and discomfort. ILS staff supported by NIEHS contract N01-ES-35504.
In Vitro and Ex Vivo Experimental Evaluation of Chemical Burns and their Decontamination: Sulfuric Acid as an Example

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Rationale and Scope: The Chemical Abstract Service (CAS) has registered more than 40 million inorganic and organic substances (1), of which about 600,000 are commonly used by industries. More than 25,000 irritant and corrosive chemicals have the potential to cause burns (2). Sulfuric acid (H₂SO₄) is widely used in industries (3). It is a strong acid that induces severe burns and can also cause thermal burns when concentrated. While decontamination with copious amounts of water has traditionally been recommended (4), an amphoteric, polyvalent, mildly hypertonic solution, Diphoterine®, has been proposed (5). In vitro and ex vivo evaluation methods are described using H₂SO₄ as an example.

Methods: The in vitro studies consisted of: 1) placing sulfuric acid on a semi-permeable membrane and evaluating diffusion through the membrane with pH measurements; 2) determining the dilution and mechanical washing effects of tap water or Diphoterine® by measuring temperature and/or pH changes. For the ex vivo studies, 30 µl of 95% sulfuric acid was placed on human skin explants; penetration, injury, and healing were evaluated histologically.

Results: In the in vitro studies, dilution was a minor effect, while mechanical washing was the major effect with both water and Diphoterine®. Diphoterine® was more efficacious than water in returning to an acceptable physiological pH. No additional heat release was observed and it required a smaller volume of Diphoterine® than water. In the ex vivo studies, tissue damage was apparent by 1 minute and involved all skin explants layers by 4 hours. No spontaneous burn healing occurred over 11 days.

Conclusions: In vitro, Diphoterine® washing was more efficacious than water. Ex vivo, the skin injury began rapidly, suggesting that washing should be initiated as soon as possible. Diphoterine® should be considered for further study and clinical use. The described methods are applicable for evaluation of other irritant or corrosive substances.

Note: All applicable ethical guidelines and regulations for the experimental use of human tissue were followed.

References

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The bovine corneal opacity and permeability (BCOP) test method is an in vitro model that provides short-term maintenance of normal physiological and biochemical function of the bovine cornea. Potential eye damage is assessed by changes in opacity and permeability to fluorescein. ICCVAM recommended that BCOP could be used to classify positive substances as ocular corrosives and severe irritants. While not a complete replacement for the rabbit eye test, BCOP can be used in a tiered-testing strategy for regulatory classification and labeling within a specific applicability domain. These recommendations were accepted by U.S. Federal agencies, and positive results from BCOP may now be used in the U.S. instead of the rabbit eye test for certain regulatory hazard classification decisions. To have the greatest impact on reducing animal use, ICCVAM, with input from stakeholders in the U.S., EU, and Japan, drafted an Organisation for Economic Co-operation and Development (OECD) test guideline that was based on the ICCVAM-recommended BCOP protocol. This protocol was developed following an international peer review evaluation with contributions from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods. OECD TG 437 has now been formally adopted by OECD and will be accepted by all 33 OECD member countries in accordance with OECD Mutual Acceptance of Data. The use of BCOP will reduce the use of rabbits for eye safety testing and eliminate such testing in animals of most substances likely to cause severe pain and discomfort. ILS staff supported by NIEHS contract N01-ES-35504.
ICCVAM Evaluation of the Usefulness and Limitations of the Cytosensor®
Microphysiometer (CM) Test Method for Ocular Safety Testing

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ICCVAM recently evaluated several in vitro test methods as potential replacements for the rabbit
eye test for identifying potential ocular hazards. None of the methods were considered adequate
as complete replacements. However, ICCVAM concluded that test substances within a defined
limited applicability domain (water-soluble surfactants, surfactant-containing formulations and
nonsurfactants) that are positive for severe effects in the Cytosensor® microphysiometer (CM)
test method can be classified as ocular corrosives/severe irritants (EPA Category I, EU R41, GHS
Category 1). False positive rates ranged from 0% (0/17, 0/18) to 10% (3/29) and false negative
rates from 9% (2/23) to 50% (6/12) depending on the hazard classification system used.
ICCVAM also concluded that test substances within an even more restricted applicability domain
(water-soluble surfactant chemicals and certain types of surfactant-containing formulations, but
not nonsurfactants) can be considered as not classified for ocular hazards (EPA Category IV, EU
Not Labeled, FHSA Not Labeled) without any further testing if they are negative in CM.
Although false positive rates were high (50% [3/6] to 69% [18/26]), false negative rates ranged
from 0% (0/27, 0/28, or 0/40) to 2% (1/46 or 1/47) depending on the hazard classification system
used. A chemical that produces a response in CM between these two extremes would require
additional testing (in vitro and/or in vivo) to establish a definitive classification. CM is not
considered adequately valid for identification of mild or moderate ocular irritants (EPA
Categories II/III, GHS Categories 2A/2B; EU R36; EPA Categories II/III). ICCVAM also
recommended a standardized CM protocol and future studies to expand the applicability domain
of CM. These recommendations have been forwarded to Federal agencies and if accepted, CM
will be the first in vitro test method available in the U.S. for identifying substances that do not
require ocular hazard labeling. ILS staff supported by NIEHS contract N01-ES-35504.
Eye injury is a leading cause of visual impairment in the U.S., with up to 50,000 new cases reported each year. To evaluate the potential of chemicals to cause eye irritation, the protocol most widely accepted by regulatory agencies is based on the rabbit eye test. Since current ocular test guidelines state that users must ensure that the topical anesthetic does not affect test results, pain medications are often not used. However, for over 25 years CPSC has recommended pre-application of a topical anesthetic for all rabbit eye toxicity studies. Therefore, ICCVAM recently conducted a comprehensive evaluation of the usefulness and limitations of routinely using topical anesthetics, systemic analgesics, and earlier more humane endpoints to minimize pain and distress in ocular safety testing. Following this evaluation, which included recommendations from an international independent peer review panel, ICCVAM concluded that a balanced preemptive pain management plan should always be used when the rabbit eye test is conducted for regulatory safety testing. This protocol should include pre-treatment with a topical anesthetic and systemic analgesic, and routine post-treatment with systemic analgesia. ICCVAM also recommends several additional humane endpoints that should be used to end studies earlier. To ensure timely and accurate detection of humane endpoints in ocular studies, ICCVAM recommends examination with a slit-lamp biomicroscope, when considered appropriate, to characterize the nature, severity, and progression of any corneal lesions. ICCVAM also recommends routine observations for clinical signs of pain and distress at least twice daily, or more often if needed. Implementation of these ICCVAM recommendations should avoid or significantly reduce pain and distress associated with ocular safety assessments while continuing to support the protection of human health. ILS staff supported by NIEHS contract N01-ES-35504.
Currently, there is no alternative (non-"in vivo") ocular irritation assay that can measure corneal tissue damage and reversibility. With the support of two Colgate-Palmolive Grants for Alternative Research, we have developed an alternative assay: Porcine Corneal Opacity Reversibility Assay (PorCORA). PorCORA measures corneal damage and recovery over extended time periods using porcine corneas excised from by-product abattoir eyes. Test articles (liquid and solid) are dosed directly onto the corneal surface, and tissue damage and recovery are assessed by sodium fluorescein (NaFL) retention in the same corneas over time (up to 21 days). We have confirmed NaFL retention results and corneal recovery in the PorCORA system via several approaches. Both fluorescence and reflective confocal microscopy confirm damage repair indicated by fluorescein retention in the cultured corneas. In addition, we have shown histological evidence that also correlates well with NaFL staining in the PorCORA assay. Here we report the results of a 32-reference chemical validation including chemicals from the following classes: acetates, acids, alcohols, alkalis, esters, hydrocarbons, inorganics, ketones, surfactants, and several solid compounds. To determine if the PorCORA system can predict European Union (EU) R41 or United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Category 1, we considered corneas that retained NaFL at 21 days post-dose to be R41 and GHS Category 1. European Centre for Ecotoxicology and Toxicology of Chemicals historical rabbit eye data was used to classify EU and GHS eye irritation for the 32 compounds tested. PorCORA predicted 11/11 compounds classified as R41 and 12/13 compounds classified as GHS Category 1. Since PorCORA can predict these categories, then compounds that cause damage that is reversible in the PorCORA system may be considered R36 or Category 2. Thus PorCORA is a highly predictive method to distinguish between ocular irritancy classifications R36 or R41 and Category 1 or 2 without the use of live animals.
The Ex Vivo Eye Irritation Test with Optical Coherence Tomography

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Rationale and Scope: The potential for chemical substances to cause corneal irritation or burns has traditionally been tested in whole-animal rabbit eyes. New models are being sought as replacements, but most do not allow assessing chronic toxicity, repeated application, or long-term evaluation. One promising new model is the rabbit cornea ex vivo eye irritation test (EVEIT)(1) with optical coherence tomography (OCT)(2-4), previously used to study hydrofluoric acid corneal penetration kinetics and comparative decontamination with water, calcium gluconate, or Hexafluorine® (most efficacious)(3).

Methods: Rabbit eyes were obtained from an abattoir and the corneas preserved ex vivo in the EVEIT system in minimal essential medium (MEM). A dropping device kept corneas moist. Substances were applied once or repeatedly to 4-5 sites per cornea with a suction device. Evaluation was with fluorescein staining and OCT. Studied were performed with: negative controls (MEM or sodium hyaluronate); positive controls (repeated 0.05% benzalkonium chloride); 0.05% benzalkonium chloride in various concentrations; unknown substances; sodium hydroxide (NaOH); sulfuric acid (H₂SO₄); trichloroacetic acid; sodium lauryl sulfate; comparative decontamination of NaOH/H₂SO₄ (normal saline/Diphoterine®); and repeated phosphate buffer solution corneal calcifications with clinical correlation.

Results: For all tested substances with either single or repeated application, EVEIT-OCT showed reproducible results.

Conclusions: The EVEIT-OCT rabbit cornea ex vivo model is useful for evaluating unknown or known substances in comparison with negative and positive controls. It can be used to assess corneal penetration and wound kinetics, and for comparison or evaluation of different eye decontamination solutions.

References
The Impact of United States Adoption of the United Nations Globally Harmonized System on the Use of *In Vitro* Methods for Ocular and Dermal Irritation and Corrosion

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Endorsed by the United Nations in 2003, the U.N. Globally Harmonized System for Classification and Labeling (GHS) is intended to harmonize hazard classification and labeling criteria throughout the world for human health and ecotoxicity endpoints. While regions such as the European Union, Canada, and United States have committed in principle to adopting the GHS in place of their own national classification systems, timelines for such adoption differ between regions and even within; for example, timelines differ in the U.S. among different regulatory agencies. One issue to be resolved is slight differences in the values of the numerical bounds separating classification categories between existing systems and the GHS.

Although the GHS was designed to correlate with existing classification systems in order to prevent retesting of substances, classification systems do have an impact on the replacement, reduction, and refinement of animals in testing, because new *in vitro* methods for skin and eye irritation are validated according to how well the methods can correctly classify substances.

This poster compares U.S. Environmental Protection Agency, U.S. Occupational Safety and Health Administration, and GHS classifications for skin and eye irritation as they relate to validated *in vitro* methods for skin and eye irritation, and discusses solutions to harmonize these classification systems. The *in vitro* methods include: the bovine corneal opacity and permeability test method, the isolated chicken eye test method, the Cytosensor® microphysiometer test method, and the fluorescein leakage test method for eye irritation; and reconstructed human epidermis and barrier models for skin irritation. Widespread adoption of GHS will help speed harmonized adoption of existing and new *in vitro* methods for relevant endpoints.