Short Time Exposure (STE) Test Method

Summary Review Document

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
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Supplements

Supplements containing files all of the available documentation related to the short time exposure (STE) test method review have been provided on the enclosed CD-ROM. These documents are also available on the NICEATM website at http://iccvam.niehs.nih.gov/STEReview.htm. Supplement A contains the Kao Corporation STE background review document (A1) and appendices (A2 to A10) as submitted. Supplement B (B1 to B3) contains the data and information used by NICEATM to conduct a technical review of the STE test method.

Supplement A
Kao Corporation Background Review Document and Appendices

List of Tables

List of Abbreviations and Acronyms

AD	Applicability domain
BCOP	Bovine corneal opacity and permeability
BRD	Background review document
CASRN	CAS Registry Number® (American Chemical Society)
CV	Coefficient of variation
EPA	U.S. Environmental Protection Agency
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
ILS	Integrated Laboratory Systems, Inc.
JaCVAM	Japanese Center for the Validation of Alternative Methods
kPa	Kilopascals
MeSH®	Medical Subject Headings (National Library of Medicine)
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NICEATM	National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NLM	National Library of Medicine
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SIRC	Statens Seruminstitut rabbit cornea
STE	Short time exposure
TG	Test Guideline
UN	United Nations

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Executive Summary

The short time exposure (STE) test method is an *in vitro* method for identifying ocular irritants. Developed by Takahashi et al. (2008), the STE test method assesses cytotoxicity in a rabbit corneal epithelial cell line (SIRC cells) through a 5-minute exposure to the test substance. In March 2011, Kao Corporation (Tochigi, Japan) submitted a background review document (BRD) titled "Current Status of *In Vitro* Test Methods for Identifying Ocular Irritants: Short Time Exposure (STE) Test" to the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NICEATM conducted a preliminary evaluation of the BRD and requested additional information, which resulted in several revisions to the BRD. Kao Corporation drafted a final BRD in May 2012 (**Supplement A**). The BRD contains all data and information that were available in the peer-reviewed literature and Kao Corporation in–house data to describe the current validation status of the STE test method, including what was known about its accuracy and reliability.

This summary review document presents an evaluation of STE test method accuracy, sensitivity, specificity, false positive rate, and false negative rate based on test substances with corresponding *in vivo* data. The analysis in a top-down and a bottom-up approach was based on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2011) and U.S. Environmental Protection Agency (EPA 2012) classification systems for eye hazard classification and labeling.

In a top-down approach, the STE test method is used to distinguish and label severe eye irritants/corrosives from all other hazard categories. Any substance not identified as a severe eye irritant/corrosive by the STE test method requires additional testing with other methods. A top-down approach requires a low false positive rate to avoid overclassification of substances. The false negative rate is not as critical because substances that test negative in the STE test method would be tested with another method.

In contrast, a bottom-up approach is used to distinguish substances not labeled as eye irritants from all other hazard categories. Any substance that tests positive in a bottom-up approach requires additional testing with other methods to determine the appropriate hazard classification and labeling. A bottom-up approach requires a low false negative rate to avoid irritants being classified and mislabeled as irritants when the correct eye hazard classification is GHS Not classified or EPA Category IV (minimal effects clearing in less than 24 hours). The false positive rate is not as critical because substances that test positive in the STE test method would be tested with another method.

The Kao Corporation BRD describes their analyses of 119 tests substances in four studies (Kojima et al. [Kao BRD]; Sakaguchi et al. 2011; Takahashi et al. 2009, 2010), with additional in-house data provided by Kao Corporation. In September 2012, Kao Corporation provided data on 52 additional surfactant or surfactant-containing substances, for a total of 169 substances with *in vitro* STE and *in vivo* rabbit eye test data (**Supplement B**).

The analyses in this report used consensus calls for both STE and rabbit eye test data when results were available from more than one laboratory or study. When equivocal results were obtained in two or more laboratories or in different studies, the more severe hazard classification was used.

Table 1 summarizes overall performance of the STE test method in a top-down approach for all substances in the database. The overall false positive rate in a top-down approach ranged from 1.2% (1/84) for GHS classification to 2.3% (2/87) for EPA classification. Exclusion of alcohols reduced the rate to 0% (data not shown). The performance of the STE test method is compared to other validated *in vitro* test methods in **Table 2**.

Table 1 Overall STE Performance in a Top-Down Approach

Regulatory System	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
GHS	120	85	102/120	53	19/36	99	83/84	1.2	1/84	47	17/36
EPA	120	87	104/120	58	19/33	98	85/87	2.3	2/87	42	14/33

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = Globally Harmonized System of Classification and Labelling of Chemicals; N = number of substances; STE = short time exposure.

Table 2 Top-Down Performance of Validated *In Vitro* Test Methods Compared to the STE Test Method

GHS	N	Accuracy		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.
BCOP	188	79	149/188	24	29/123	15	10/65
ICE	144	83	120/144	8.0	9/114	50	15/30
CM	82	90	74/82	2.0	1/48	21	7/34
STE	120	85	102/120	1.2	1/84	47	17/36

Abbreviations: BCOP = bovine corneal opacity and permeability; CM = Cytosensor microphysiometer; GHS = Globally Harmonized System of Classification and Labelling of Chemicals; ICE = isolated chicken eye; N = number of substances.

The STE overall performance in a bottom-up approach is shown in **Table 3.** The overall false negative rate in a bottom-up approach ranged from 12.3% (9/73) for GHS classification to 24.7% (24/97) for EPA classification.

Table 3 Overall STE Performance in a Bottom-Up Approach

Regulatory System	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
GHS	129	85	109/129	88	64/73	80	45/56	20	11/56	12	9/73
EPA	129	80	103/129	75	73/97	94	30/32	6.3	2/32	25	24/97

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = Globally Harmonized System of Classification and Labelling of Chemicals; N = number of substances; STE = short time exposure.

The applicability domain was evaluated to reduce the false positive rate and increase performance for both GHS and EPA classifications in a bottom-up approach. Improvements in the applicability domain were determined by analyzing assay performance by chemical class and physical properties. As a result, two applicability domains were evaluated based on excluding certain chemical and product classes, or physical characteristics. Applicability domain one excludes liquids with vapor pressures ≥6 kilopascals (kPa) solid alcohols, hydrocarbons, and salts while applicability domain two excludes liquids with vapor pressures ≥6 kilopascals (kPa) and nonsurfactant solids (**Table 4)**. The performance of the STE test method is compared to other validated *in vitro* test methods in **Table 5**.

Table 4 Overall STE Performance in a Bottom-Up Approach After Excluding Discordant Categories

Regulatory System	N	Accuracy		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.
Applicability Domain 1 - Exclusion of liquids with vapor pressures ≥6 kilopascals (kPa) solid alcohols, hydrocarbons, and salts							
GHS	94	90	85/94	18	8/45	2	1/49
EPA	94	83	78/94	7.7	2/26	21	14/68
Applicability Domain 2 - Exclusion of liquids with vapor pressures ≥6 kilopascals (kPa) and nonsurfactant solids							
GHS	101	90	91/101	19	9/47	1.9	1/54
EPA	101	85	86/101	7.1	2/28	18	13/73

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = Globally Harmonized System of Classification and Labelling of Chemicals; No. = number; STE = short time exposure.

Table 5 Bottom-Up Performance of Validated *In Vitro* Test Methods Compared to the STE Test Method

GHS	N	Accuracy		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.
BCOP	188	66	125/188	69	63/91	0	0/97
CM	53	68	36/53	68	17/25	0	0/28
STE – AD1	94	91	85/94	18	8/45	2	1/49
STE – AD2	101	90	91/101	19	9/47	1.9	1/54

Abbreviations: AD = applicability domain; BCOP = bovine corneal opacity and permeability; CM = Cytosensor microphysiometer; N = number of substances; STE = short time exposure.

This evaluation of the STE performance shows that this method is able to distinguish substances as severe irritants or corrosives (i.e., GHS Category 1 or EPA Category I) from all other hazard categories (GHS Category 2A, 2B, Not Classified or EPA Category II, III, IV) in a top-down approach, with false positive rates ranging from 1.2% (1/84) to 2.3% (2/87) for the GHS and EPA classification systems, respectively. Exclusion of discordant chemical classes (e.g., alcohols, ethers, hydrocarbons, or nonionic surfactants) reduced the false positive rate to 0%. In a bottom-up approach to distinguish substances that were either not classified or minimal irritants (i.e., GHS Not Classified or EPA Category IV) from all other hazard categories (i.e., GHS Category 1, 2A, 2B or EPA Category I, II, III), the STE false negative rates ranged from 12.3% (9/73) to 24.7% (24/97). The range of false negative rates in a bottom-up approach was decreased to 2% (1/49) and 21% (14/68) for the GHS and EPA classification systems, respectively, when liquids with vapor pressures >6kPa solid alcohols, hydrocarbons, and salts were excluded. The range of false negative rates in a bottom-up approach was decreased to 1.9% (1/54) and 18% (13/73) for the GHS and EPA classification systems, respectively, when liquids with vapor pressures >6kPa and nonsurfactant solids were excluded.

This SRD along with the original Kao BRD and other supporting documentation were forward by NTP to four external scientific reviewers. The reviewers were provided a list of questions that included a request to comment on the adequacy of the database used for evaluating STE, the adequacy of the performance evaluation, and to provide comments for regulators using the test

method. In response, the reviewers indicated that the database of compounds was generally sufficient and the review thorough. A summary of reviewer comments is provided in **Section 5.0**.

1.0 Introduction and Background

The Draize rabbit eye test has been the primary method used to determine the ocular irritation potential of chemicals (Draize et al. 1944). However, public interest in animal welfare has increased the pressure to develop non-animal alternatives. The development of alternative methods is also accelerating due to regulations banning animal ocular irritation tests for cosmetics in the European Union (Directive 2003/15/EC; European Union 2003). As a result, numerous alternative ocular irritation methods that use cell lines and tissues are being developed around the world (Balls et al. 1999; Eskes et al. 2005; Ohno et al. 1999). The test guidelines for the bovine corneal opacity and permeability (BCOP) test method and isolated chicken eye (ICE) test method were accepted by the Organisation for Economic Co-operation and Development (OECD) for predicting severe ocular irritation (OECD 2012a, 2012b). However, no other test guidelines have been accepted for *in vitro* ocular irritation tests.

The short time exposure (STE) test method is an alternative ocular irritation method developed by Kao Corporation (Takahashi et al. 2008). The STE test method uses a cultured cell line (SIRC cells) derived from rabbit cornea and uses shorter exposure times than many other cytotoxicity-based methods. Generally, cytotoxicity tests using cultured cells have the advantage of being simple, quick procedures with a low evaluation cost. The facility requirements necessary to conduct the STE test include a standard laboratory setup for cell culture. The cornea is one of the main targets during accidental eye exposures, and damage to the cornea can result in visual impairment. A final advantage of the STE test method is that it can be used to evaluate poorly water-soluble chemicals like toluene, octanol, and hexanol by using mineral oil as the vehicle (Takahashi et al. 2008).

The STE test method involves exposing SIRC cells to 5% and 0.05% concentrations of test substance for 5 minutes. Following exposure to 5% test substance concentration, substances that reduce cell viability below 70% are classified as irritants. Using this classification scheme, Kao Corporation assessed the performance of the STE test method in a bottom-up approach to distinguish substances not labeled as irritants from all other categories. Kao Corporation also proposed a second approach to establish an ocular irritation potency ranking that differentiates severely irritating substances from mild and moderate irritants. This approach uses a point system based on the test concentration and relative viability resulting from an exposure to 5% or 0.05% of test material (Takahashi et al. 2008). This second approach was used to review the STE test method in a top-down approach to distinguish corrosives/severe irritants from all other categories.

In March 2011, the Japanese Center for the Validation of Alternative Methods (JaCVAM), as part of the International Cooperation on Alternative Test Methods agreement, requested that the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) conduct a technical review of the STE test method. In support of the review, Kao Corporation submitted their STE test method BRD and subsequently provided NICEATM with a revised May 2012 BRD (**Supplement A**). The BRD contains STE and rabbit eye test data for 119 substances from four *in vitro–in vivo* comparative studies, with additional in-house data on 23 substances provided by Kao Corporation. After the preliminary analysis, additional data were requested and provided for 52 surfactants or surfactant-containing formulations that increased the STE database to 169 substances.

To assess the ability of the STE test method to predict the regulatory hazard classification identified in the rabbit eye test, the STE rank results were converted to Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and U.S. Environmental Protection Agency (EPA) classifications (UN 2011, EPA 2012). An STE rank of 1 (nonirritant), 2 (mild or moderate irritant), or 3 (severe irritant) was converted to GHS Not Classified, Category 2A/2B, or Category 1, respectively,

or to the U.S. EPA Label Review Manual classification of eye irritation as Category IV, Category III/II, or Category I, respectively.

STE test method performance was also evaluated in a top-down approach (i.e., distinguishing GHS Category 1 or EPA Category I substances from those in all other categories) or in a bottom-up approach (i.e., distinguishing GHS Not Classified or EPA Category IV substances from all other categories) for substances with corresponding *in vitro–in vivo* data. For a top-down approach, 120 substances had corresponding *in vitro* and *in vivo* classification data using the GHS or EPA classification systems, respectively, that were suitable for accuracy analysis. For a bottom-up approach, 129 substances had suitable in *vitro–in vivo* data for GHS and EPA classifications.

A variety of chemical categories were tested in the STE test method, and the chemical categories with the greatest amount of test data are alcohols, carboxylic acids, esters, ethers/polyethers, heterocyclic compounds, ketones/lactones, onium compounds, and salts. Physical properties of these substances have also been evaluated (pH, solids, liquids, and surfactants [nonionic, anionic, cationic]). This summary review document describes evaluations of the STE test method performance in a top-down or bottom-up approach.

2.0 STE Test Method Database

The May 2012 BRD submitted by Kao Corporation (**Supplement A**) includes information on the STE test method, the test method protocol, and data and performance analyses based on:

- Takahashi et al. (2009) – Prevalidation study 1 on 44 substances
- Takahashi et al. (2010) – Prevalidation study 2 on 70 substances
- Sakaguchi et al. (2011) – Phase I validation study on 25 substances
- Kojima et al. (Kao BRD) – Phase II validation study on 40 substances, then combined with Phase I substances for a total of 63 substances
- Kao in-house data on 22 of 23 substances in the original BRD

Additional information on the test substances are found in **Supplement B**. **Supplement B1** contains the *in vivo* data used to develop consensus *in vivo* classifications for substances evaluated in the STE test method. **Supplement B2** shows the test substances along with CAS Registry Number® (American Chemical Society), concentration tested, STE test data (mean viability value, standard deviation, number of replicates), category classification, and the reference. **Supplement B3** provides the same information but indicates the consensus STE classification.

The STE database includes test substances in the Kao Corporation BRD, with additional data on 52 surfactants and surfactant-containing formulations provided by Kao Corporation. However, the database used to assess performance consists of consensus classifications when a single substance was tested in multiple laboratories or in different studies. *In vivo* data are typically generated by testing neat chemicals. Twenty-three substances that were tested in the STE test method at a concentration less than 100% and that did not produce a severe irritant effect were excluded from these analyses because a mild/moderate irritant or nonirritant classification of a diluted chemical may be classified as a severe irritant when tested neat *in vitro*.

Chemicals that directly reduce MTT in the absence of cells have been shown to artificially inflate viability measures and underpredict cytotoxicity (Huang 2004; Sims and Plattner 2009). Kao Corporation assessed chemicals for their ability to directly reduce MTT by incubating the test substances with MTT and visually inspecting for color development. Test substances that were identified as direct MTT reducers were removed from top-down analysis and those classified as STE nonirritants were removed from bottom-up analysis, as these could be false negative.

Finally, *in vivo* data were analyzed to calculate the appropriate ocular irritation hazard classification. These data include cornea, iris, and conjunctiva scores for each animal at 24, 48, and 72 hours following test substance administration and/or assessment of lesions at 7, 14, and 21 days. Some test substances had insufficient *in vivo* data to assign a hazard classification. Thus, these substances were not used to evaluate STE accuracy and reliability.

The STE database contains 169 test substances representing a variety of chemical classes and physicochemical properties. **Table 2-1** provides information on the test substances evaluated in a top-down approach to identify severe eye irritants or corrosives. **Table 2-2** provides information evaluated in a bottom-up approach to identify GHS Not Classified or EPA Category IV substances. These substances had corresponding *in vivo* data, were assigned a GHS (UN 2011) or EPA (EPA 2012) eye hazard classification, and met other assay criteria as discussed in **Section 3.2**. **Table 2-3** shows the substances used to assess the STE test method in a bottom-up approach applicability domain one excluding liquids with vapor pressure >6 kilopascals alcohols, hydrocarbons, and salts and applicability domain two excluding liquids with vapor pressure >6 kilopascals and nonsurfactant solids.

Table 2-1 Test Substances Used to Evaluate STE Performance in a Top-Down Approach[a]

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
1-Bromo-4-chlorobutane	6940-78-9	Liquid	0.164	100%	Not Classified	Category IV	1
1-Dodecanaminium, N-(2-hydroxy-3-sulfopropyl)-N,N-dimethyl-, inner salt	13197-76-7	Liquid	1.16E-21	100%	Category 1	Category I	2
1-Methylpropyl benzene	135-98-8	Liquid	0.176	100%	Not Classified	Category IV	1
1-Octanol	111-87-5	Liquid	0.013	100%	Category 2A	Category II	2
1,3-Di-isopropylbenzene	99-62-7	Liquid	0.041	100%	Not Classified	Category IV	1
1,5-Hexadiene	592-42-7	Liquid	28.6	100%	Not Classified	Category III	1
1,9-Decadiene	1647-16-1	Liquid	0.320	100%	Not Classified	Category IV	1
2-Benzyloxyethanol	622-08-2	Liquid	2.9E-4	100%	Category 2A	Category II	2
2-Ethoxyethyl acetate (Cellosolve acetate)	111-15-9	Liquid	0.397	100%	Not Classified	Category III	1
2-Ethyl-1-hexanol	104-76-7	Liquid	0.025	100%	Category 2A	Category II	2
2-Ethylhexyl p-dimethylamino benzoate	21245-02-3	Liquid	4.72E-06	100%	Not Classified	Category IV	1
2-Methyl-1-pentanol	105-30-6	Liquid	0.191	100%	Category 2B	Category III	2
2-Methylbutyric acid	116-53-0	Liquid	0.149	100%	Category 1	Category I	2
2-Methylpentane	107-83-5	Liquid	27.8	100%	Not Classified	Category IV	1
2-Naphthalenesulfonic acid,6-hydroxy-,monosodium salt, polymer with formaldehyde and hydroxymethylbenzenesulfonic aid monosodium salt	85255-76-1	Liquid	NA	100%	Category 1	Category II	2
2,2-Dimethyl-3-pentanol	3970-62-5	Liquid	0.413	100%	Not Classified	Category III	1

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
2,4-Pentanediol	625-69-4	Liquid	7.3E-03	100%	Not Classified	Category IV	1
2,5-Dimethyl-2,5-hexanediol	110-03-2	Solid	5.78E-04	100%	Category 1	Category I	1
3-Methoxy-1,2-propanediol	623-39-2	Liquid	1.92E-03	100%	Not Classified	Category IV	1
3-Methylhexane	589-34-4	Liquid	8.29	100%	Not Classified	Category IV	1
3,3-Dimethylpentane	562-49-2	Liquid	10.1	100%	Not Classified	Category IV	1
Acetone	67-64-1	Liquid	33.2	100%	Category 2A	Category II	2
Acid red 92	18472-87-2	Solid	5.71E-24	100%	Category 1	Category I	3
Acrylic acid homopolymer sodium salt	9003-04-7	Solid	4.56E-04	100%	Not Classified	Category IV	1
Ammonium nitrate	6484-52-2	Solid	4.48E-16	100%	Category 2B	Category III	1
Benzalkonium chloride	8001-54-5	Liquid	NA	100%	Category 1	Category I	3
Benzalkonium chloride (10%)	63449-41-2	Solid	NA	10%	Category 1	Category I	3
Benzene, 1,1'-oxybis-, tetrapropylene derivatives, sulfonated, sodium salts	119345-04-9	Solid	NA	100%	Category 1	Category I	3
Benzyl alcohol	100-51-6	Liquid	7.14E-03	100%	Category 1	Category I	2
Body shampoo A	NA	Liquid	NA	100%	Category 2A	Category II	2
Butanol	71-36-3	Liquid	1.04	100%	Category 1	Category I	2
Butyl acetate	123-86-4	Liquid	1.59	100%	Not Classified	Category III	1
Butyl cellosolve	111-76-2	Liquid	0.0633	100%	Category 1	Category II	2
Butylnaphthalenesulfonic acid sodium salt	25638-17-9	Solid	NA	100%	Category 1	Category I	2
Butyrolactone	96-48-0	Liquid	0.0394	100%	Category 2A	Category II	2

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Camphene	79-92-5	Solid	0.237	100%	Category 2B	Category III	1
Cetylpyridinium bromide (10%)	140-72-7	Solid	3.47E-07	10%	Category 1	Category I	3
Cetylpyridinium chloride	6004-24-6	Solid	NA	10%	Category 1	Category I	3
Cetyltrimethylammonium bromide (10%)	57-09-0	Solid	NA	10%	Category 1	Category I	3
Cyclohexanol	108-93-0	Liquid	0.087	100%	Category 1	Category I	2
Cyclohexanone	108-94-1	Liquid	0.539	100%	Not Classified	Category III	2
Cyclopentanol	96-41-3	Liquid	0.307	100%	Category 2B	Category II	2
Di-n-propyl disulphide	629-19-6	Liquid	0.0664	100%	Not Classified	Category IV	1
Di(2-Ethylhexyl) sodium sulfosuccinate	577-11-7	Solid	1.63E-15	10%	Category 1	Category I	3
Di(propylene glycol) propyl ether	29911-27-1	Liquid	2.38E-04	100%	Category 2B	Category III	2
Diisobutyl ketone	108-83-8	Liquid	0.287	100%	Not Classified	Category IV	1
Dimethyl sulfoxide	67-68-5	Liquid	0.0829	100%	Not Classified	Category III	1
Dodecane	112-40-3	Liquid	0.0315	100%	Not Classified	Category III	1
Domiphen bromide	538-71-6	Solid	NA	10%	Category 1	Category I	3
Ethanol	64-17-5	Liquid	812	100%	Category 2A	Category I	1
Ethyl 2-methylacetoacetate	609-14-3	Liquid	0.0915	100%	Category 2B	Category III	2
Ethyl acetate	141-78-6	Liquid	13.1	100%	Not Classified	Category III	2
Ethyl trimethyl acetate	3938-95-2	Liquid	2.24	100%	Not Classified	Category III	1
Ethylhexyl salicylate	118-60-5	Liquid	9.51E-07	100%	Not Classified	Category IV	1
Glycerol	56-81-5	Liquid	1.06E-05	100%	Not Classified	Category IV	1
Glycidyl methacrylate	106-91-2	Liquid	0.0829	100%	Not Classified	Category III	2

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Imidazole	288-32-4	Solid	5.78E-04	100%	Category 1	Category I	2
Iso-octyl acrylate	29590-42-9	Liquid	0.0204	100%	Not Classified	Category IV	1
Isobutanal	78-84-2	Liquid	21.9	100%	Category 2B	Category III	2
Isobutyl alcohol	78-83-1	Liquid	1.78	100%	Category 1	Category I	2
Isopropyl alcohol	67-63-0	Liquid	6.61	100%	Category 2A	Category III	1
Isopropyl bromide	75-26-3	Liquid	28.5	100%	Not Classified	Category IV	1
Isopropyl myristate	110-27-0	Liquid	1.08E-04	100%	Not Classified	Category IV	1
Lactic acid	50-21-5	Liquid	3.81E-03	100%	Category 1	Category I	2
Lauryldimethylamine oxide	1643-20-5	Solid	1.68E-15	100%	Category 1	Category I	3
Lotion A	NA	Liquid	NA	100%	Not Classified	Category IV	1
m-Phenylene diamine	108-45-2	Solid	251E-04	100%	Category 1	Category I	2
Methoxyethyl acrylate	3121-61-7	Liquid	0.598	100%	Category 1	≥Category III	2
Methyl acetate	79-20-9	Liquid	7.03	100%	Category 2A	Category II	1
Methyl amyl ketone	110-43-0	Liquid	0.655	100%	Not Classified	Category III	1
Methyl cyanoacetate	105-34-0	Liquid	0.047	100%	Category 2A	Category II	2
Methyl cyclopentane	96-37-7	Liquid	17.8	100%	Not classified	Category III	1
Methyl ethyl ketone (2-Butanone)	78-93-3	Liquid	13.1	100%	Category 2A	Category III	2
Methyl isobutyl ketone	108-10-1	Liquid	2.90	100%	Not Classified	Category III	1
Methyl trimethyl acetate	598-98-1	Liquid	4.76	100%	Not Classified	Category IV	1
Myristyl alcohol	112-72-1	Solid	269E-05	100%	Category 2A	Category III	1
n-Hexanol	111-27-3	Liquid	0.117	100%	Category 2A	Category II	2
n-Hexyl bromide	111-25-1	Liquid	0.541	100%	Not Classified	Category IV	1

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
n-Octyl bromide	111-83-1	Liquid	0.0691	100%	Not Classified	Category IV	1
n,n-Dimethylguanidine sulfate	598-65-2	Solid	4.04	100%	Not Classified	Category III	1
Naphthalenesulfonic acid, butyl-, polymer with formaldehyde and 2-naphthalenesulfonic acid, sodium salt	188070-49-7	Solid	NA	100%	Category 2A	Category II	2
Polyethylene glycol 400	25322-68-3	Liquid	NA	100%	Not Classified	Category IV	1
Polyethyleneglycol monolaurate (10 E.O.)	9004-81-3	Liquid	0	100%	Not Classified	Category IV	2
Polyoxyethylene hydrogenated castor oil (60E.O.)	61788-85-0	Solid	NA	100%	Not Classified	Category IV	1
Polyoxyethylene(10) poly oxypropylene(1.5) lauryl-myristyl ether	68439-51-0	Liquid	NA	100%	Category 1	Category I	3
Polyoxyethylene(13) (mo no-, di-, tri-) styrenated phenyl ether	104376-75-2	Liquid	NA	100%	Not Classified	Category III	3
Polyoxyethylene(14) tribe nzylated phenyl ether	116998-28-8	Liquid	NA	100%	Not Classified	Category IV	1
Polyoxyethylene(160) sor bitan triisostearate	54392-28-8	Solid	NA	100%	Not Classified	Category IV	1
Polyoxyethylene(19) (mo no-, di-, tri-) styrenated phenyl ether	104376-75-2	Liquid	NA	100%	Not Classified	Category II	2
Polyoxyethylene(23) laury l ether	9002-92-0	Solid	2.03E-13	100%	Category 2A	Category III	2

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Polyoxyethylene(40) hydrogenated castor oil	61788-85-0	Liquid	NA	100%	Not Classified	Category IV	1
Potassium laurate	10124-65-9	Solid	0	10%	Category 1	Category I	3
Potassium oleate	143-18-0	Solid	4.93E-10	100%	Not Classified	Category III	2
Promethazine hydrochloride	58-33-3	Solid	0	100%	Category 1	Category I	3
Propasol solvent P	1569-01-3	Liquid	0.180	100%	Category 2B	Category II	2
Propylene glycol	57-55-6	Liquid	0.0148	100%	Not Classified	Category IV	1
Pyridine	110-86-1	Liquid	2.58	100%	Category 1	Category I	2
Rinse A	NA	Liquid	NA	100%	Not Classified	Category III	2
Rinse B	NA	Liquid	NA	100%	Category 2B	Category III	2
Rinse C	NA	Liquid	NA	100%	Not Classified	Category IV	1
Rinse D	NA	Liquid	NA	100%	Not Classified	Category III	1
Shampoo A	NA	Liquid	NA	100%	Category 2A	Category II	2
Shampoo B	NA	Liquid	NA	100%	Category 1	Category I	2
Shampoo C	NA	Liquid	NA	100%	Category 2A	Category II	2
Shampoo D	NA	Liquid	NA	100%	Category 2A	Category II	2
Sodium 2-naphthalenesulfonate	532-02-5	Solid	NA	100%	Not Classified	Category III	2
Sodium hydroxide	1310-73-2	Solid	6.53E-21	10%	Category 1	Category I	3
Sodium lauryl sulfate	151-21-3	Solid	2.40E-13	100%	≥Category 2A	Category III	3
Sodium lauryl sulfate (15%)	151-21-3	Solid	NA	15%	Category 1	Category I	3
Sodium monochloroacetate	3926-62-3	Solid	4.23E-09	100%	Category 2B	Category III	2

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Sodium polyoxyethylene(3) lauryl ether sulfate	9004-82-4	Liquid	2.27E-13	100%	Category 1	Category I	3
Sodium salicylate	54-21-7	Solid	4.84E-12	100%	Category 1	Category I	1
Sorbitan monolaurate	1338-39-2	Liquid	1.25E-15	100%	Not Classified	Category IV	2
Stearyltrimethylammonium chloride	112-03-8	Solid	NA	10%	Category 1	Category I	3
Styrene	100-42-5	Liquid	0.673	100%	Not Classified	Category III	1
Toluene	108-88-3	Liquid	3.16	100%	≥Category 2B	Category III	1
Triethanolamine	102-71-6	Liquid	4.51E-07	100%	Not Classified	Category III	1
Triethanolamine polyoxyethylene(3.0) lauryl ether sulfate	27028-82-6	Liquid	2.50E-10	100%	Category 1	Category I	3
Triton X-100	9002-93-1	Liquid	0	100%	Category 1	Category I	3
Triton X-100 (10%)	9002-93-1	Liquid	9.32E-04	10%	Category 1	Category I	2
Tween 20	9005-64-5	Liquid	0	100%	Not Classified	Category III	2
Tween 80	9005-65-6	Liquid	0	100%	Not Classified	Category IV	1

Abbreviations: CASRN = CAS Registry Number® (American Chemical Society); EPA = U.S. Environmental Protection Agency; GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals; JaCVAM = Japanese Center for the Validation of Alternative Methods; kPa = kilopascals; NA = not available; STE = short time exposure.

[a] A top-down approach is used to distinguish severe eye irritants or corrosives (i.e., GHS Category 1, EPA Category I, or STE Rank 3) from all other hazard or no hazard categories (i.e., GHS Category 2A, 2B, Not Classified; EPA Category II, III, IV; or STE Rank 1 or 2).

[b] Vapor pressure is expressed in kilopascals at 25°C. Vapor pressures were found using the Hazardous Substances Data Bank (HSDB® [U.S. National Library of Medicine]), available at http://toxnet.nlm.nih.gov (accessed 2/25/2013) or from ChemSpider (available at www.chemspider.com [accessed 2/25/2013]). If actual values were not available, predicted values were obtained from the U.S. EPA EPI (Estimation Programs Interface) Suite™ for Microsoft® Windows, v. 4.11) or ACD/Labs' ACD/PhysChem Suite available at http://www.acdlabs.com/products/pc_admet/physchem/physchemsuite/ (accessed 2/25/2013). Data from the EPI Suite and ACD/PhysChem Suite programs were also available in ChemSpider.

[c] The concentration as tested in the rabbit eye test, based on NICEATM data. For substances tested at 100%, the starting material was tested neat/undiluted.

[d] The consensus classification of two or more studies. When there was no consensus using either the GHS (UN 2011) or EPA (EPA 2012) eye hazard classification system (e.g., one GHS Category 2A and one GHS Category 2B), the more hazardous classification (i.e., GHS Category 2A) was used as the consensus classification.

[e] STE rank scores from Kao Corporation were equated to the GHS or EPA classification of eye hazard (i.e., UN 2011 and EPA 2012) such that an STE rank of 3 was considered a severe eye irritant or corrosive (i.e., GHS Category 1 or EPA Category I); an STE rank of 2 was considered a moderate to mild eye irritant (i.e., GHS Category 2A or 2B or EPA Category II or III); and an STE rank of 1 was considered to be equivalent to GHS Not Classified or EPA Category IV (minimal effects clearing in less than 24 hours).

Table 2-2 Test Substances Used to Evaluate STE Performance in a Bottom-Up Approach[a]

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
1-Bromo-4-chlorobutane	6940-78-9	Liquid	0.164	100%	Not Classified	Category IV	1
1-Dodecanaminium, N-(2-hydroxy-3-sulfopropyl)-N,N-dimethyl-, inner salt	13197-76-7	Liquid	1.16E-21	100%	Category 1	Category I	2
1-Methylpropyl benzene	135-98-8	Liquid	0.176	100%	Not Classified	Category IV	1
1-Octanol	111-87-5	Liquid	0.0132	100%	Category 2A	Category II	2
1,3-Di-isopropylbenzene	99-62-7	Liquid	0.041	100%	Not Classified	Category IV	1
1,5-Hexadiene	592-42-7	Liquid	28.6	100%	Not Classified	Category III	1
1,9-Decadiene	1647-16-1	Liquid	0.320	100%	Not Classified	Category IV	1
2-Benzyloxyethanol	622-08-2	Liquid	294E-04	100%	Category 2A	Category II	2
2-Ethoxyethyl acetate (Cellosolve acetate)	111-15-9	Liquid	0.397	100%	Not Classified	Category III	1
2-Ethyl-1-hexanol	104-76-7	Liquid	0.245	100%	Category 2A	Category II	2
2-Ethylhexyl p-dimethylamino benzoate	21245-02-3	Liquid	4.72E-06	100%	Not Classified	Category IV	1
2-Methyl-1-pentanol	105-30-6	Liquid	0.191	100%	Category 2B	Category III	2
2-Methylbutyric acid	116-53-0	Liquid	0.149	100%	Category 1	Category I	2
2-Methylpentane	107-83-5	Liquid	27.8	100%	Not Classified	Category IV	1
2-Naphthalenesulfonic acid,6-hydroxy-,monosodium salt, polymer with formaldehyde and hydroxymethylbenzenesulfonic aid monosodium salt	85255-76-1	Liquid	NA	100%	Category 1	Category II	2

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
2,2-Dimethyl-3-pentanol	3970-62-5	Liquid	0.413	100%	Not Classified	Category III	1
2,4-Pentanediol	625-69-4	Liquid	7.3E-03	100%	Not Classified	Category IV	1
2,5-Dimethyl-2,5-hexanediol	110-03-2	Solid	578E-04	100%	Category 1	Category I	1
3-Methoxy-1,2-propanediol	623-39-2	Liquid	1.92E-03	100%	Not Classified	Category IV	1
3-Methylhexane	589-34-4	Liquid	8.29	100%	Not Classified	Category IV	1
3,3-Dimethylpentane	562-49-2	Liquid	10.1	100%	Not Classified	Category IV	1
Acetic acid	64-19-7	Liquid	2.29	10%	Category 1	Category I	2
Acetone	67-64-1	Liquid	33.2	100%	Category 2A	Category II	2
Acid red 92	18472-87-2	Solid	5.71E-24	100%	Category 1	Category I	3
Acrylic acid homopolymer sodium salt	9003-04-7	Solid	4.56E-04	100%	Not Classified	Category IV	1
Ammonium nitrate	6484-52-2	Solid	4.48E-16	100%	Category 2B	Category III	1
Benzalkonium chloride	8001-54-5	Liquid	NA	100%	Category 1	Category I	3
Benzalkonium chloride (10%)	63449-41-2	Solid	NA	10%	Category 1	Category I	3
Benzene, 1,1'-oxybis-, tetrapropylene derivatives, sulfonated, sodium salts	119345-04-9	Solid	NA	100%	Category 1	Category I	3
Benzyl alcohol	100-51-6	Liquid	7.14E-03	100%	Category 1	Category I	2
Body shampoo A	NA	Liquid	NA	100%	Category 2A	Category II	2
Butanol	71-36-3	Liquid	1.04	100%	Category 1	Category I	2
Butyl acetate	123-86-4	Liquid	1.595	100%	Not Classified	Category III	1
Butyl cellosolve	111-76-2	Liquid	0.0633	100%	Category 1	Category II	2
Butylnaphthalenesulfonic acid sodium salt	25638-17-9	Solid	NA	100%	Category 1	Category I	2
Butyrolactone	96-48-0	Liquid	0.0394	100%	Category 2A	Category II	2
Calcium thioglycolate	5793-98-6	Solid	4.20E-03	100%	Category 1	Category I	2

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Camphene	79-92-5	Solid	0.237	100%	Category 2B	Category III	1
Cetyl trimethyl ammonium chloride	112-02-7	Liquid	NA	5%	Category 1	Category I	2
Cetylpyridinium bromide	140-72-7	Solid	3.47E-07	10%	Category 1	Category I	3
Cetylpyridinium chloride	6004-24-6	Solid	NA	10%	Category 1	Category I	3
Cetyltrimethylammonium bromide	57-09-0	Solid	NA	10%	Category 1	Category I	3
Cetyltrimethylammonium bromide (10%)	57-09-0	Solid	NA	10%	Category 1	Category I	3
Cyclohexanol	108-93-0	Liquid	0.0866	100%	Category 1	Category I	2
Cyclohexanone	108-94-1	Liquid	539	100%	Not Classified	Category III	2
Cyclopentanol	96-41-3	Liquid	307	100%	Category 2B	Category II	2
Di-n-propyl disulphide	629-19-6	Liquid	0.0664	100%	Not Classified	Category IV	1
Di(2-Ethylhexyl) sodium sulfosuccinate	577-11-7	Solid	1.63E-15	10%	Category 1	Category I	3
Di(propylene glycol) propyl ether	29911-27-1	Liquid	2.38E-04	100%	Category 2B	Category III	2
Diethylethanolamine	100-37-8	Liquid	0.0863	100%	Category 1	Category I	2
Diisobutyl ketone	108-83-8	Liquid	0.287	100%	Not Classified	Category IV	1
Dimethyl sulfoxide	67-68-5	Liquid	0.0829	100%	Not Classified	Category III	1
Distearyldimethylammonium chloride	107-64-2	Solid	2.55E-15	100%	Category 1	Category I	2
Dodecane	112-40-3	Liquid	0.0315	100%	Not Classified	Category III	1
Domiphen bromide	538-71-6	Solid	NA	10%	Category 1	Category I	3
Ethanol	64-17-5	Liquid	8.12	100%	Category 2A	Category I	1
Ethyl 2-methylacetoacetate	609-14-3	Liquid	0.0915	100%	Category 2B	Category III	2
Ethyl acetate	141-78-6	Liquid	13.1	100%	Not Classified	Category III	2

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Ethyl trimethyl acetate	3938-95-2	Liquid	2.240	100%	Not Classified	Category III	1
Ethylhexyl salicylate	118-60-5	Liquid	9.51E-07	100%	Not Classified	Category IV	1
Glycerol	56-81-5	Liquid	1.06E-05	100%	Not Classified	Category IV	1
Glycidyl methacrylate	106-91-2	Liquid	0.0829	100%	Not Classified	Category III	2
Imidazole	288-32-4	Solid	5.78E-04	100%	Category 1	Category I	2
Iso-octyl acrylate	29590-42-9	Liquid	0.0204	100%	Not Classified	Category IV	1
Isobutanal	78-84-2	Liquid	21.9	100%	Category 2B	Category III	2
Isobutyl alcohol	78-83-1	Liquid	1.78	100%	Category 1	Category I	2
Isopropyl alcohol	67-63-0	Liquid	6.61	100%	Category 2A	Category III	1
Isopropyl bromide	75-26-3	Liquid	28.5	100%	Not Classified	Category IV	1
Isopropyl myristate	110-27-0	Liquid	.108E-04	100%	Not Classified	Category IV	1
Lactic acid	50-21-5	Liquid	3.81E-03	100%	Category 1	Category I	2
Lauric acid	143-07-7	Solid	2.13E-09	100%	≥Category 2A	≥Category II	2
Lauryldimethylamine oxide	1643-20-5	Solid	1.68E-15	100%	Category 1	Category I	3
Lotion A	NA	Liquid	NA	100%	Not Classified	Category IV	1
m-Phenylene diamine	108-45-2	Solid	2.51E-04	100%	Category 1	Category I	2
Methoxyethyl acrylate	3121-61-7	Liquid	0.598	100%	Category 1	≥Category III	2
Methyl acetate	79-20-9	Liquid	7.03	100%	Category 2A	Category II	1
Methyl amyl ketone	110-43-0	Liquid	0.655	100%	Not Classified	Category III	1
Methyl cyanoacetate	105-34-0	Liquid	0.0469	100%	Category 2A	Category II	2
Methyl cyclopentane	96-37-7	Liquid	17.8	100%	Not Classified	Category III	1
Methyl ethyl ketone (2-Butanone)	78-93-3	Liquid	13.1	100%	Category 2A	Category III	2
Methyl isobutyl ketone	108-10-1	Liquid	2.90	100%	Not Classified	Category III	1
Methyl trimethyl acetate	598-98-1	Liquid	4.76	100%	Not Classified	Category IV	1

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Myristyl alcohol	112-72-1	Solid	2.69E-05	100%	Category 2A	Category III	1
n-Butanal	123-72-8	Liquid	14.4	100%	Category 2B	Category III	2
n-Hexanol	111-27-3	Liquid	0.117	100%	Category 2A	Category II	2
n-Hexyl bromide	111-25-1	Liquid	0.541	100%	Not Classified	Category IV	1
n-Octyl bromide	111-83-1	Liquid	0.0691	100%	Not Classified	Category IV	1
n,n-Dimethylguanidine sulfate	598-65-2	Solid	4.04	100%	Not Classified	Category III	1
Naphthalenesulfonic acid, butyl-, polymer with formaldehyde and 2-naphthalenesulfonic acid, sodium salt	188070-49-7	Solid	NA	100%	Category 2A	Category II	2
Polyethylene glycol 400	25322-68-3	Liquid	NA	100%	Not Classified	Category IV	1
Polyethyleneglycol monolaurate (10 E.O.)	9004-81-3	Liquid	0	100%	Not Classified	Category IV	2
Polyoxyethylene hydrogenated castor oil (60E.O.)	61788-85-0	Solid	NA	100%	Not Classified	Category IV	1
Polyoxyethylene(10) polyoxy propylene(1.5) lauryl-myristyl ether	68439-51-0	Liquid	NA	100%	Category 1	Category I	3
Polyoxyethylene(13) (mono-, di-, tri-)styrenated phenyl ether	104376-75-2	Liquid	NA	100%	Not Classified	Category III	3
Polyoxyethylene(14) tribenzy lated phenyl ether	116998-28-8	Liquid	NA	100%	Not Classified	Category IV	1
Polyoxyethylene(160) sorbita n triisostearate	54392-28-8	Solid	NA	100%	Not Classified	Category IV	1

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Polyoxyethylene(19) (mono-, di-, tri-) styrenated phenyl ether	104376-75-2	Liquid	NA	100%	Not Classified	Category II	2
Polyoxyethylene(20) hydrogenated tallow amine	61790-82-7	Solid	NA	100%	≥Category 2A	≥Category II	3
Polyoxyethylene(23) lauryl ether	9002-92-0	Solid	2.03E-13	100%	Category 2A	Category III	2
Polyoxyethylene(40) hydrogenated castor oil	61788-85-0	Liquid	NA	100%	Not Classified	Category IV	1
Potassium laurate	10124-65-9	Solid	0	10%	Category 1	Category I	3
Potassium oleate	143-18-0	Solid	4.93E-10	100%	Not Classified	Category III	2
Promethazine hydrochloride	58-33-3	Solid	0	100%	Category 1	Category I	3
Propasol solvent P	1569-01-3	Liquid	0.180	100%	Category 2B	Category II	2
Propylene glycol	57-55-6	Liquid	0.0148	100%	Not Classified	Category IV	1
Pyridine	110-86-1	Liquid	2.58	100%	Category 1	Category I	2
Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides	68424-85-1	Solid	NA	1%	Category 1	Category I	2
Quaternary ammonium compounds, di-C12-15-alkyldimethyl, chlorides	68910-56-5	Solid	NA	10%	Category 1	Category I	2
Rinse A	NA	Liquid	NA	100%	Not Classified	Category III	2
Rinse B	NA	Liquid	NA	100%	Category 2B	Category III	2
Rinse C	NA	Liquid	NA	100%	Not Classified	Category IV	1
Rinse D	NA	Liquid	NA	100%	Not Classified	Category III	1
Shampoo A	NA	Liquid	NA	100%	Category 2A	Category II	2
Shampoo B	NA	Liquid	NA	100%	Category 1	Category I	2
Shampoo C	NA	Liquid	NA	100%	Category 2A	Category II	2

Test Substance	CASRN	Physical Form as Tested	Vapor Pressure (kPa 25°C)[b]	*In Vivo* Conc (%)[c]	GHS Consensus[d]	EPA Consensus[d]	Overall Consensus STE Rank[e]
Shampoo D	NA	Liquid	NA	100%	Category 2A	Category II	2
Sodium 2-naphthalenesulfonate	532-02-5	Solid	NA	100%	Not Classified	Category III	2
Sodium hydroxide	1310-73-2	Solid	6.53E-22	10%	Category 1	Category I	3
Sodium lauryl sulfate	151-21-3	Solid	2.40E-13	100%	≥Category 2A	Category III	3
Sodium lauryl sulfate (15%)	151-21-3	Solid	NA	15%	Category 1	Category I	3
Sodium monochloroacetate	3926-62-3	Solid	4.23E-09	100%	Category 2B	Category III	2
Sodium polyoxyethylene(3) lauryl ether sulfate	9004-82-4	Liquid	2.27E-13	100%	Category 1	Category I	3
Sodium salicylate	54-21-7	Solid	4.84E-12	100%	Category 1	Category I	1
Sorbitan monolaurate	1338-39-2	Liquid	1.25E-15	100%	Not Classified	Category IV	2
Stearyltrimethylammonium chloride	112-03-8	Solid	NA	10%	Category 1	Category I	3
Styrene	100-42-5	Liquid	0.673	100%	Not Classified	Category III	1
Sucrose fatty acid ester	NA	Solid	NA	100%	≥Category 2A	≥Category II	2
Toluene	108-88-3	Liquid	3.160	100%	≥Category 2B	Category III	1
Triethanolamine	102-71-6	Liquid	4.51E-07	100%	Not Classified	Category III	1
Triethanolamine polyoxyethylene(3.0) lauryl ether sulfate	27028-82-6	Liquid	2.50E-10	100%	Category 1	Category I	3
Triton X-100	9002-93-1	Liquid	0	100%	Category 1	Category I	3
Tween 20	9005-64-5	Liquid	0	100%	Not Classified	Category III	2
Tween 80	9005-65-6	Liquid	0	100%	Not Classified	Category IV	1
Xylene	1330-20-7	Liquid	0.883	100%	Not Classified	Category II	1

Abbreviations: CASRN = CAS Registry Number® (American Chemical Society); EPA = U.S. Environmental Protection Agency; GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals; JaCVAM = Japanese Center for the Validation of Alternative Methods; kPa = kilopascals; NA = not available; STE = short time exposure.

[a] A bottom-up approach is used to distinguish GHS Not Classified or EPA Category IV (minimal effects clearing in less than 24 hours) and STE Rank 1 from all other hazard categories (i.e., GHS Category 1, 2A, 2B; EPA Category I, II, III; or STE Rank 2 and 3).

[b] Vapor pressure is expressed in kilopascals at 25°C. Vapor pressures were found using the Hazardous Substances Data Bank (HSDB® [U.S. National Library of Medicine]), available at http://toxnet.nlm.nih.gov (accessed 2/25/2013) or from ChemSpider (available at www.chemspider.com [accessed 2/25/2013]). If actual values were not available, predicted values were obtained from the U.S. EPA EPI (Estimation Programs Interface) Suite™ for Microsoft® Windows, v. 4.11) or ACD/Labs' ACD/PhysChem Suite available at http://www.acdlabs.com/products/pc_admet/physchem/physchemsuite/ (accessed 2/25/2013). Data from the EPI Suite and ACD/PhysChem Suite programs were also available in ChemSpider.

[c] The concentration as tested in the rabbit eye test, based on NICEATM data. For substances tested at 100%, the starting material was tested neat/undiluted.

[d] The consensus classification of two or more studies. When there was no consensus using either the GHS (UN 2011) or EPA (EPA 2012) eye hazard classification system (e.g., one GHS Category 2A and one GHS Category 2B), the more hazardous classification (i.e., GHS Category 2A) was used as the consensus classification.

[e] STE rank scores from Kao Corporation were equated to the GHS or EPA classification of eye hazard (i.e., UN 2011 and EPA 2012) such that an STE rank of 3 was considered a severe eye irritant or corrosive (i.e., GHS Category 1 or EPA Category I); an STE rank of 2 was considered a moderate to mild eye irritant (i.e., GHS Category 2A or 2B or EPA Category II or III); and an STE rank of 1 was considered to be equivalent to GHS Not Classified or EPA Category IV (minimal effects clearing in less than 24 hours).

Table 2-3 Test Substances Used to Evaluate STE Performance in a Bottom-Up Approach Within the Defined Applicability Domain[a,b]

Substance	CASRN	App Domain[b]	Physical Form as Tested	Vapor Pressure (kPa 25ºC)[c]	NICEATM *In Vivo* Conc[d] (%)	GHS-NICEATM Consensus[e]	EPA-NICEATM Consensus[e]	JaCVAM Overall Consensus STE Rank[f]
1-Bromo-4-chlorobutane	6940-78-9	1,2	Liquid	1.640E-01	100%	Not classified	Category IV	1
1-Dodecanaminium, N-(2-hydroxy-3-sulfopropyl)-N,N-dimethyl-, inner salt	13197-76-7	1,2	Liquid	1.160E-21	neat	Category 1	Category I	2
1-Methylpropyl benzene	135-98-8	1,2	Liquid	1.760E-01	100%	Not classified	Category IV	1
1-Octanol	111-87-5	1,2	Liquid	1.320E-02	100%	Category 2A	Category II	2
1,3-Di-isopropylbenzene	99-62-7	1,2	Liquid	4.100E-02	100%	Not classified	Category IV	1
1,9-Decadiene	1647-16-1	1,2	Liquid	3.200E-01	100%	Not classified	Category IV	1
2-Benzyloxyethanol	622-08-2	1,2	Liquid	2.940E-04	100%	Category 2A	Category II	2
2-Ethoxyethyl acetate (Cellosolve acetate)	111-15-9	1,2	Liquid	3.970E-01	100%	Not classified	Category III	1
2-Ethyl-1-hexanol	104-76-7	1,2	Liquid	2.460E-02	100%	Category 2A	Category II	2

Substance	CASRN	App Domain[b]	Physical Form as Tested	Vapor Pressure (kPa 25ºC)[c]	NICEATM *In Vivo* Conc[d] (%)	GHS-NICEATM Consensus[e]	EPA-NICEATM Consensus[e]	JaCVAM Overall Consensus STE Rank[f]
2-Ethylhexyl p-dimethylamino benzoate	21245-02-3	1,2	Liquid	4.720E-06	100%	Not classified	Category IV	1
2-Methyl-1-pentanol	105-30-6	1,2	Liquid	1.910E-01	100%	Category 2B	Category III	2
2-Methylbutyric acid	116-53-0	1,2	Liquid	1.490E-01	100%	Category 1	Category I	2
2-Naphthalenesulfonic acid,6-hydroxy-,monosodium salt, polymer with formaldehyde and hydroxymethylbenzenesulfonic aid monosodium salt	85255-76-1	1,2	Liquid	NA	neat	Category 1	Category II	2
2,2-Dimethyl-3-pentanol	3970-62-5	1,2	Liquid	4.130E-01	100%	Not classified	Category III	1
2,4-Pentanediol	625-69-4	1,2	Liquid	7.300E-03	100%	Not classified	Category IV	1
3-Methoxy-1,2-propanediol	623-39-2	1,2	Liquid	1.920E-03	100%	Not classified	Category IV	1
Acid red 92	18472-87-2	1	Solid	5.710E-24	100%	Category 1	Category I	3
Acrylic acid homopolymer sodium salt	9003-04-7	2	Solid	4.560E-04	neat	Not classified	Category IV	1
Benzalkonium chloride	8001-54-5	1,2	Liquid	NA	100%	Category 1	Category I	3
Benzalkonium chloride (10%)	63449-41-2	2	Solid	NA	10	Category 1	Category I	3
Benzene, 1,1'-oxybis-, tetrapropylene derivs., sulfonated, sodium salts	119345-04-9	2	Solid	NA	neat	Category 1	Category I	3
Benzyl alcohol	100-51-6	1,2	Liquid	7.140E-03	100%	Category 1	Category I	2
Body shampoo A	NA	1,2	Liquid	NA	100	Category 2A	Category II	2
Butanol	71-36-3	1,2	Liquid	1.040E+00	100%	Category 1	Category I	2
Butyl acetate	123-86-4	1,2	Liquid	1.587E+00	100%	Not classified	Category III	1

Substance	CASRN	App Domain[b]	Physical Form as Tested	Vapor Pressure (kPa 25ºC)[c]	NICEATM *In Vivo* Conc[d] (%)	GHS-NICEATM Consensus[e]	EPA-NICEATM Consensus[e]	JaCVAM Overall Consensus STE Rank[f]
Butyl cellosolve	111-76-2	1,2	Liquid	6.330E-02	100%	Category 1	Category II	2
Butylnaphthalenesulfonic acid sodium salt	25638-17-9	2	Solid	NA	neat	Category 1	Category I	2
Butyrolactone	96-48-0	1,2	Liquid	3.940E-02	100%	Category 2A	Category II	2
Cetylpyridinium bromide (10%)	140-72-7	2	Solid	3.470E-07	10%	Category 1	Category I	3
Cetylpyridinium chloride	6004-24-6	2	Solid	NA	10%	Category 1	Category I	3
Cetyltrimethylammonium bromide (10%)	57-09-0	2	Solid	NA	10%	Category 1	Category I	3
Calcium thioglycolate	5793-98-6	1	Solid	4.200E-03	100%	Category 1	Category I	2
Cyclohexanol	108-93-0	1,2	Liquid	8.660E-02	100%	Category 1	Category I	2
Cyclohexanone	108-94-1	1,2	Liquid	5.390E-01	100%	Not classified	Category III	2
Cyclopentanol	96-41-3	1,2	Liquid	3.070E-01	100%	Category 2B	Category II	2
Di-n-propyl disulphide	629-19-6	1,2	Liquid	6.640E-02	100%	Not classified	Category IV	1
Di(2-Ethylhexyl) sodium sulfosuccinate	577-11-7	1,2	Solid	1.630E-15	10%	Category 1	Category I	3
Di(propylene glycol) propyl ether	29911-27-1	1,2	Liquid	2.380E-04	100%	Category 2B	Category III	2
Diethylethanolamine	100-37-8	1,2	Liquid	8.630E-02	100%	Category 1	Category I	2
Diisobutyl ketone	108-83-8	1,2	Liquid	2.870E-01	100%	Not classified	Category IV	1
Dimethyl sulfoxide	67-68-5	1,2	Liquid	8.290E-02	100%	Not classified	Category III	1
Distearyldimethylammonium chloride	107-64-2	1,2	Solid	2.550E-15	100%	Category 1	Category I	2
Dodecane	112-40-3	1,2	Liquid	3.150E-02	100%	Not classified	Category III	1

Substance	CASRN	App Domain[b]	Physical Form as Tested	Vapor Pressure (kPa 25ºC)[c]	NICEATM *In Vivo* Conc[d] (%)	GHS-NICEATM Consensus[e]	EPA-NICEATM Consensus[e]	JaCVAM Overall Consensus STE Rank[f]
Domiphen bromide	538-71-6	1,2	Solid	NA	10%	Category 1	Category I	3
Ethyl 2-methylacetoacetate	609-14-3	1,2	Liquid	9.150E-02	100%	Category 2B	Category III	2
Ethyl trimethyl acetate	3938-95-2	1,2	Liquid	2.240E+00	100%	Not classified	Category III	1
Ethylhexyl salicylate	118-60-5	1,2	Liquid	9.510E-07	100%	Not classified	Category IV	1
Glycerol	56-81-5	1,2	Liquid	1.060E-05	100%	Not classified	Category IV	1
Glycidyl methacrylate	106-91-2	1,2	Liquid	8.290E-02	100%	Not classified	Category III	2
Imidazole	288-32-4	1	Solid	5.780E-04	100%	Category 1	Category I	2
Iso-octyl acrylate	29590-42-9	1,2	Liquid	2.040E-02	100%	Not classified	Category IV	1
Isobutyl alcohol	78-83-1	1,2	Liquid	1.780E+00	100%	Category 1	Category I	2
Isopropyl myristate	110-27-0	1,2	Liquid	1.080E-04	100%	Not classified	Category IV	1
Lactic acid	50-21-5	1,2	Liquid	3.810E-03	100%	Category 1	Category I	2
Lauric acid	143-07-7	1,2	Solid	2.130E-09	neat	≥Category 2A	≥Category II	2
Lauryldimethylamine oxide	1643-20-5	2	Solid	1.680E-15	neat	Category 1	Category I	3
Lotion A	NA	1,2	Liquid	NA	100	Not classified	Category IV	1
m-Phenylene diamine	108-45-2	1	Solid	2.510E-04	100%	Category 1	Category I	2
Methoxyethyl acrylate	3121-61-7	1,2	Liquid	5.980E-01	100%	Category 1	≥Category III	2
Methyl amyl ketone	110-43-0	1,2	Liquid	6.550E-01	100%	Not classified	Category III	1
Methyl cyanoacetate	105-34-0	1,2	Liquid	4.690E-02	100%	Category 2A	Category II	2
Methyl isobutyl ketone	108-10-1	1,2	Liquid	2.900E+00	100%	Not classified	Category III	1
Methyl trimethyl acetate	598-98-1	1,2	Liquid	4.760E+00	100%	Not classified	Category IV	1

Substance	CASRN	App Domain[b]	Physical Form as Tested	Vapor Pressure (kPa 25ºC)[c]	NICEATM *In Vivo* Conc[d] (%)	GHS-NICEATM Consensus[e]	EPA-NICEATM Consensus[e]	JaCVAM Overall Consensus STE Rank[f]
n-Hexanol	111-27-3	1,2	Liquid	1.170E-01	100%	Category 2A	Category II	2
n-Hexyl bromide	111-25-1	1,2	Liquid	5.410E-01	100%	Not classified	Category IV	1
n-Octyl bromide	111-83-1	1,2	Liquid	6.910E-02	100%	Not classified	Category IV	1
Naphthalenesulfonic acid, butyl-, polymer with formaldehyde and 2-naphthalenesulfonic acid, sodium salt	188070-49-7	2	Solid	NA	neat	Category 2A	Category II	2
n,n-Dimethylguanidine sulfate	598-65-2	1	Solid	4.040E+00	100%	Not classified	Category III	1
Polyethylene glycol 400	25322-68-3	1,2	Liquid	NA	100%	Not classified	Category IV	1
Polyethyleneglycol monolaurate (10 E.O.)	9004-81-3	1,2	Liquid	0.000E+00	100%	Not classified	Category IV	2
Polyoxyethylene hydrogenated castor Oil (60E.O.)	61788-85-0	2	Solid	NA	100%	Not classified	Category IV	1
Polyoxyethylene(10) polyoxypropylene(1.5) lauryl-myristyl ether	68439-51-0	1,2	Liquid	NA	neat	Category 1	Category I	3
Polyoxyethylene(13) (mono-, di-, tri-)styrenated phenyl ether	104376-75-2	1,2	Liquid	NA	neat	Not classified	Category III	3
Polyoxyethylene(14) tribenzylated phenyl ether	116998-28-8	1,2	Liquid	NA	neat	Not classified	Category IV	1
Polyoxyethylene(160) sorbitan triisostearate	54392-28-8	1,2	Solid	NA	neat	Not classified	Category IV	1
Polyoxyethylene(19) (mono-, di-, tri-)styrenated phenyl ether	104376-75-2	1,2	Liquid	NA	neat	Not classified	Category II	2
Polyoxyethylene(20) hydrogenated tallow amine	61790-82-7	1,2	Solid	NA	neat	≥Category 2A	≥Category II	3
Polyoxyethylene(23) lauryl ether	9002-92-0	2	Solid	2.030E-13	neat	Category 2A	Category III	2

Substance	CASRN	App Domain[b]	Physical Form as Tested	Vapor Pressure (kPa 25°C)[c]	NICEATM *In Vivo* Conc[d] (%)	GHS-NICEATM Consensus[e]	EPA-NICEATM Consensus[e]	JaCVAM Overall Consensus STE Rank[f]
Polyoxyethylene(40) hydrogenated castor oil	61788-85-0	1,2	Liquid	NA	neat	Not classified	Category IV	1
Potassium laurate	10124-65-9	1,2	Solid	0.000E+00	10%	Category 1	Category I	3
Potassium oleate	143-18-0	2	Solid	4.930E-10	neat	Not classified	Category III	2
Promethazine hydrochloride	58-33-3	1	Solid	0.000E+00	100%	Category 1	Category I	3
Propasol solvent P	1569-01-3	1,2	Liquid	1.800E-01	100%	Category 2B	Category II	2
Propylene glycol	57-55-6	1,2	Liquid	1.480E-02	100%	Not classified	Category IV	1
Pyridine	110-86-1	1,2	Liquid	2.580E+00	100%	Category 1	Category I	2
Rinse A	NA	1,2	Liquid	NA	100	Not classified	Category III	2
Rinse B	NA	1,2	Liquid	NA	100	Category 2B	Category III	2
Rinse C	NA	1,2	Liquid	NA	100	Not classified	Category IV	1
Rinse D	NA	1,2	Liquid	NA	100	Not classified	Category III	1
Shampoo A	NA	1,2	Liquid	NA	100	Category 2A	Category II	2
Shampoo B	NA	1,2	Liquid	NA	100	Category 1	Category I	2
Shampoo C	NA	1,2	Liquid	NA	100	Category 2A	Category II	2
Shampoo D	NA	1,2	Liquid	NA	100	Category 2A	Category II	2
Sodium hydroxide	1310-73-2	1	Solid	6.530E-22	10%	Category 1	Category I	3
Sodium lauryl sulfate	151-21-3	2	Solid	2.400E-13	100%	≥Category 2A	Category III	3
Sodium lauryl sulfate (15%)	151-21-3	2	Solid	NA	15	Category 1	Category I	3
Sodium polyoxyethylene(3) lauryl ether sulfate	9004-82-4	1,2	Liquid	2.270E-13	neat	Category 1	Category I	3

Substance	CASRN	App Domain[b]	Physical Form as Tested	Vapor Pressure (kPa 25°C)[c]	NICEATM *In Vivo* Conc[d] (%)	GHS-NICEATM Consensus[e]	EPA-NICEATM Consensus[e]	JaCVAM Overall Consensus STE Rank[f]
Sorbitan monolaurate	1338-39-2	1,2	Liquid	1.250E-15	neat	Not classified	Category IV	2
Stearyltrimethylammonium chloride	112-03-8	1,2	Solid	NA	10%	Category 1	Category I	3
Styrene	100-42-5	1,2	Liquid	6.730E-01	100%	Not classified	Category III	1
Sucrose fatty acid ester	NA	1,2	Solid	NA	100%	≥Category 2A	≥Category II	2
Toluene	108-88-3	1,2	Liquid	3.160E+00	100%	≥Category 2B	Category III	1
Triethanolamine	102-71-6	1,2	Liquid	4.510E-07	100%	Not classified	Category III	1
Triethanolamine polyoxyethylene(3.0) lauryl ether sulfate	27028-82-6	1,2	Liquid	2.500E-10	neat	Category 1	Category I	3
Triton X-100	9002-93-1	1,2	Liquid	0.000E+00	100%	Category 1	Category I	3
Tween 20	9005-64-5	1,2	Liquid	0.000E+00	100%	Not classified	Category III	2
Tween 80	9005-65-6	1,2	Liquid	0.000E+00	100%	Not classified	Category IV	1
Xylene	1330-20-7	1,2	Liquid	8.826E-01	100%	Not classified	Category II	1

Abbreviations: App = applicability; CASRN = CAS Registry Number® (American Chemical Society); EPA = U.S. Environmental Protection Agency; GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals; JaCVAM = Japanese Center for the Validation of Alternative Methods; kPa = kilopascals; NA = not available; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; STE = short time exposure.

[a] A bottom-up approach is used to distinguish GHS Not Classified or EPA Category IV (minimal effects clearing in less than 24 hours) and STE Rank 1 from all other hazard categories (i.e., GHS Category 1, 2A, 2B; EPA Category I, II, III; or STE Rank 2 and 3).

[b] There are two defined applicability domains (AD) for the STE test method. AD 1 (n=94 substances) includes all substances with vapor pressures ≤6 kilopascals but excludes solids that are alcohols, hydrocarbons, or salts. AD 2 (n = 101 substances) includes all liquids with vapor pressures ≤6 kilopascals and solid surfactants or surfactant-containing formulations (i.e., nonsurfactant solids and substances with vapor pressures >6 kilopascals are excluded).

[c] Vapor pressure is expressed in kilopascals at 25°C. Vapor pressures were found using the Hazardous Substances Data Bank (HSDB® [U.S. National Library of Medicine]), available at http://toxnet.nlm.nih.gov (accessed 2/25/2013) or from ChemSpider (available at www.chemspider.com [accessed 2/25/2013]). If actual values were not available, predicted values were obtained from the U.S. EPA EPI (Estimation Programs Interface) Suite™ for Microsoft® Windows, v. 4.11) or ACD/Labs' ACD/PhysChem Suite available at http://www.acdlabs.com/products/pc_admet/physchem/physchemsuite/ (accessed 2/25/2013). Data from the EPI Suite and ACD/PhysChem Suite programs were also available in ChemSpider.

[d] The concentration as tested in the rabbit eye test, based on NICEATM data.

[e] The consensus classification of two or more studies. When there was no consensus using either the GHS (UN 2011) or EPA (EPA 2012) eye hazard classification system (e.g., one GHS Category 2A and one GHS Category 2B), the more hazardous classification (i.e., GHS Category 2A) was used as the consensus classification.

[f] STE rank scores were equated to the GHS or EPA classification of eye hazard (i.e., UN 2011 and EPA 2012) such that an STE rank of 3 was considered a severe eye irritant or corrosive (i.e., GHS Category 1 or EPA Category I); an STE rank of 2 was considered a moderate to mild eye irritant (i.e., GHS Category 2A or 2B or EPA Category II or III); and an STE rank of 1 was considered to be equivalent to GHS Not Classified or EPA Category IV (minimal effects clearing in less than 24 hours).

3.0 STE Test Method Performance

Test method performance is typically evaluated by calculating the following (ICCVAM 2003):

- Accuracy (concordance): the proportion of correct outcomes (positive and negative) of a test method
- Sensitivity: the proportion of all positive substances that are classified correctly as positive
- Specificity: the proportion of all negative substances that are classified correctly 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

The STE test method performance was evaluated for each study, and the data set is provided in **Supplement B**. An overall STE ocular irritation classification was assigned for each test substance in the database based on the majority of ocular irritation classification calls. When a test substance had an even number of different irritation classifications (e.g., two tests classified a substance as a moderate irritant and two tests classified a substance as a severe irritant), the more severe hazard classification was used for its overall classification (e.g., severe irritant). Using the consensus ocular irritation classification for each substance, the STE test method was evaluated in a top-down approach to distinguish ocular corrosives and severe irritants (i.e., GHS Category 1 or EPA Category I) from all other categories (i.e., GHS Category 2A, 2B, Not Classified or EPA Category II, III, IV). The STE test method was also evaluated in a bottom-up approach to identify GHS Not Classified substances or EPA Category IV (minimally irritant) substances from all other irritant categories (i.e., GHS Category 1, 2A, or 2B or EPA Category I, II, or III).

The overall accuracy of the STE test method in a top-down approach ranged from 70% to 96%, and the accuracy in a bottom-up approach ranged from 80% to 85% depending on the classification. The predictive capacity of the STE test method was assessed by identifying the chemical classes or physical properties that increased the false positive rate in a top-down approach and those that increased the false negative rate in a bottom-up approach. Excluding discordant chemical classes or physical properties optimized the applicability domain for a top-down or bottom-up approach.

3.1 GHS Classification System: STE Performance in a Top-Down Approach

The performance of the STE test method was evaluated for GHS ocular hazard classification in a top-down approach. STE accuracy, sensitivity, specificity, false positive rate, and false negative rate were determined based on available *in vivo* reference data for the test substances. Test substances that were identified as direct MTT reducers were removed from the analyses. These include two substances from Kojima et al. (Kao BRD), two substances from Takahashi et al. (2010), and one substance from the Kao in-house studies. These analyses were performed for each of the five studies as well as for 120 unique substances from these five studies that remained after duplicates were removed and consensus classifications were assigned (**Table 3-1**). The GHS classification for each test substance is listed in **Supplement B**.

Table 3-1 STE Performance for GHS Classification in a Top-Down Approach

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate[a]		False Negative Rate[b]	
		%	No.[c]	%	No.[c]	%	No.[c]	%	No.[c]	%	No.[c]
Kojima et al. (Kao BRD)	30	70	21/30	10	1/10	100	20/20	0	0/20	90	9/10
Sakaguchi et al. 2011	23	96	22/23	80	4/5	100	18/18	0	0/18	20	1/5
Takahashi et al. 2009	37	84	31/37	65	11/17	100	20/20	0	0/20	35	6/17
Takahashi et al. 2010	47	83	39/47	58	11/19	100	28/28	0	0/28	42	8/19
Kao In-House	22	96	21/22	0	0/1	100	21/21	0	0/21	100	1/1
Kao New Surfactants	39	69	27/39	45	9/20	95	18/19	5.3	1/19	55	11/20
Unique Substances[d]	120	85	102/120	53	19/36	99	83/84	1.2	1/84	47	17/36

Abbreviations: BRD = background review document; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2011); N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

[d] Substances from all of the above studies remaining after duplicates were removed and consensus classifications were assigned.

3.1.1 STE Discordant Results for GHS Classification in a Top-Down Approach

The STE results that were discordant with *in vivo* results were analyzed further. These analyses were performed on specific categories of chemicals, as well as on certain physicochemical properties potentially relevant to ocular toxicity testing (e.g., surfactants, pH, physical form).

Several trends were noted in STE performance among these subgroups of substances (**Table 3-2**). Only one of 84 substances was overpredicted (i.e., false positive) and slightly affected the overprediction of its constituent chemical classes (3.6% to 8.3% overprediction). The chemical categories of substances that were most consistently underpredicted (i.e., false negatives) by the STE test method were alcohols and carboxylic acids. Of the 17 underpredicted substances, 7 were alcohols, 4 were carboxylic acids, and 3 were salts. Additional chemical categories represented among the underpredicted substances were esters (2) and heterocyclic compounds (2).

With regard to the physical form of the substances underpredicted by the STE test method, 12 were liquids and 5 were solids. Considering the proportion of the total available database, solids (16%; 5/31) and liquids (13%; 12/89) were underpredicted at a similar rate by the STE test method.

Table 3-2 STE False Positive and False Negative Rates by Chemical Category and Properties of Interest for GHS Classification in a Top-Down Approach

Category	N	False Positive Rate[a]		False Negative Rate[b]	
		%	No.[c]	%	No.[c]
Overall	120	1.2	1/84	47	17/36
Chemical Category[d]					
Alcohol	39	3.6	1/28	64	7/11
Amine/Amidine	8	0	0/2	17	1/6
Carboxylic acid	21	0	0/14	57	4/7
Ester	17	0	0/14	67	2/3
Ether/Polyether	16	8.3	1/12	0	0/4
Heterocyclic compound	9	0	0/3	33	2/6
Hydrocarbon	23	5.0	1/20	33	1/3
Ketone	8	0	0/8	-	0/0
Onium compound	10	0	0/1	11	1/9
Salt	17	0	0/6	27	3/11
Properties of Interest					
Liquids	89	1.4	1/72	71	12/17
Solids	31	0	0/12	26	5/19
Surfactants – Total	44	4.2	1/24	20	4/20
-nonionic	14	8.3	1/12	0	0/2
-anionic	11	0	0/3	25	2/8
-cationic	7	-	0/0	0	0/7
-ampholyic	2	-	0/0	50	1/2
pH – Total[e]	27	0	0/10	41	7/17
-acidic (pH < 7.0)	19	0	0/8	36	4/11
-basic (pH > 7.0)	7	0	0/1	50	3/6
-equals 7	1	0	0/1	-	0/0
Vapor Pressure – Total	90	0	0/66	58	14/24
>6kPa	13	0	0/13	-	0/0
≤6kPa	77	0	0/53	58	14/24

Abbreviations: GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2011); kPa = kilopascals; N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

[d] One or more chemical categories were assigned to each test substance based on the chemical categories outlined in the tree structure provided for that chemical in the National Library of Medicine's Medical Subject Headings (MeSH®) for inorganic or organic chemicals when available (http://www.nlm.nih.gov/mesh) and on the presence of common organic functional groups (i.e., ketones) if that functional group was not available in the MeSH tree structure.

[e] Total number of substances with pH data available.

Table 3-3 shows the STE test method performance in a top-down approach when problematic categories are excluded that gave the most discordant results in the GHS classification system. In general, exclusion of alcohols, ethers/polyethers, hydrocarbons, or nonionic surfactants individually reduced false positive rates to 0% and marginally reduced or slightly increased false negative rates. The performance of validated *in vitro* methods for GHS classification is included for comparison.

Table 3-3 STE Performance for GHS Classification in a Top-Down Approach After Excluding Discordant Categories

Method Evaluated	Accuracy		False Positive Rate[a]		False Negative Rate[b]	
	%	No.[c]	%	No.[c]	%	No.[c]
STE Overall	85	102/120	1.2	1/84	47	17/36
STE w/o Alcohols	87	71/82	0	0/56	42	11/26
STE w/o Ethers/Polyethers	84	87/104	0	0/72	53	17/32
STE w/o Hydrocarbons	84	81/97	0	0/64	49	16/33
STE w/o Nonionic surfactants	84	89/106	0	0/73	50	17/34
BCOP	79	149/188	24	29/123	15	10/65
ICE	83	120/144	8	9/114	50	15/30
CM	90	74/82	2	1/48	21	7/34

Abbreviations: BCOP = bovine corneal opacity and permeability; CM = Cytosensor microphysiometer; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2011); ICE = isolated chicken eye; N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

3.2 GHS Classification System: STE Performance in a Bottom-Up Approach

The performance of the STE test method was evaluated for GHS ocular hazard classification in a bottom-up approach. STE accuracy, sensitivity, specificity, false positive rate, and false negative rate were determined based on available *in vivo* reference data for the test substances. Test substances that were identified as direct MTT reducers and classified as STE nonirritants were removed from the bottom-up analysis, as these could be false negative. These include two substances from Kojima et al. (Kao BRD), two substances from Takahashi et al. (2010), and one substance from the Kao in-house studies. These analyses were performed for each of the five studies as well as for 129 unique substances from these five studies that remained after duplicates were removed and consensus classifications were assigned (**Table 3-4**). The GHS classification for each test substance is listed in **Supplement B**.

Table 3-4 STE Performance for GHS Classification in a Bottom-Up Approach

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate[a]		False Negative Rate[b]	
		%	No.[c]	%	No.[c]	%	No.[c]	%	No.[c]	%	No.[c]
Kojima et al. (Kao BRD)	31	71	22/31	73	19/26	60	3/5	40	2/5	27	7/26
Sakaguchi et al. 2011	24	88	21/24	77	10/13	100	11/11	0	0/11	23	3/13
Takahashi et al. 2009	39	87	34/39	85	23/27	92	11/12	8.3	1/12	15	4/27
Takahashi et al. 2010	52	83	43/52	85	28/33	79	15/19	21	4/19	15	5/33
Kao In-House	22	96	21/22	100	1/1	95	20/21	4.8	1/21	0	0/1
Kao New Surfactants	34	85	29/34	100	22/22	58	7/12	42	5/12	0	0/22
Unique Substances[d]	129	85	109/129	88	64/73	80	45/56	20	11/56	12	9/73

Abbreviations: BRD = background review document; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2011); N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

[d] Substances from all of the above studies remaining after duplicates were removed and consensus classifications were assigned.

3.2.1 STE Discordant Results for GHS Classification in a Bottom-Up Approach

The STE results that were discordant with *in vivo* results were analyzed further. These analyses were performed on specific categories of chemicals, as well as on certain physicochemical properties potentially relevant to ocular toxicity testing (e.g., surfactants, pH, physical form).

Several trends were noted in STE performance among these subgroups of substances (**Table 3-5**). The overall false positive rate was 20%. The chemical categories of substances that the STE test method most consistently underpredicted for GHS classification (i.e., false negatives) were salts (13%; 2/15), hydrocarbons (33%; 2/6), and alcohols (16%; 4/25).

With regard to the physical form of the substances underpredicted by the STE test method, four were liquids and five were solids. Considering the proportion of the total available database, solids (14%; 5/37) appear more likely than liquids (4.3%; 4/92) to be underpredicted by the STE test method.

Table 3-5 STE False Positive and False Negative Rates by Chemical Category and Properties of Interest for GHS Classification in a Bottom-Up Approach

Category	N	False Positive Rate[a]		False Negative Rate[b]	
		%	No.[c]	%	No.[c]
Overall	129	20	11/56	12	9/73
Chemical Category[d]					
Alcohol	41	31	5/16	16	4/25
Amine/Amidine	17	0	0/2	0	0/15
Carboxylic acid	33	28	3/11	9.1	2/22
Ester	18	46	5/11	14	1/7
Ether/Polyether	16	38	3/8	0	0/8
Heterocyclic compound	10	50	1/2	0	0/8
Hydrocarbon	24	17	3/18	33	2/6
Ketone	8	20	1/5	0	0/3
Onium compound	12	-	0/0	8.3	1/12
Salt	18	67	2/3	13	2/15
Properties of Interest					
Liquids	92	18	9/50	9.5	4/42
Solids	37	33	2/6	16	5/31
Surfactants – Total	49	41	7/17	0	0/32
-nonionic	16	46	5/11	0	0/5
-anionic	12	50	1/2	0	0/10
-cationic	8	-	0/0	0	0/8
pH – Total[e]	33	25	2/8	13	3/24
-acidic (pH < 7.0)	23	17	1/6	18	3/17
-basic (pH > 7.0)	8	0	0/1	0	0/7
-equals 7	1	0	0/1	-	0/0
Vapor Pressure – Total	97	16	7/44	17	9/53
>6kPa	14	14	1/7	43	3/7
≤6kPa	83	16	6/37	13	6/46

Abbreviations: GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2011); kPa = kilopascals; N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = The proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

[d] One or more chemical categories were assigned to each test substance based on the chemical categories outlined in the tree structure provided for that chemical in the National Library of Medicine's Medical Subject Headings (MeSH®) for inorganic or organic chemicals when available (http://www.nlm.nih.gov/mesh) and on the presence of common organic functional groups (i.e., ketones) if that functional group was not available in the MeSH tree structure.

[e] Total number of substances with pH data available.

Table 3-6 shows the STE test method performance in a bottom-up approach when problematic categories are excluded that gave the most discordant results in the GHS classification system. In

general, exclusion of alcohols, hydrocarbons, salts, or solids individually resulted in small changes in assay performance. However, two applicability domains were evaluated based on excluding certain chemical and product classes, or physical characteristics. When substances with high vapor pressures, solid alcohols, hydrocarbons, and salts were excluded, the false negative rate was reduced to 2.0% (1/49). When substances with high vapor pressures and nonsurfactant solids were excluded, the false negative rate was reduced to 1.9% (1/54). The single false negative substance using the restricted applicability domains was toluene. In the NICEATM database, an *in vivo* study from the European Centre for Ecotoxicology and Toxicology of Chemicals classifies toluene as GHS Not Classified (ECETOC 1998), whereas a study submitted to the EPA under the Toxic Substances Control Act (TSCA) classifies it as GHS Category 2B (eye irritation data made available by the U.S. Environmental Protection Agency). These data suggest toluene is a mild ocular irritant and mitigates concern about the false negative classification. The performance of validated *in vitro* methods for GHS classification is included in **Table 3-6** for comparison.

Table 3-6 STE Performance for GHS Classification in a Bottom-Up Approach After Excluding Discordant Categories

Method Evaluated	Accuracy		False Positive Rate[a]		False Negative Rate[b]	
	%	No.[c]	%	No.[c]	%	No.[c]
STE Overall	85	109/129	20	11/56	12	9/73
STE w/o Alcohols	89	78/89	15	6/40	10	5/49
STE w/o Hydrocarbons	86	90/105	21	8/38	10	7/67
STE w/o Salts	86	95/111	17	9/53	12	7/58
STE w/o Solids	86	79/92	18	9/50	9.5	4/42
STE w/o Vapor Pressure >6kPa, solid alcohols, hydrocarbons, and salts	90	85/94	18	8/45	2.0	1/49
STE w/o Vapor Pressure >6kPa and Nonsurfactant Solids	90	91/101	19	9/47	1.9	1/54
BCOP	66	125/188	69	63/91	0	0/97
CM	68	36/53	68	17/25	0	0/28

Abbreviations: BCOP = bovine corneal opacity and permeability; CM = Cytosensor microphysiometer; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2011); kPa = kilopascals; N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

3.3 EPA Classification System: STE Performance in a Top-Down Approach

The performance of the STE test method was evaluated for EPA ocular hazard classification in a top-down approach. STE accuracy, sensitivity, specificity, false positive rate, and false negative rate were determined based on available *in vivo* reference data for the test substances. Test substances that were identified as direct MTT reducers were removed from the analyses. These include two substances from Kojima et al. (Kao BRD), two substances from Takahashi et al. (2010), one substance from the Kao in-house studies, and five substances from the combined overall data set. These analyses were performed for each of the five studies as well as for the overall data set of 120 test substances from all these studies (**Table 3-7**). The EPA classification for each test substance is listed in **Supplement B**.

Table 3-7 STE Performance for EPA Classification in a Top-Down Approach

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate[a]		False Negative Rate[b]	
		%	No.[c]	%	No.[c]	%	No.[c]	%	No.[c]	%	No.[c]
Kojima et al. (Kao BRD)	30	70	21/30	11	1/9	95	20/21	4.8	1/21	89	8/9
Sakaguchi et al. 2011	24	92	22/24	67	4/6	100	18/18	0	0/18	33	2/6
Takahashi et al. 2009	38	82	31/38	61	11/18	100	20/20	0	0/20	39	7/18
Takahashi et al. 2010	49	82	40/49	58	11/19	97	29/30	3.3	1/30	42	8/19
Kao In-House	21	100	21/21	-	0/0	100	21/21	0	0/21	-	0/0
Kao New Surfactants	39	72	28/39	47	9/19	95	19/20	5.0	1/20	53	10/19
Overall	120	87	104/120	58	19/33	98	85/87	2	2/87	42	14/33

Abbreviations: EPA = U.S. Environmental Protection Agency (EPA 2012); N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

3.3.1 STE Discordant Results for EPA Classification in a Top-Down Approach

The STE results that were discordant with *in vivo* results were analyzed further. These analyses were performed on specific categories of chemicals, as well as on certain physicochemical properties potentially relevant to ocular toxicity testing (e.g., surfactants, pH, physical form).

Several trends were noted in STE performance among these subgroups of substances (**Table 3-8**). Two of 87 substances were overpredicted (i.e., false positives) and affected its representative chemical classes, with false positive rates ranging from 4.8% to 12.5%. The chemical categories of substances that were most consistently underpredicted for EPA classification (i.e., false negatives) were alcohols (64%; 7/11) and carboxylic acids (50%; 3/6). Of the 14 underpredicted substances, seven were alcohols, three were carboxylic acids, two were heterocyclic compounds, and two were salts.

With regard to the physical form of the substances overpredicted by the STE test method, 1.4% (1/74) were liquids and 7.7% (1/13) were solids. With regard to the physical form of the substances underpredicted by the STE test method, 10 were liquids and 5 were solids. Considering the proportion of the total available database, solids (16%; 5/32) appear more likely than liquids (11%; 10/89) to be underpredicted by the STE test method.

Table 3-8 STE False Positive and False Negative Rates by Chemical Category and Properties of Interest for EPA Classification in a Top-Down Approach

Category	N	False Positive Rate[a]		False Negative Rate[b]	
		%	No.[c]	%	No.[c]
Overall	120	2.3	2/87	42	14/33
Chemical Category[d]					
Alcohol	40	6.9	2/29	64	7/11
Amine/Amidine	8	0	0/2	17	1/6
Carboxylic acid	20	0	0/14	50	3/6
Ester	16	0	0/15	0	0/1
Ether/Polyether	16	8.3	1/12	0	0/4
Heterocyclic compound	9	0	0/3	33	2/6
Hydrocarbon	24	4.8	1/21	33	1/3
Ketone	8	0	0/8	-	0/0
Onium compound	10	0	0/1	11	1/9
Salt	18	12.5	1/8	20	2/10
Properties of Interest					
Liquids	89	1.4	1/74	67	10/15
Solids	32	7.7	1/13	26	5/19
Surfactants – Total	45	7.7	2/26	16	3/19
-nonionic	14	8.3	1/12	0	0/2
-anionic	12	20	1/5	14	1/7
-cationic	7	-	0/0	0	0/7
-ampholytic	2	-	0/0	50	1/2
pH – Total[e]	28	10	1/10	44	8/18
-acidic (pH < 7.0)	20	13	1/8	42	5/12
-basic (pH > 7.0)	7	0	0/1	50	3/6
-equals 7	1	0	0/1	-	0/0
Vapor Pressure – Total	91	1.5	1/68	57	13/23
>6kPa	13	0	0/12	100	1/1
≤6kPa	78	1.8	1/56	55	12/22

Abbreviations: EPA = U.S. Environmental Protection Agency (EPA 2012); kPa = kilopascals; N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

[d] One or more chemical categories were assigned to each test substance based on the chemical categories outlined in the tree structure provided for that chemical in the National Library of Medicine's Medical Subject Headings (MeSH®) for inorganic or organic chemicals when available (http://www.nlm.nih.gov/mesh) and on the presence of common organic functional groups (i.e., ketones) if that functional group was not available in the MeSH tree structure.

[e] Total number of substances with pH data available.

Table 3-9 shows the STE test method in a top-down approach when problematic categories are excluded that gave the most discordant results for the EPA classification system. Exclusion of

alcohols reduced the false positive rate from 2.3% (2/87) to 0% (0/58) with only a slight reduction in the false negative rate (42%, 14/33 to 38%, 9/24). In general, removal of other individual chemical classes with false positive rates produced higher false negative rates.

Table 3-9 STE Performance for EPA Classification in a Top-Down Approach After Excluding Discordant Categories

Method Evaluated	Accuracy		False Positive Rate[a]		False Negative Rate[b]	
	%	No.[c]	%	No.[c]	%	No.[c]
STE Overall	87	104/120	2.3	2/87	42	14/33
STE w/o Alcohols	89	73/82	0	0/58	38	9/24
STE w/o Ethers/Polyethers	85	89/105	1.3	1/75	50	15/30
STE w/o Hydrocarbons	85	82/97	1.5	1/66	45	14/31
STE w/o Salts	86	89/103	1.3	1/79	54	13/24
STE w/o Solids	88	78/89	1.4	1/74	67	10/15

Abbreviations: EPA = U.S. Environmental Protection Agency (EPA 2012); STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

3.4 EPA Classification System: STE Performance in a Bottom-Up Approach

The performance of the STE test method was evaluated for EPA ocular hazard classification in a bottom-up approach to identify EPA Category IV substances (i.e., minimal effects clearing in less than 24 hours). STE accuracy, sensitivity, specificity, false positive rate, and false negative rate were determined based on available *in vivo* reference data for the test substances. Test substances that were identified as direct MTT reducers and classified as STE nonirritants were removed from bottom-up analysis, as these could be false negative. These include two substances from Kojima et al. (Kao BRD) and two substances from Takahashi et al. (2010). These analyses were performed for each of the five studies as well as for the overall data set of 129 test substances from all these studies (**Table 3-10**). The EPA classification for each test substance is listed in **Supplement B.**

Table 3-10 STE Performance for EPA Classification in a Bottom-Up Approach

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate[a]		False Negative Rate[b]	
		%	No.[c]	%	No.[c]	%	No.[c]	%	No.[c]	%	No.[c]
Kojima et al. (Kao BRD)	31	77	24/31	75	21/28	100	3/3	0	0/3	25	7/28
Sakaguchi et al. 2011	24	67	16/24	56	10/18	100	6/6	0	0/6	44	8/18
Takahashi et al. 2009	39	80	31/39	75	24/32	100	7/7	0	0/7	25	8/32
Takahashi et al. 2010	52	77	40/52	74	31/42	90	9/10	10	1/10	26	11/42
Kao In-House	22	68	15/22	22	2/9	100	13/13	0	0/13	78	7/9

Kao New Surfactants	34	94	32/34	96	26/27	86	6/7	14	1/7	3.7	1/27
Overall	129	80	103/129	75	73/97	94	30/32	6.3	2/32	25	24/97

Abbreviations: BRD = background review document; EPA = U.S. Environmental Protection Agency (EPA 2012); N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

3.4.1 STE Discordant Results for EPA Classification in a Bottom-Up Approach

The STE results that were discordant with *in vivo* results were analyzed further. These analyses were performed on specific categories of chemicals, as well as on certain physicochemical properties potentially relevant to ocular toxicity testing (e.g., surfactants, pH, physical form).

Several trends were noted in STE performance among these subgroups of substances (**Table 3-11**). Two substances were overpredicted. The overall false positive rate was 6.3% (2/32), with alcohols (20% 2/10), esters (33%; 2/6), and heterocyclic compounds (50%; 1/2) overpredicted. The chemical categories of substances that the STE test method most consistently underpredicted for EPA classification (i.e., false negatives) were hydrocarbons, ketones, and esters. Of the 24 underpredicted substances, seven were alcohols, five were carboxylic acids, and six were hydrocarbons. Additional chemical categories represented among the underpredicted substances were amines/amidines (2), esters (3), ethers/polyethers (1), ketones (3), onium compounds (1), and salts (2).

With regard to the physical form of the substances in a bottom-up approach, two liquids were overpredicted (6.9%; 2/29), which was 2.2% (2/92) of the entire database. With regard to the physical form of the substances underpredicted by the STE test method, 18 were liquids and six were solids. Considering the proportion of the total available database, liquids (20%; 18/92) appear more likely than solids (16%; 6/37) to be underpredicted by the STE test method.

Table 3-11 STE False Positive and False Negative Rates by Chemical Category and Properties of Interest for EPA Classification in a Bottom-Up Approach

Category	N	False Positive Rate[a]		False Negative Rate[b]	
		%	No.[c]	%	No.[c]
Overall	129	6.3	2/32	25	24/97
Chemical Category[d]					
Alcohol	41	20	2/10	23	7/31
Amine/Amidine	11	-	0/0	18	2/11
Carboxylic acid	33	0	0/5	18	5/28
Ester	18	33	2/6	25	3/12
Ether/Polyether	16	0	0/4	8.3	1/12
Heterocyclic compound	10	50	1/2	0	0/8
Hydrocarbon	24	0	0/11	46	6/13
Ketone	8	-	0/0	38	3/8
Onium compound	12	-	0/0	8.3	1/12
Salt	18	0	0/1	12	2/17

Properties of Interest					
Liquids	92	6.9	2/29	29	18/63
Solids	37	0	0/3	18	6/34
Surfactants – Total	49	18	2/11	2.6	1/38
-nonionic	16	25	2/8	0	0/8
-anionic	12	0	0/1	0	0/11
-cationic	8	-	0/0	0	0/8
pH – Total[e]	32	17	1/6	15	4/26
-acidic (pH < 7.0)	23	17	1/6	18	3/17
-basic (pH > 7.0)	8	-	0/0	12	1/8
-equals 7	1	-	0/0	100	1/1
Vapor Pressure – Total	97	8.0	2/25	32	23/72
>6kPa	14	0	0/4	50-	5/10
<6kPa	83	9.5	2/21	29	18/62

Abbreviations: EPA = U.S. Environmental Protection Agency (EPA 2012); kPa = kilopascals; N = number of substances; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

[d] One or more chemical categories were assigned to each test substance based on the chemical categories outlined in the tree structure provided for that chemical in the National Library of Medicine's Medical Subject Headings (MeSH®) for inorganic or organic chemicals when available (http://www.nlm.nih.gov/mesh) and on the presence of common organic functional groups (i.e., ketones) if that functional group was not available in the MeSH tree structure.

[e] Total number of substances with pH data available.

Table 3-12 shows the STE test method performance in a bottom-up approach when problematic categories are excluded that gave the most discordant results in the EPA classification system. In general, exclusion of alcohols, hydrocarbons, salts, or solids individually resulted in small changes in assay performance. However, two applicability domains were evaluated based on excluding certain chemical and product classes, or physical characteristics. When substances with high vapor pressures, solid alcohols, hydrocarbons, and salts were excluded, the false negative rate was slightly reduced to 21% (14/68). When substances with high vapor pressures, and nonsurfactant solids were excluded, the false negative rate was slightly reduced to 18% (13/73).

Table 3-12 STE Performance for EPA Classification in a Bottom-Up Approach After Excluding Discordant Categories

Method Evaluated	Accuracy		False Positive Rate[a]		False Negative Rate[b]	
	%	No.[c]	%	No.[c]	%	No.[c]
STE Overall	80	103/129	6.3	2/32	25	24/97
STE w/o Alcohols	89	72/89	0	0/22	25	17/67
STE w/o Hydrocarbons	81	85/105	9.5	2/21	21	18/84
STE w/o Solids	78	72/92	6.9	2/29	29	18/63
STE w/o Salts	78	87/111	6.5	2/31	28	22/80
STE w/o Vapor Pressure >6kPa, solid alcohols, hydrocarbons, and salts	83	78/94	7.7	2/26	21	14/68
STE w/o Vapor Pressure >6kPa and Nonsurfactant Solids	85	86/101	7.1	2/28	18	13/73

Abbreviations: EPA = U.S. Environmental Protection Agency (EPA 2012); kPa = kilopascals; STE = short time exposure.

[a] False positive rate = the proportion of all negative substances that are falsely identified as positive *in vitro*.

[b] False negative rate = the proportion of all positive substances that are falsely identified as negative *in vitro*.

[c] Data used to calculate the percentage.

4.0 STE Test Method Reliability

Test method reliability (intralaboratory repeatability and intra- and interlaboratory reproducibility) is an essential element of an evaluation of assay performance (ICCVAM 2003). Repeatability refers to the closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period (ICCVAM 1997, 2003). Intralaboratory reproducibility refers to the extent to which qualified personnel within the same laboratory can replicate results using a specific test protocol at different times. Interlaboratory reproducibility refers to the extent to which different laboratories can replicate results using the same protocol and test chemicals and indicates the extent to which a test method can be transferred successfully among laboratories. A reliability assessment includes (1) reviewing the rationale for selecting the substances used to evaluate test method reliability, (2) discussing the extent to which the substances tested represent the range of possible test outcomes and the properties of the various substances for which the test method is proposed for use, and (3) performing a quantitative and/or qualitative analysis of repeatability and intra- and interlaboratory reproducibility.

Background information, data, and the performance (i.e., accuracy and reliability) analyses of the STE test method conducted by Kao Corporation are provided in the BRD (**Supplement A)**. STE test data were available for replicates within individual experiments repeated three times for each test substance in two to five different laboratories. Coefficient of variation (CV) analyses were performed on within-experiment and between-laboratory STE data, using the cell viability value obtained for each test substance within each of the two to five testing laboratories.

The %CV values for intralaboratory reliability for substances classified as nonirritants ranged from 0.3% to 23.5% in the four studies evaluated. Substances classified *in vitro* as irritants tended to have greater %CV values, as expected, because the cell viability for these chemicals was often quite low. Further, the mean viability for the positive control, 0.01% sodium lauryl sulfate, was 41.7% (N = 71) with %CV of 24.7%.

In terms of interlaboratory agreement, the laboratories recorded 100% agreement for 83% to 100% of the substances for GHS classification and 87% to 100% of the substances for EPA classification.

5.0 Peer Review Summary

To ensure the completeness of the NICEATM STE performance review, NTP provided the STE Summary Review Document along with the original Kao BRD and other supporting documentation to four external scientific reviewers who were asked to:

- Comment on whether the protocol is complete and adequate
- Comment on the adequacy of the database used for evaluating STE
- Provide any additional published STE studies or data
- Comment on the adequacy of the test method reliability
- Comment on the adequacy of the performance evaluation
- Provide any additional comments on the protocol or analysis
- Provide comments for regulatory agencies considering using data from this test method

The reviewers commented that the evaluation of the STE test method performance was thoroughly conducted. The reviewers remarked that the evaluation not only examined performance of the test method based upon the entire set of chemicals but also had a secondary assessment of STE performance with select chemical classes removed from the applicability domain. Given the thoroughness of the review, the reviewers stated that no other analysis is necessary. As with the performance analysis, the reviewers commented that the reliability analysis was thorough with no need for additional analysis.

The reviewers commented that the STE database was adequate for its intended purpose and added that they were not aware of additional STE data that could be used in this evaluation. The reviewers did however suggest that the STE database would require further development if the test method were to be used in the evaluation of pesticides.

The reviewers made a number of comments directed towards regulatory agencies considering using data obtained from the STE method:

When compared to other *in vitro* or *in vivo* assays currently available for eye irritation assessment, STE has a number of advantages, including time and cost required to do the assay, the use of a cell line rather than *ex vivo* tissue, its ability to assess poorly water-soluble substances, a low false positive rate, and protocol simplicity

The analysis highlights that the performance of a test method is dependent upon the classification system to which it is being compared. Specifically, STE “false negatives”, after all poorly compatible substances are excluded, are EPA Cat III: mild irritants.

Acceptance and use of data from the STE method is suggested by the developers as part of an *in vitro* / *ex vivo* battery of tests designed to offer an alternative to the *in vivo* OECD Test Guideline 405 Acute Eye Irritation/Corrosion assay in rabbits. As such, results from the STE when considered alone would most often be seen as “screening data” and best interpreted as part of a systematic evaluation of hazard.

There are likely to be circumstances in which the predictive nature of the STE assay is unknown (i.e., new chemical domains, mixtures, etc.). Submission of useful STE data relies on careful and

consistent conduct of the assay and verification of the performance of the STE method when extended beyond the currently available chemical domain or space.

At present, there is no *in vitro* alternative test to definitively and accurately distinguish non-irritant and irritant chemicals. In a bottom-up approach aimed at identifying non-irritants, it is important to reduce the false negative substances as much as possible. The false negative rate of BCOP and ICE are 0%, but their false positive rates are high, 68 to 69%. While the false negative rate in STE is not 0%, when either applicability domain is adopted, the rate is 1.9 to 2%. Test systems that obtain high accuracy, low false positive rate, and low false negative rate are desirable and based upon its performance, STE is suitable for use in a bottom-up approach.

As a result of the reviewers comments, minor edits were made to the NICEATM SRD including updating OECD TG references and clarifying the evaluation of direct MTT reducers. The reviewers also had a number of comments and suggestions for the STE protocol that were compiled and provided to Kao Corporation.

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