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Interagency Coordinating Committee on the Validation of Alternative Methods

ICCVAM Evaluation of In Vitro Methods to Identify Ocular Corrosives and Severe Irritants: Report from the Expert Panel

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ExxonMobil Biomedical Sciences, Inc.

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Alexandria, VA

National Toxicology Program

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Scheffer Tseng, M.D., Ph.D., Director, Ocular Surface Research and Education Foundation, Miami, Florida
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Expert Panel Questions

1. Evaluate the extent and adequacy to which each of the applicable ICCVAM validation and acceptance criteria have been addressed
2. Develop conclusions and recommendations on:
   - Current usefulness and limitations of each of the four test methods for identifying ocular corrosives and severe/irreversible irritants
   - The test method protocol that should be used for future testing and validation studies
   - The adequacy of proposed optimization and/or validation studies
   - The adequacy of reference substances proposed for future validation studies
3. In the second meeting, the Panel was asked:
   - to determine if the information provided in the BRD Addendum were appropriate for inclusion in the accuracy and reliability re-analyses, and
   - if any changes to the original recommendations established at the January 11-12, 2005 meeting were warranted based on the updated information detailed in the BRD Addendum.
   - to consider the adequacy of the revised proposed list of reference substances.

Abbreviations and Definitions

- EPA = U.S. Environmental Protection Agency
- EU = European Union
- GHS = United Nations Globally Harmonized System for Classification and Labeling of Chemicals
- n = Number of substances in the database (variations in the number of substances vary by classification system due to the extent and type of individual animal data available).
- Performance calculated using the overall *in vitro* classification based on the majority and/or most severe classification among the multiple testing laboratories and tests (for substances tested multiple times in a laboratory).
  - Accuracy (concordance): the proportion of correct outcomes (positive and negative) of a test method
  - Sensitivity: the proportion of all positive substances that are classified as positive
  - Specificity: the proportion of all negative substances that are classified as negative
  - Positive predictivity: the proportion of correct positive responses among substances testing positive
  - Negative predictivity: the proportion of correct negative responses among substances testing negative
  - False positive rate: the proportion of all negative substances that are falsely identified as positive
  - False negative rate: the proportion of all positive substances that are falsely identified as negative.

Recommendations Generic to the Majority of *In Vitro* Test Methods

- Histopathology should be added to the organotypic assays.
  - Validation is not required for the addition of histopathology
  - NICEATM/ICCVAM should facilitate the development of a histopathology scoring system for corneal damage (with visual aids)
- Any optimization and validation studies should use existing animal data, if available
- Additional animal studies should only be conducted if important data gaps are identified and such studies should be carefully designed to maximize the amount of pathophysiological information obtained (e.g., wound healing)
  - *Minority opinion: as sufficient data should be available, additional animal testing for this purpose is not needed – Dr. Stephens*
- Reference substances should be identified that can be used as part of performance standards
- *Minority Opinion* - Drs. Stephens and Theran believe that the term "accuracy" is inappropriately used, and that it is more appropriate to use the term "consistency with *in vivo* data" when comparing test results.

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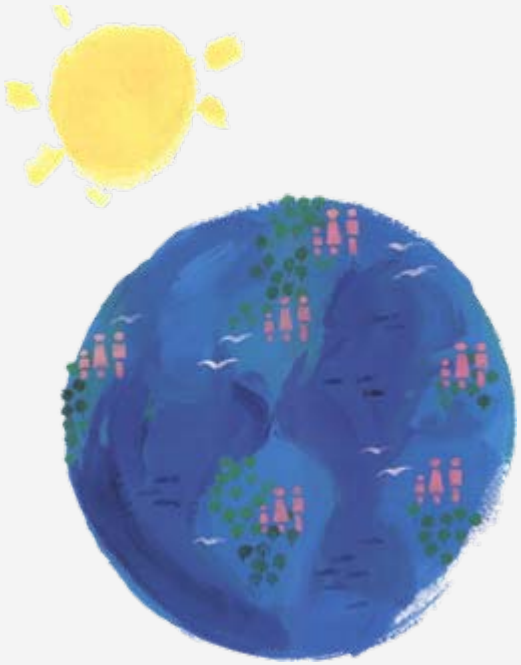
Bovine Corneal Opacity and Permeability (BCOP) Test Method



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BCOP Accuracy Reanalysis - GHS

Statistic	Old (n=120)	New (n=147)
Accuracy	79% (95/120)	81% (119/147)
Sensitivity	76% (32/142)	84% (36/43)
Specificity	81% (63/78)	80% (83/104)
Positive Predictivity	69% (34/49)	63% (36/57)
Negative Predictivity	86% (61/71)	92% (83/90)
False Positive Rate	19% (15/78)	20% (21/104)
False Negative Rate	24% (10/42)	16% (7/43)
Total *In Vivo* Severe Substances Used for Analysis	42	43
Total *In Vivo* Nonsevere Substances Used for Analysis	78	104

BCOP Technical Summary - Overall

Statistic	Ocular Hazard Classification System		
	GHS (n=147)	EPA (n=143)	EU (n=143)
Accuracy	81% (119/147)	79% (113/143)	80% (114/143)
Sensitivity	84% (36/43)	75% (30/40)	82% (33/40)
Specificity	80% (83/104)	81% (83/103)	79% (81/103)
False Positive Rate	20% (21/104)	19% (20/103)	21% (22/103)
False Negative Rate	16% (7/43)	25% (10/40)	18% (7/40)

BCOP data from the following studies were pooled for this analysis: Gautheron et al. (1994), Balls et al. (1995), Swanson et al. (1995), Gettings et al. (1996), Southee (1998), Swanson and Harbell (2000), Bailey et al. (2004).

BCOP Technical Summary - Chemical Class Analysis (GHS)

Chemical Class	N	False Positive Rate		False Negative Rate	
		%	No.	%	No.
Overall	***147***	***20***	***21/104***	***16***	***7/43***
Alcohols	18	53	8/15	67	2/3
Amines/Amidines	8	0	0/4	0	0/4
Carboxylic acids	15	38	3/8	14	1/7
Esters	12	12	1/8	0	0/4
Ethers/Polyethers	6	0	0/5	0	0/1
Heterocycles	12	33	2/6	17	1/6
Hydrocarbons	12	8	1/12	-	0/0
Ketones	10	40	4/10	-	0/0
Onium compounds	11	0	0/3	0	0/8

Only those chemical classes represented by ≥ 5 substances were included in this analysis

BCOP Technical Summary - Properties of Interest Analysis

Property of Interest	N	False Positive Rate		False Negative Rate	
		%	No.	%	No.
Overall	***147***	***20***	***21/104***	***16***	***7/43***
Liquids	92	26	18/68	4	1/24
Solids	32	10	2/20	42	5/12
Surfactants - Total	35	5	1/21	7	1/14
-nonionic	5	0	0/4	0	0/1
-anionic	3	0	0/2	100	1/1
-cationic	6	0	0/1	0	0/7
pH - Total	28	-	-	21	5/24
-acidic (<7.0)	11	-	-	18	2/11
-basic (>7.0)	15	-	-	23	3/13
-neutral (pH=7.0)	2	-	-	-	-

Expert Panel Recommended Use of the Current BCOP Test Method

- For the purpose of detecting severe eye irritants in the testing scheme outlined in the BRD, the BCOP test as presented is useful in identifying ocular corrosives and severe irritants, with the following exceptions:
  - Alcohols, ketones, and solids are problematic
  - Histopathological examination must be added, unless the substance is from a class of materials known to be accurately predicted using only opacity and permeability in the BCOP assay
- There is a need to confirm that the BCOP identifies substances known to cause serious eye injury in humans
- *Minority Opinion*
  - Dr. Freeman expressed no opinion as to whether the BCOP assay had met the validation criteria as set forth in the ICCVAM Submission Guidelines (2003). This is because the question of whether these validation criteria had been met never reached a conclusive decision by the Panel.
- Additional data did not change the Panel's conclusions

Expert Panel Recommended BCOP Test Method Protocol

- The Panel agreed with the BRD-proposed BCOP test method protocol:
  - Negative, positive and benchmark controls should be included
  - Eyes from young adult cattle should be used but also consider the use of younger animals
  - Users should be aware of the risk of BSE and other zoonoses and use proper precautions
  - 0.9% NaCl should be used as the standard diluent and rinse
  - Osmolarity and pH of test solutions should be determined
  - The larger holder designed by Ubels should be used to eliminate the crush zone
  - The calculated total score should be further evaluated
  - The media used to bathe the eyes should be optimized
  - The rinsing procedures should be optimized
  - The use of antibiotics during eye transportation should be discouraged

Expert Panel Recommended Optimization and Validation Studies for BCOP

- Optimization studies will be necessary to ensure any changes to the protocol will not increase the variability of the test method
- The following may be satisfied by the anticipated submission of additional data:
  - Protocol for solids (improved exposure methods)
  - Protocol for alcohols and ketones (3 minute exposure time)

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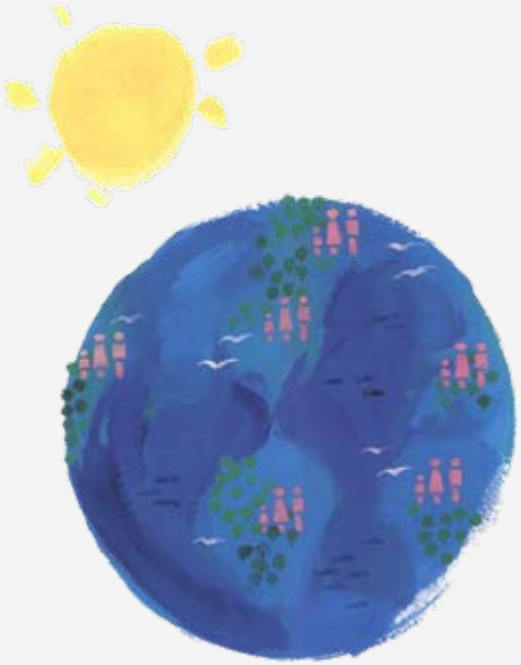
Isolated Chicken Eye (ICE) Test Method



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ICE Accuracy Reanalysis - GHS

Statistic	Old (n=92)	New (n=144)
Accuracy	82% (75/192)	83% (120/144)
Sensitivity	60% (15/25)	50% (15/30)
Specificity	90% (60/67)	92% (105/114)
Positive Predictivity	68% (15/22)	63% (15/24)
Negative Predictivity	86% (60/70)	88% (105/120)
False Positive Rate	10% (7/67)	8% (9/114)
False Negative Rate	40% (10/25)	50% (15/30)
Total *In Vivo* Severe Substances Used for Analysis	25	30
Total *In Vivo* Nonsevere Substances Used for Analysis	67	114

ICE Technical Summary - Overall

Statistic	Ocular Hazard Classification System		
	GHS (n=144)	EPA (n=145)	EU (n=154)
Accuracy	83% (120/144)	84% (122/145)	87% (134/154)
Sensitivity	50% (15/30)	52% (15/29)	59% (19/32)
Specificity	92% (105/114)	92% (107/116)	94% (115/122)
False Positive Rate	8% (9/114)	8% (9/116)	6% (7/122)
False Negative Rate	50% (15/30)	48% (14/29)	41% (13/32)

Data was obtained from Prinsen and Koëter (1993), Balls et al. (1995), Prinsen (1996), Prinsen (2000), and Prinsen (2005).

ICE Technical Summary - Chemical Class Analysis (GHS)

Chemical Class	N	False Positive Rate		False Negative Rate	
		%	No.	%	No.
Overall	***144***	***8***	***9/114***	***50***	***15/30***
Alcohols	12	50	5/10	50	1/2
Amines/Amidines	5	0	0/2	33	1/3
Carboxylic acids	10	0	0/3	43	3/7
Esters	9	13	1/8	0	0/1
Heterocycles	9	0	0/2	33	2/6
Onium compounds	8	0	0/2	33	2/6

Only those chemical classes represented by ≥ 5 substances were included in this analysis

ICE Technical Summary - Properties of Interest Analysis

Property of Interest	N	False Positive Rate		False Negative Rate	
		%	No.	%	No.
Overall	***144***	***8***	***9/114***	***50***	***15/30***
Liquids	108	10	9/90	44	8/18
Solids	36	0	0/24	58	7/12
Surfactants - Total	21	0	0/12	56	5/9
-nonionic	4	0	0/3	100	1/1
-anionic	2	0	0/1	100	1/1
-cationic	7	0	0/1	33	2/6
pH - Total	20	-	-	40	8/20
-acidic (<7.0)	12	-	-	33	4/12
-basic (>7.0)	8	-	-	50	4/8

Expert Panel Recommended Use of the Current ICE Test Method

- The ICE test method appears to be useful in the identification of ocular corrosives/severe irritants in a tiered-testing strategy, with the following limitations:
  - Alcohols tend to be overpredicted
  - Surfactants tend to be underpredicted
  - Solids and insoluble substances may be problematic as they may not come in adequate contact with the corneal surface (leading to underprediction)
  - The low overall false positive rate (8-10%) means that the ICE test can be used at present to screen for severe eye irritants.
    - However, given the high false positive rate (50%) calculated for a small number of alcohols (n=10), caution should be observed when evaluating ICE test results with these types of substances.
- Additional data did not change Panel conclusions

Expert Panel Recommended ICE Test Method Protocol

- The Panel agreed with the BRD-proposed ICE test method protocol
  - The only difference from previous protocols is the inclusion of a positive control and additional test eyes for the negative control (n=3 eyes for each test substance and controls)
- The appropriateness of using only three eyes per test substance has not been formally evaluated
- Recommended protocol improvements:
  - Maintain the eyes in a horizontal position throughout the assay
  - Define a standardized scoring scheme for histopathology using the formal language of pathology to describe any effects (with an accompanying atlas)
  - Identify the appropriate circumstances under which histopathology would be warranted
  - Provide reference photographs for all subjective endpoints
  - Install centering lights on the optical pachymeter to enhance reproducibility of corneal thickness measurements
  - The protocol must specify that universal safety precautions be observed when handling chemical and biological materials.

Expert Panel Recommended Optimization and Validation Studies for ICE

- Optimization studies should focus on:
  - The ICE decision criteria in order to reduce the false negative rate
  - Determining the optimum number of eyes to be tested
  - Evaluating the impact of delayed use of chicken eyes on assay performance
  - Expanding the capacity of the custom superfusion apparatus
  - Defining the most appropriate assay medium

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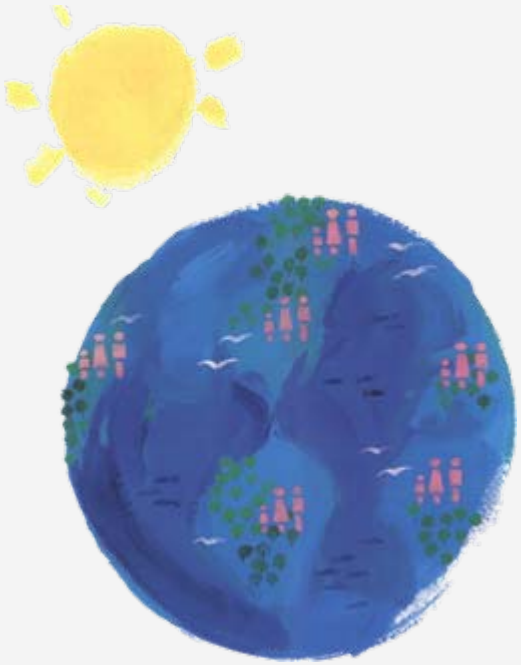
Isolated Rabbit Eye (IRE) Test Method



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IRE Accuracy Reanalysis - GHS

Statistic	Old (n=36)*	New (n=76)*
Accuracy	78% (28/36)	68% (52/76)
Sensitivity	100% (12/12)	100% (33/33)
Specificity	67% (16/24)	44% (19/43)
Positive Predictivity	58% (11/19)	58% (33/57)
Negative Predictivity	100% (17/17)	100% (19/19)
False Positive Rate	33% (8/24)	56% (24/43)
False Negative Rate	0% (0/12)	0% (0/33)
Total *In Vivo* Severe Substances Used for Analysis	12	33
Total *In Vivo* Nonsevere Substances Used for Analysis	24	43

* Old refers to analysis conducted with data from Guerriero et al. (2004) data only; New refers to "Expanded Data Set" discussed previously; 38 substances from Guerriero et al. (2004) and 38 substances from Balls et al. (1995) and Gettings et al. (1996).

IRE Technical Summary - Overall

Statistic	Study	Ocular Hazard Classification System		
		GHS (n=38/76)	EPA (n=38/76)	EU (n=38/80)
Accuracy	Guerriero et al. (2004)	79% (30/38)	79% (30/38)	79% (30/38)
	Expanded Data Set[1]	68% (52/76)	66% (50/76)	70% (56/80)
Sensitivity	Guerriero et al. (2004)	100% (11/11)	100% (11/11)	100% (11/11)
	Expanded Data Set	100% (33/33)	100% (31/31)	100% (37/37)
Specificity	Guerriero et al. (2004)	70% (19/27)	70% (19/27)	70% (19/27)
	Expanded Data Set	44% (19/43)	42% (19/45)	44% (19/45)
False Positive Rate	Guerriero et al. (2004)	30% (8/27)	30% (8/27)	30% (8/27)
	Expanded Data Set	56% (24/43)	58% (26/45)	56% (24/43)
False Negative Rate	Guerriero et al. (2004)	0% (0/11)	0% (0/11)	0% (0/11)
	Expanded Data Set	0% (0/33)	0% (0/31)	0% (0/37)

[1]Substances in CEC (1991), Balls et al. (1995), and Gettings et al. (1996) identified as corrosives/severe irritants using the Guerriero et al. (2004) decision criteria were added to the substances tested by Guerriero et al (2004). Substances identified as nonsevere irritants in these studies could not be used in the accuracy analysis, since only one to three of the four ocular endpoints were used and any missing endpoint might have resulted in a severe irritant classification.

IRE Technical Summary - Chemical Class Analysis (GHS)

Chemical Class	N	False Positive Rate		False Negative Rate	
		%	No.	%	No.
Overall	***76***	***56***	***24/43***	***0***	***0/33***
Alcohols	11	60	6/10	0	0/1
Amides	5	0	0/3	0	0/2
Amines	9	60	3/5	0	0/4
Carboxylic acids	5	67	2/3	0	0/2
Esters	6	67	4/6	-	0/0
Ethers	8	40	2/5	0	0/3
Formulations	12	100	2/2	0	0/10
Heterocycles	16	50	4/8	0	0/8
Ketones	6	67	4/6	-	0/0
Onium compounds	9	33	1/3	0	0/6
Sulfur compounds	7	20	1/5	0	0/2

Only those chemical classes represented by ≥ 5 substances were included in this analysis.
Expanded Data Set" discussed previously; 38 substances from Guerriero et al. (2004) and 38 substances from Balls et al. (1995) and Gettings et al. (1996).

IRE Technical Summary - Properties of Interest Analysis

Property of Interest	N	False Positive Rate		False Negative Rate	
		%	No.	%	No.
Overall	***76***	***56***	***24/43***	***0***	***0/33***
Liquids	43	83	19/23	0	0/20
Solids	33	25	5/20	0	0/13
Surfactants - Total	10	50	2/4	0	0/6
-nonionic	3	50	1/2	0	0/1
-anionic	-	-	-	-	-
-cationic	7	100	1/1	0	0/6
pH - Total	27	-	-	-	-
-acidic (<7.0)	18	20	2/10	0	0/8
-basic (>7.0)	7	33	2/6	0	0/1

Expert Panel Recommended Use of the Current IRE Test Method

- The test method appears to be useful in a tiered-testing strategy for identification of severe irritants/corrosives
  - Addition of fluorescein penetration and epithelial integrity improve accuracy, but limited data was available for analysis with this protocol (n=36 substances for GHS and EPA analyses)
  - Decision for use of test method in a tiered-testing strategy requires a larger set of data (increased n) to corroborate the accuracy results and provide a reliability assessment
- Additional data did not change Panel conclusions

Expert Panel Recommended IRE Test Method Protocol

- The recommended standardized protocol was appropriate, with revision:
  - The rabbit source should be defined
  - The use of eyes from rabbits used for other studies should be evaluated with the EPA
  - The prediction model and the rationale for its use should be better defined
  - Users should be alert for potential zoonoses from tissue handling

Expert Panel Recommended Future Optimization and Validation Studies for IRE

- The false positive rate (56%) needs to be reduced without unacceptably increasing the current false negative rate (0%)

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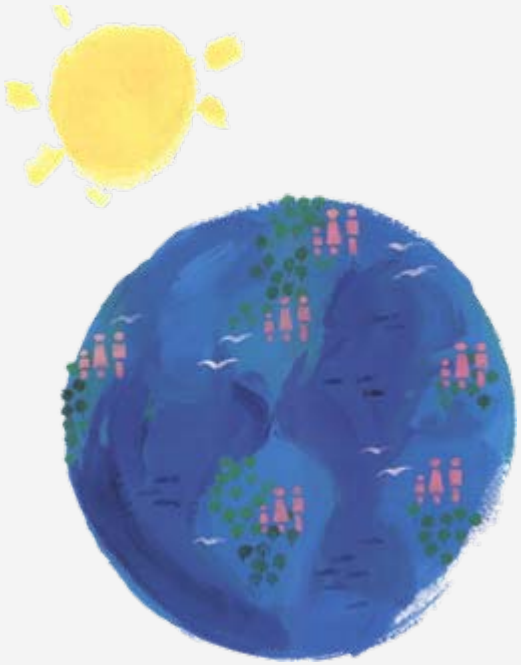
Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) Test Method



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HET-CAM Accuracy Reanalysis - GHS

Statistic	Old (n=55)*	New (n=101)*
Accuracy	85% (4/52)	68% (69/101)
Sensitivity	100% (12/12)	70% (28/40)
Specificity	80% (32/40)	67% (41/61)
Positive Predictivity	60% (12/20)	58% (28/48)
Negative Predictivity	100% (32/32)	77% (41/53)
False Positive Rate	20% (8/40)	33% (20/61)
False Negative Rate	0% (0/12)	30% (12/40)
Total *In Vivo* Severe Substances Used for Analysis	12	40
Total *In Vivo* Nonsevere Substances Used for Analysis	40	61

*Old refers to analysis for IS(B) analysis method (Gettings et al. data); New refers to analysis for IS(B)-10 analysis method (substances tested *in vitro* at 10% and compared to substances tested undiluted *in vivo*.

HET-CAM Technical Summary - Overall

Statistic	IS(B)-10 Analysis Method			IS(B)-100 Analysis Method		
	GHS (n=101)	EPA (n=98)	EU (n=95)	GHS (n=143)	EPA (n=138)	EU (n=178)
Accuracy	68% (69/101)	65% (64/98)	67% (64/95)	53% (76/143)	51% (70/138)	54% (96/178)
Sensitivity	70% (28/40)	68% (21/31)	70% (23/55)	85% (35/41)	87% (26/30)	89% (31/35)
Specificity	67% (41/61)	64% (43/67)	66% (41/62)	40% (41/102)	41% (44/108)	45% (65/143)
False Positive Rate	33% (20/41)	36% (24/67)	34% (21/61)	60% (61/102)	59% (64/108)	55% (78/143)
False Negative Rate	30% (12/40)	32% (10/31)	30% (10/33)	15% (6/35)	13% (4/30)	11% (4/35)

IS(B) = Kalweit et al. (1989, 1990)

HET-CAM Technical Summary - Chemical Class Analysis (GHS)

Chemical Class	N	False Positive Rate		False Negative Rate	
		%	No.	%	No.
IS (B)-10 Method					
Overall	***101***	***33***	***20/61***	***30***	***12/40***
Alcohols	16	89	8/9	25	2/7
Amines	7	60	3/5	50	1/2
Aldehydes	5	0	0/4	100	1/1
Ethers	14	50	5/10	50	2/4
Formulations	24	0	0/8	44	7/16
Heterocycles	7	86	6/7	-	0/0
Organic salts	7	57	4/7	-	0/0
IS (B)-100 Method					
Overall	***143***	***60***	***61/102***	***15***	***6/41***
Alcohols	26	94	16/17	11	1/9
Aldehydes	6	80	4/5	0	0/1
Amines	10	83	5/6	50	2/4
Esters	14	83	10/12	0	0/2
Ethers	18	57	8/14	25	1/4
Formulations	27	26	6/23	0	0/4
Heterocycles	13	78	7/9	50	2/4
Inorganic salts	5	100	2/2	0	0/3
Ketones	6	67	4/6	-	0/0
Organic salts	9		6/7	0	0/2

Only those chemical classes represented by ≥ 5 substances were included in this analysis

HET-CAM Technical Summary - Properties of Interest Analysis

Property of Interest	N	False Positive Rate		False Negative Rate	
		%	No.	%	No.
IS (B)-10 Method					
Overall	***101***	***33***	***20/61***	***30***	***12/40***
Liquids/Solutions	35	19	3/16	37	7/19
Solids	27	58	11/19	13	1/8
Unknowns	39	23	6/26	31	4/13
Surfactant-based Formulations	24	0	0/8	44	7/16
pH - Total -acidic (<7.0) -basic (>7.0)	35 24 11	58 50 80	11/19 7/14 4/5	13 20 0	2/16 2/10 0/6
IS (B)-100 Method					
Overall	***143***	***60***	***61/102***	***15***	***6/41***
Liquids	63	67	36/54	0	0/9
Solids	43	67	16/24	26	5/19
Unknowns	37	38	9/24	8	1/13
pH - Total -acidic (<7.0) -basic (>7.0)	35 23 12	68 69 67	13/19 9/13 4/6	13 10 17	2/16 1/10 1/6
Surfactants - Total -nonionic -anionic -cationic	3 3 0 0	66 66 - -	2/3 2/3 0/0 0/0	- - - -	- - - -

Expert Panel Recommended Use of the Current HET-CAM Test Method

- Based on the revised analysis, the IS(B) analysis method (according to Kalweit) is not sufficiently predictive of ocular corrosives and severe irritants

Expert Panel Proposed Recommended HET-CAM Test Method Protocol

- Agreed with recommended test method protocol for the testing of liquids
- Procedures for evaluating solids should be revised to discuss applying and removing solids for testing
- Additional endpoints (e.g., trypan blue absorption, antibody staining, histology, membrane changes) could be evaluated to increase the accuracy and reliability of method

Expert Panel Proposed Future Optimization and Validation Studies for HET-CAM

- Retrospective optimization studies should be conducted to reduce the false positive and false negative rates
- Once an optimized test method protocol is developed, small scale validation studies may be needed

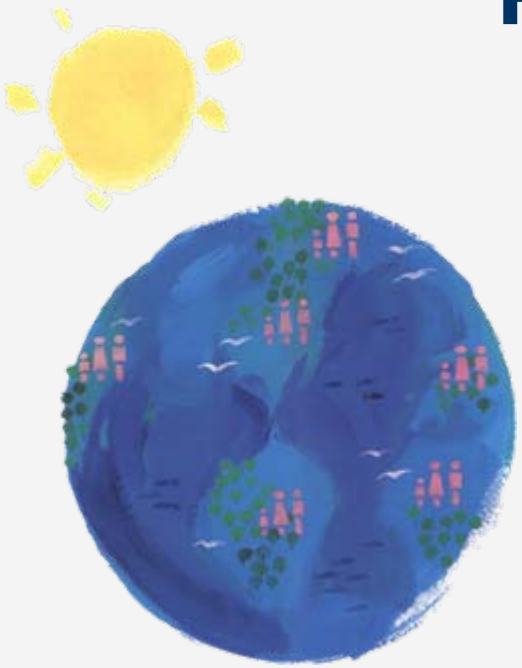
NICEATM

National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods

Recommended Reference Substances



National Toxicology Program

NIEHS

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Reference Substances

- Should cover the range of ocular responses
  - Induce very severe responses within a relatively short period, as well as those where the response is delayed
  - Adversely affect the cornea, iris, and/or conjunctiva
  - Induce persistent and non-persistent lesions
- Should represent a range of known or anticipated mechanisms or modes of action
- Should represent a diverse population of chemical classes and physicochemical properties
- Should be based on high quality *in vivo* rabbit eye test method studies
- Should have a well-defined chemical composition
- Should have been tested at a defined concentration and purity
- Should be readily available

1st Proposed Reference Substance List

- The list of 89 substances included:
  - 48 GHS Category 1 substances
  - 26 GHS Category 2 substances
  - 15 GHS nonirritant substances
  - 26 chemical classes
  - 29 product classes
  - 67 liquids
  - 22 solids
- Individual rabbit data are available for each substance

Proposed Reference Substances - 1st Set of Panel Recommendations

- Adequacy and Completeness of the List of Reference Substances
  - The list of recommended substances is comprehensive
  - The substances appear to be readily available and in acceptably pure form.
  - The range of possible ocular toxicity responses in terms of severity and types of lesions appears to be adequately represented.
  - However, while it is recognized the selection of reference substances is in part limited by the availability of *in vivo* reference data, comments and recommendations for the list include:
    - The current list has entirely too many substances and is unwieldy
    - Surfactants are over-represented and thus could be reduced in number
    - More inorganic substances should be added to the list if feasible
    - Substances known to induce severe lesions, *in vivo*, in the eyes of humans should be included, even in the absence of rabbit data
- For all validation studies, Material Safety Data Sheets (MSDS) for the recommended substances should be provided (e.g., a coded MSDS); also do prestudy safety briefing

2nd Proposed Reference Substance List

- The revised list of 122 substances included:
  - 79 GHS Category 1 substances
    - 10 substances classified as severe irritants based on human data
  - 28 GHS Category 2 substances
  - 15 GHS nonirritant substances
  - 34 chemical classes
  - 29 product classes
  - 79 liquids
  - 43 solids
- Individual rabbit data are available for each substance

Proposed Reference Substances - 2nd Set of Panel Recommendations

- The Panel still considered the list too large if the list is intended to be the minimum number of substances that should be used for validation of a new test method BUT it is appropriate if it is a list of candidate chemicals from which to select sets of chemicals to be used in validation studies.
- The Panel recommended that a focus on mechanism of action may reduce the number of substances needed to evaluate the relevance and reliability of a proposed test method.
- The Panel recommended that the highest purity level available from major suppliers for each substance be used and ideally, information on major impurities provided.