The Impact of US Adoption of the UN Globally Harmonized System on the Use of In Vitro Methods for Ocular and Dermal Irritation and Corrosion

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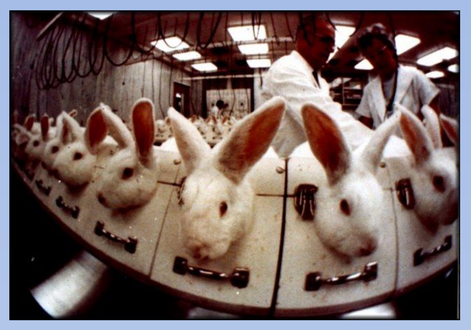


Abstract

Endorsed by the United Nations in 2003, the UN Globally Harmonized System for classification and labeling is intended to harmonize hazard classification and labeling criteria throughout the world for human health and ecotoxicity endpoints. While GHS was designed to correlate with existing classification systems and the European Union, Canada, and United States have committed in principle to adopting GHS in place of their own national classification systems, differences among classification systems have delayed adoption of GHS by various agencies. Harmonization with GHS impacts classification the replacement, reduction, and refinement of animals in testing since *in vitro* methods for skin and eye irritation have been and are currently being validated according to GHS classification. This poster compares US EPA, US OSHA and GHS classifications for skin and eye irritation as they relate to validated *in vitro* methods for skin and eye irritation and discusses methods to harmonize these classification systems. The 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.

Harmonization of Labeling Schemes

The primary objective of hazard classification and communication systems is to provide information to protect human health and the environment. Hazard classifications are also used to inform users of chemicals so that measures can be taken to minimize risk. Because of differences in use and exposure, hazard classification systems have historically varied with little or no consistency within or between different countries. Inconsistencies arising from these differences can lead to confusion regarding potential hazards and safe use of chemicals.

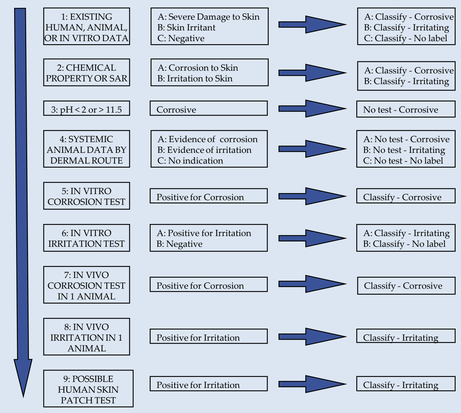
Through the United Nations, discussions regarding the international harmonization of classification and labeling of chemicals began in the early 1950's. The Organization for Economic Cooperation and Development (OECD) became involved in this project in the early 1990's resulting in the publication of the Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances in 1998, which has subsequently been revised (1).

GHS has been adopted in the EU and in some other regions; however, efforts to adopt GHS internationally are ongoing. Barriers to adoption of GHS include differing methods of assessing biological endpoints, use of different number of classification groups, differing cut-off values for classification, and differences in labeling systems. This presentation focuses on differences between US EPA, OSHA, and GHS classification and labeling schemes.

1. United Nations (UN) (2007). Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Second revised edition, UN New York and Geneva, 2007. Available at: [http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html]

Dermal

Figure A. Tiered testing and evaluation of dermal irritation potential



Adapted from OECD TG404: Acute Dermal Irritation/Corrosion (2002).
Note: At any time, user should review all available data from preceding steps to determine whether classification can be made.

Table 1: GHS Categories for Skin Corrosion/irritation

Class	Test results	
1: Corrosive	exposure	observation
1a	≤ 3 minutes	≤ 1 hour
1b	> 3 minutes - ≤ 1 hour	≤ 14 days
1c	> 1 hour - ≤ 4 hours	≤ 14 days
2. Irritant	(1) Mean value of > 2.3 - < 4.0 for erythema/eschar or for edema in at least 2 of 3 tested animals, or (2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, or (3) In some cases where there is pronounced variability of response among animals, with very definite positive in a single animal but less than the criteria above.	
3. Mild Irritant	Mean value of > 1.5 - < 2.3 for erythema/eschar or for edema from gradings in at least 2 of 3 tested animals or, if reactions are delayed, from grades on 3 consecutive days after the onset of dermal reactions.	

Table 2: EPA Categories for Skin Corrosion/irritation

Category	Test results / PII score*
I . Corrosive	> 7.0
II . Severe irritant	5.0 - ≤ 7.0
III . Moderate	2.0 - ≤ 5.0
IV. No/mild	0 - < 2.0

*Separately add each animal's erythema and edema scores for each interval (1, 24, 48 and 72 hours) and divide by (the number of test sites X 4 intervals), or add the scores for all animals at each interval and divide by (the number of test sites X 4 intervals)

Scoring: two separate scores, erythema and edema:
0 No erythema/edemea
1 Very slight erythema/edema (barely perceptible)
2 Well defined erythema/slight edema
3 Moderate erythema /edema
4 Severe erythema /ed

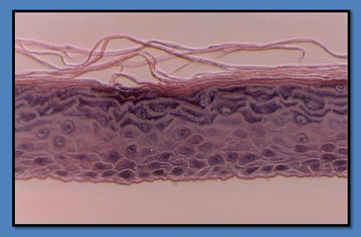


Table 3: *In vitro* approaches for skin irritation

Skin Corrosion	OECD TG 431 (2004), EU B.40bis (2004)	Skin corrosive or non-corrosive
	EPISKIN™	Furthermore, EPISKIN™ is able to distinguish corrosive 1A and 1B
	EpiDerm™	
	SkinEthic™ RHE	
	EST-1000	
	OECD TG 430 (2004), EU B.40 (2004)	Skin corrosive or non-corrosive
	Transcutaneous Electrical	
	Resistance Test (TER)	
	OECD TG 435 (2006)	Skin corrosive 1A, 1B and 1C or non-corrosive
	Corrositex®	
Skin Irritation	OECD TG 439 (2010), EU B.46 (2009)	No classification or irritant cat. 2
	EPISKIN™ SIT	
	EpiDerm™ EPI-200-SIT	
	SkinEthic™ SIT-24b	

United Nations (UN) (2007). Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Second revised edition, UN New York and Geneva, 2007. Available at: [http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html]

EC-ECVAM (2009). Statement on the "Performance under UN GHS of three *in vitro* assays for skin irritation testing and the adaptation of the Reference Chemicals and Defined Accuracy Values of the ECVAM skin irritation Performance Standards", issued by the ECVAM Scientific Advisory Committee (ESAC30), 9 April 2009. Available under "*Publications*" at: [http://ecvam.jrc.ec.europa.eu]

OECD (2004). Test Guideline 404. OECD Guideline for the Testing of Chemicals. Acute dermal irritation/corrosion. Updated Guideline, adopted April 24, 2002. Available at: [http://www.oecd.org/env/testguidelines]

Table 4: Comparison of GHS, EPA and OSHA Classifications

GHS	Draize score	EPA Category	PII	OSHA category
I Corrosive	≥ 4	**I Danger**	> 7.0	"Corrosive:" Causes visible destruction , or irreversible alterations.
II Irritant	≥ 2.3 and ≤ 4	**II Warning**	5.0 - ≤ 7.0	"Irritant" Not corrosive, but which causes a reversible inflammatory effect.
III Mild irritant (optional)	≥ 1.5 and < 2.3	**III Caution**	2.0 - ≤ 5.0	No classification
No label	< 1.5	**IV (Caution Optional)**	0 - < 2.0	No classification

Effect on human health protection of adopting GHS in the US

OSHA classification is consistent with GHS – no effect. What information changes if EPA were to adopt GHS?

1. Distinction between Category II (PII 5 – 7) and Category III (PII 2 – 5)

2. Differences between:
   a. GHS Category II (Draize 2.3 - 4.0) and combined EPA II/III (PII 2.0 – 7.0)
   b. GHS no label (Draize < 2.3) and EPA no label (PII < 2.0)

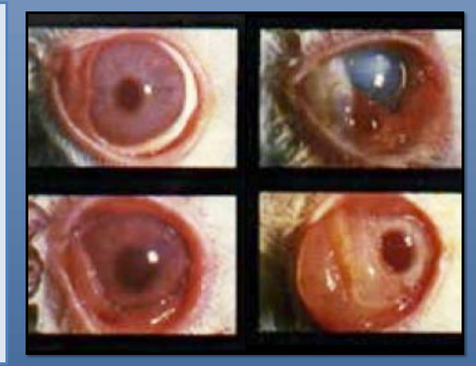
Would these changes be significant regarding protection?

Dermal and Eye Corrosion and Irritation Testing: historical animal-based testing

The determination of acute eye and skin irritation is included in international regulatory requirements for the testing of chemicals because of the possibility of exposure during the production, transport, marketing, and disposal of products. In 1992, OECD first published Test Guidelines for accute dermal and eye corrosion and irritation (1,2). These animal tests are quite painful, and the results are not strongly correlative with effects in humans; therefore, alternative tests are being developed to replace the use of animals for these endpoints.

Given the staged manner in which in alternative tests have been developed and validated over time and the desire to use additional physico-chemical or other existing information to avoid animal tests, staged testing strategies have been recommended (figures A and B).

1. OECD (2004). Test Guideline 404. OECD Guideline for the Testing of Chemicals. Acute dermal irritation/corrosion. Updated Guideline, adopted April 24, 2002. Available at: [http://www.oecd.org/env/testguidelines]
2. Organization for Economic Coordination and Development (OECD). OECD Guideline for the Testing of Chemicals 405: Acute Eye Irritation/Corrosion. 2002. Available at: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg405.pdf (accessed August 12, 2011).



Ocular

Eye Corrosion and Irritation

Eye corrosion is the production of tissue damage in the eye which is not fully reversible within 21 days of exposure. Eye irritation is the production of changes in the eye which are fully reversible within 21 days of exposure (1).

In vitro approaches to eye irritation

Two *ex vivo* methods have been adopted by OECD for the assessment of severe eye irritation (corrosion), and two *in vitro* methods are in the draft TG stage (Table 5). The Cytosensor Microphysiometer (CM) method can also detect non-irritant surfactants (i.e. GHS "no-label") using a "bottom-up" approach (2).

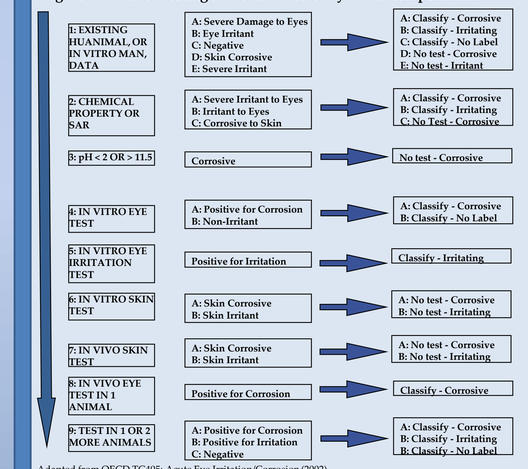
The EpiOcular reconstructed human corneal model is currently undergoing validation for eye irritation at ECVAM. It appears to be able to classify substances into all four US EPA categories and three GHS categories (3).

In addition to the testing strategy in Figure B, the "top-down/bottom-up" approach has recently been validated with the BCOP, CM, and Fluorescein Leakage (FL) methods for certain classes of chemicals (Figure C). In principle, substances that are not classified as either severe irritants or non-irritants could be considered irritants, although this has not been recommended (2, 4).

In 2009, the US EPA began a pilot program to allow classification of antimicrobial cleaning products using the BCOP, the CM, and the EpiOcular test methods, allowing complete replacement of the Draize eye test for this product class.

1. Organization for Economic Coordination and Development (OECD). OECD Guideline for the Testing of Chemicals 405: Acute Eye Irritation/Corrosion. 2002. Available at: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg405.pdf (accessed August 12, 2011).
2. EC-ECVAM (2009). Statement on the "Scientific Validity of Cytotoxicity/Cell Function Based In Vitro Assays for Eye Irritation Testing," issued by the ECVAM Scientific Advisory Committee (ESAC31), 10 July 2009. Available under "Publications" at: [http://ecvam.jrc.ec.europa.eu]
3. Jones et al. (2001) Comparative evaluation of five in vitro tests for assessing the eye irritation potential of hair-care products. Altern Lab Anim. 29(6):669-92.
4. Stern et al. (1998) Evaluation of the EpiOcular Tissue Model as an Alternative to the Draize Eye Irritation Test. Toxicol In Vitro. 12(4):455-61.

Figure B. Tiered testing and evaluation of eye irritation potential



Adapted from OECD TG405: Acute Eye Irritation/Corrosion (2002).
Note: At any time, user should review all available data from preceding steps to determine whether classification can be made.

Table 6: Comparison of EPA, GHS and OSHA Classification

	US EPA (in vivo)	UN GHS (in vivo)	OSHA Hazard Communication Requirements
"Corrosive"	Category I: At least one instance of irreversible destruction of ocular tissue or corneal involvement or irritation persisting for more than 21 days	Category 1: At least one instance of irreversible eye damage during 21-day period	A chemical that causes visible destruction of, or irreversible alterations in, living tissue by chemical action at the site of contact.
"Irritant"	Category II: 1/3 animals showing eye irritation which reverses from 7-21 days	Category 2: 2/3 animals showing eye irritation which reverses after 21 days	A chemical, which is not corrosive, but which causes a reversible inflammatory effect on living tissue by chemical action at the site of contact.
"Irritant"	Category III: 1/3 animals showing eye irritation which reverses within 7 days	Category 2B (optional): effects reverse within 7 days	N/A
No hazard	Category IV: minimal or no irritation clearing within 24 hours	No significant effects	N/A

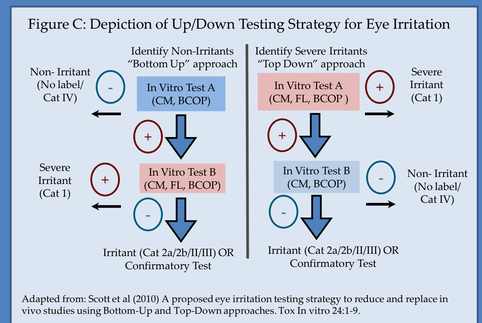
*In both US EPA and UN GHS systems, "irritation" is defined as a score of any of the following: corneal opacity or iritis of >/= 1 or conjuctival redness or edema of >/= 2. However, in GHS scores are a mean of scores at 24, 48, and 72 hours, while the EPA system uses absolute scores at any time point.

Table 5: In vitro methods assessing eye irritation

		GHS Classification	EPA Classification	Applicability and limitations
Identification of serious eye irritation		Positive results lead to Category 1 classification, whereas negative results require further testing	Category 1	BCOP, ICE: Positive results obtained with alcohols or ketones should be interpreted cautiously due to risk of over-prediction. High false negative rate. CM and FL: Applicable to water soluble substances and mixtures. High false negative rate.
OECD TG 437 (2009) Bovine Cornea Opacity Test (BCOP)	IVIS >/= 55.1			
OECD TG 438 (2009) Isolated chicken eye test (ICE)	Class IV in 2/3 eyes			
Validated in 2009: Cytosensor Microphysiometer (CM)	MRD50 </= 2 mg/mL			
Fluorescein Leakage (FL)	FL20 </= 100 mg/mL			
Identification of non irritants to the eye		MRD50 >10 mg/mL Negative results lead to no classification, whereas positive results require further testing	MRD50 >80 mg/mL = Category IV	CM: Applicable to water-soluble surfactants and water-soluble surfactant containing mixtures. High false positive rate. BCOP: High false positive rate.
Validated in 2009: Cytosensor Microphysiometer (CM)	MRD50 >10 mg/mL MRD50 >80 mg/mL			
Peer-reviewed in 2009: Bovine Cornea Opacity Test (BCOP)				

IVIS: In Vitro Irritancy Score
FL20: Fluorescein leakage of 20%
MRD50: Test chemical concentration that results in reduction of cell metabolic rate of 50%

Figure C: Depiction of Up/Down Testing Strategy for Eye Irritation



Adapted from: Scott et al (2010) A proposed eye irritation testing strategy to reduce and replace in vivo studies using Bottom-Up and Top-Down approaches. Tox In vitro 24:1-9.