

Reference Substances for the Validation of *In Vitro* Ocular Toxicity Test Methods for the Evaluation of Ocular Corrosives and Severe Irritants

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Abstract

NICEATM evaluated four in vitro ocular test methods (the Bovine Corneal Opacity and Permeability, the Isolated Chicken Eye, the Isolated Rabbit Eye, and the Hen's Egg Test - Chorioallantoic Membrane) for their ability to identify substances that cause severe irritation or corrosion. During these evaluations, a list of 122 proposed reference substances was developed for potential optimization and/or validation studies with in vitro ocular toxicity tests. The substances also may be used for development of performance standards for use in the validation of test methods that are functionally and mechanistically similar to a validated in vitro ocular test method and for proficiency testing. Based on the ICCVAM Submission Guidelines, these substances are intended to: 1) represent the range of ocular responses (i.e., corrosive/severe irritant vs. nonsevere irritant/nonirritant) to be detected; 2) represent the classes of chemicals to be tested; 3) represent the range of known or anticipated mechanisms or modes of action to be tested; 4) have produced high quality Draize in vivo rabbit eye test method studies and/or in humans; 5) have well-defined chemical composition; 6) be available from commercial sources; and 7) not be associated with excessive hazard or costs (purchase and/or disposal). Based on the United Nations Globally Harmonized ocular hazard classification system, this list contains 79 Category 1, 15 Category 2A, 13 Category 2B, and 15 nonirritating substances (79 liquids, 34 solids), and covers 34 chemical classes and 29 product classes.

Introduction

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) evaluated four in vitro ocular test methods (the Bovine Corneal Opacity and Permeability, the Isolated Chicken Eye, the Isolated Rabbit Eye, and the Hen's Egg Test – Chorioallantoic Membrane) for their ability to identify substances that cause severe irritation or corrosion. During these evaluations, a list of proposed reference substances was developed for potential optimization and/or validation studies with in vitro ocular toxicity tests. NICEATM initially compiled a database of existing in vivo ocular toxicity data for over 950 substances. Commercially available substances for which adequate in vivo data were available to assign an ocular irritancy hazard classification based on the United Nations Globally Harmonized System (GHS; UN 2003) were identified as candidate substances. The GHS classification system was used for selecting candidates because it has been internationally harmonized through the UN, and is anticipated to be implemented globally sometime in the future. From these candidates, a list of proposed reference substances was developed, in conjunction with the ICCVAM Ocular Toxicity Working Group (OTWG). The proposed reference substances are intended to:

- represent the range of ocular responses (i.e., corrosive/severe irritant; nonsevere irritant/noncorrosive) that the test method is expected to be capable of predicting
- represent the range of chemical/product classes and physicochemical properties (e.g., solid, liquid) that the test method is expected to be capable of testing
- represent the range of known or anticipated mechanisms or modes of action for severe/irreversible ocular irritation or
- have high-quality in vivo rabbit eye test method studies available that follow Organization for Economic Cooperation and Development (OECD) Test Guideline (TG) 405 and preferably conducted in compliance with Good Laboratory Practices (GLP) guidelines
- have a well-defined chemical composition
- be tested at a defined concentration and at a defined purity¹
- be readily available

¹Information on purity and the concentration tested were not available for all Category 1 substances lacking purity information were included because testing at a potentially higher concentration would not likely alter their classification as a GHS Category 1 substance although it might alter the criteria by which they were classified as an ocular corrosive/severe irritant. Where information on purity was lacking, an assumption was made that testing would have been conducted with a relatively pure substance. For substances included because they cause severe ocular effects in humans but lacked appropriate in vivo rabbit eye test data, information on concentration and purity were not available.

Proposed Reference Substances

For the detection of ocular corrosives and severe irritants, the list of reference substances needs to include those that:

- induce very severe responses within a relatively short time period, as well as those where the toxic response is delayed
- adversely affect the cornea, iris, and/or conjunctiva
- induce persistent versus non-persistent lesions (when assessed at 21 days post treatment)
- represent diverse chemical classes and physicochemical

To cover all of these needs, the reference list contains 122 substances (Table 1). The total number of proposed reference substances reflects the need to ensure, to the extent possible, that the substances covered the range of responses of interest chemical/product classes and physicochemical properties of interest, and known or anticipated mechanisms or modes of action for severe/irreversible ocular irritation or corrosion.

As shown in **Table 2**, the list includes 79 GHS Category 1 substances (10 of which were classified as severe irritants based on human data only), 28 GHS Category 2 substances (15 Category 2A, 13 GHS Category 2B) and 15 nonirritants. These 122 substances cover 34 chemical classes (Table 3) and 24 product classes (Table 4) and include 79 substances tested in liquid form and 43 tested as solids (Table 3). The GHS Category 1 substances that are included in the list cover the entire range of responses that could result in a corrosive/severe irritant classification, based on both persistence and severity of the resulting lesion (Table 5).

Ocular Expert Panel Recommendations

In conjunction with ICCVAM, NICEATM convened an expert panel meeting on January 11-12, 2005, to independently assess the validation status of the four *in vitro* test methods previously mentioned. Additional data were made available subsequent to this meeting that mandated reconvening the expert panel on September 19, 2005. The reports from each of these meetings are available at http://iccvam.niehs.nih.gov/methods/eyeirrit.htm. The Panel made the following recommendations related to the proposed reference substances:

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

ICCVAM Recommendations

ICCVAM reviewed the Expert Panel's report, public comments received, and the recommendations of the OTWG. Based on these sources, ICCVAM made the following recommendations with relation to the list of proposed reference substances:

- ICCVAM endorsed the proposed list of 122 reference substances described above.
- ICCVAM endorsed the use of the reference substance list as a source for generating a subset of substances to be used for evaluating *in vitro* ocular toxicity test methods on a scientifically sound case-by-case basis.
- It was recommended that the subset of substances that are developed from the reference substance list comprise a scientifically sound distribution of substances among various properties including, but not limited to, chemical class, product class, physical form, irritancy severity classification, mechanism of action, physical and chemical characteristics, and molecular weight.
- In situations where a listed substance is not available, other substances of the same class for which there is high quality in vivo reference data may be used.

Table 1. Proposed List of Substances for Validation Studies of *In Vitro* Test Methods for the Identification of Ocular **Corrosives/Severe Irritants (Organized** by GHS Hazard Classification Category, Physical Form, and Substance Name)

	ny						
Substance	CAS RN	MW	Cat. 1 Subcat. ¹	Chemical Class	Product Class	Conc. Tested	Physical Form
GHS Category 1							
2,2-Dimethyl butanoic acid	595-37-9	116.2	1	Acid (Organic) [Carboxylic acid] ²	Pharmaceutical	undiluted	Liquid
2-Benzyl-4-chlorophenol	120-32-1	218.7	1	Phenol	-	100%	Liquid
2-Methylbutyric acid	116-53-0	102.1	0	Acid (Organic) [Carboxylic acid]	Solvent	100%	Liquid
3,4-Dichlorophenyl isocyanate	102-36-3	188.1	1	Isocyanates	-	100%	Liquid
4-Tert-butylcatechol	98-29-3	166.2	4	Phenol	- Industrial Chamical Tahanatan	85%	Liquid
Acetic acid	64-19-7	60.1	4	Acid (Organic) [Carboxylic acid]	Industrial Chemical, Laboratory Chemical	10%	Liquid
Aluminum chloride	16603-84-	98.9	4	Salt, Inorganic	-	-	Liquid
Benzalkonium chloride	8001-54-5	471.5	3	Onium Compound	Surfactant, Cationic	5%	Liquid
Benzalkonium chloride	8001-54-5	471.5	4	Onium Compound	Surfactant, Cationic	10%	Liquid
Bis-(3-aminopropyl) tetramethyl disiloxane	2469-55-8	248.5	4	Amine, Amidine, Organosilicon Compound	Industrial Chemical	undiluted	Liquid
Benzenesulfonyl chloride	98-09-9	176.6	4	Acyl Halide, Sulfur Compound,	<u>-</u>		Liquid
Benzethonium chloride	121-54-0	448.1	4	Organic Amine, Onium Compound		10%	Liquid
Butyl cellosolve	111-76-2	118.2	4	Alcohol, Ether	Solvent	undiluted	Liquid
Cetylpyridinium bromide	140-72-7	384.4	2	Onium Compound, Heterocyclic	Surfactant, Cationic	6%	Liquid
Catalogui dinigan basani da	140.72.7	204.4	4	Compound Onium Compound, Heterocyclic	Surfactort Cationia	100/	
Cetylpyridinium bromide	140-72-7	384.4	4	Compound	Surfactant, Cationic	10%	Liquid
Cetyltrimethylammonium bromide	57-09-0	364.4	4	Salt, Organic, Onium Compound	Cosmetic	10%	Liquid
Cyclohexanol	108-93-0	100.2	2	Alcohol	Solvent	undiluted	Liquid
	3173-53-3	125.2	4	Isocyanates	-	undiluted	Liquid
Di(2-ethylhexyl)sodium sulfosuccinate	577-11-7	444.6	4	Ester, Sulfur Compound, Organic, Salt, Organic	Cleaning agent, Wetting Agent, Solubilizer, Adjuvant	10%	Liquid
Diethylaminopropionitrile	5351-04-2	126.2	4	Amine, Nitrile	-	undiluted	Liquid
Diethylethanolamine	100-37-8	117.9	4	Amine, Alcohol	-	25%	Liquid
Domiphen bromide	538-71-6	414.5	4	Onium Compound	Anti-infective	10%	Liquid
gamma-Aminopropyltriethoxy	919-30-2	221.4	1	Amine, Amidine, Organosilicon	Industrial Chemical	undiluted	Liquid
Hydroxyethyl acrylate	818-61-1	116.1	4	Compound Alcohol, Ester		undiluted	Liquid
iso-Butanol	78-83-1	74.1	1	Alcohol	_	-	Liquid
Lactic acid	50-21-5	90.1	4	Acid (Organic) [Carboxylic acid],	Cosmetic	undiluted	Liquid
				Alcohol Ester,			
	3121-61-7	130.1	4	Ether	-	undiluted	Liquid
Methylpentynol	77-75-8	98.1	4	Alcohol	-	-	Liquid
Methylthioglycolate	2365-48-2	106.1	4	Ester, Sulfur Compound, Organic	Industrial Chemical	undiluted	Liquid
N,N,N',N'-Tetramethyl hexanediamine	111-18-2	172.31	4	Amine	-	100%	Liquid
n-Butanol	71-36-3	74.0	4	Alcohol	-	-	Liquid
n-Octylamine	111-86-4	129.2	4	Amine	-	100%	Liquid
Organofunctional Silane 45-49	82985-35-1	341.6	1	Amine, Organosilicon Compound	Polish	100%	Liquid
Phosphorodicloridic acid, ethyl ester	1498-51-7	162.9	4	Ester, Organophosphorus Compound	-	100%	Liquid
	10124-65-9	238.4	4	Salt, Organic,	_	10%	Liquid
Protectol PP	80-54-6	204.31	1	Acid (Organic) [Carboxylic Acid] Aldehyde	_	100%	Liquid
				-	Pharmaceutical Intermediate,		
Pyridine	110-86-1	79.1	4	Heterocyclic Compound	Pesticide Intermediate, Solvent	undiluted	Liquid
Sodium hydroxide	1310-73-2	40.0	4	Alkali	Caustic Agent	10%	Liquid
Surfonic N-102	9016-45-9	308.5	4	Heterocyclic Compound, Alcohol, Ether	-	100%	Liquid
Tetraethylene glycol diacrylate	17831-71-9	302.32	4	Ether, Nitro Compound	-	100%	Liquid
Tetrahydrofuran	109-99-9	72.1	4	Heterocyclic Compound, Ether	-	100%	Liquid
Trichloroacetic acid	76-03-9	163.4	4	Acid (Organic) [Carboxylic Acid]	Herbicide,	30%	Liquid
	76-02-8	163.4		, , , , , , , , , , , , , , , , , , , ,	Caustic Agent		
Trichloroacetyl chloride			4	Acyl Halide	-	-	Liquid
Triton X-100	9002-93-1	250.4	4	Ether Heterocyclic Compound, Salt,	Surfactant, Non-ionic	100%	Liquid
1.2 Diiminahanz (f)	41253-21-8		4	Organic	-	neat	Solid
isoindoline	65558-69-2		4	Amine, Heterocyclic Compound	-	100%	Solid
1-Naphthalene acetic acid	86-87-3	186.2	4	Acid (Organic) [Carboxylic Acid]	Pesticide	neat	Solid
2,5-Dimethylhexanediol	110-03-2	146.2	1	Alcohol	Chemical Intermediate	neat	Solid
2-Benzyl-4-chlorophenol	120-32-1	218.69		Phenol	-	100%	Solid
2-Hydroxyisobutyric acid	594-61-6	104.1	4	Acid (Organic) [Carboxylic Acid]	-	-	Solid
2-Hydroxyisobutyric acid ethylester	80-55-7	132.2	2	Alcohol, Ester	-	-	Solid
2-Nitro-4-thiocyanoaniline	54029-45-7	195.2	0	Nitro Compound, Amine, Sulfur Compound, Organic	-	100%	Solid
4-(1,1,3,3- Tetramethylbutyl)phenol	140-66-9	206.3	2	Phenol	-	100%	Solid
4-Chloro-methanilic acid	98-36-2	207.6	4	Amine, Sulfur Compound, Organic	-	-	Solid
4-Cinoro-incularinic acid		473.4	4	Amine, Quinone, Salt, Organic	Industrial Chemical	neat	Solid
Acid blue 40	6424-85-7		4	Acid (Organic) [Carboxylic Acid]	-	-	Solid
	328-50-7	146.1				100%	Solid
Acid blue 40		146.1 291.52	4	Salt, Inorganic	-	10070	
Acid blue 40 alpha-Ketoglutaric acid	328-50-7		4	Salt, Inorganic Acid (Organic) [Carboxylic Acid], Phenol	-	-	Solid
Acid blue 40 alpha-Ketoglutaric acid Antimony oxide	328-50-7 1309-64-4	291.52		Acid (Organic) [Carboxylic Acid],	- Pesticide		Solid Solid
Acid blue 40 alpha-Ketoglutaric acid Antimony oxide beta-Resorcylic acid	328-50-7 1309-64-4 89-86-1	291.52 154.1	4	Acid (Organic) [Carboxylic Acid], Phenol	-	-	
Acid blue 40 alpha-Ketoglutaric acid Antimony oxide beta-Resorcylic acid Captan 90-concentrate (solid) Chlorhexidine	328-50-7 1309-64-4 89-86-1 133-06-2 55-56-1	291.52 154.1 300.6 505.4	4 4	Acid (Organic) [Carboxylic Acid], Phenol Heterocyclic Compound Amidine Acid (Organic) [Carboxylic Acid],	Pesticide Disinfectant, Anti-Infective	neat neat	Solid Solid
Acid blue 40 alpha-Ketoglutaric acid Antimony oxide beta-Resorcylic acid Captan 90-concentrate (solid) Chlorhexidine Dibenzoyl-L-tartaric acid	328-50-7 1309-64-4 89-86-1 133-06-2 55-56-1 2743-38-6	291.52 154.1 300.6 505.4 358.3	4	Acid (Organic) [Carboxylic Acid], Phenol Heterocyclic Compound Amidine Acid (Organic) [Carboxylic Acid], Ester	Pesticide Disinfectant, Anti-Infective Chemical Intermediate	neat	Solid Solid
Acid blue 40 alpha-Ketoglutaric acid Antimony oxide beta-Resorcylic acid Captan 90-concentrate (solid) Chlorhexidine	328-50-7 1309-64-4 89-86-1 133-06-2 55-56-1	291.52 154.1 300.6 505.4 358.3	4 4	Acid (Organic) [Carboxylic Acid], Phenol Heterocyclic Compound Amidine Acid (Organic) [Carboxylic Acid],	Pesticide Disinfectant, Anti-Infective	neat neat	Solid Solid
Acid blue 40 alpha-Ketoglutaric acid Antimony oxide beta-Resorcylic acid Captan 90-concentrate (solid) Chlorhexidine Dibenzoyl-L-tartaric acid Dibenzoyl-L-tartaric acid	328-50-7 1309-64-4 89-86-1 133-06-2 55-56-1 2743-38-6	291.52 154.1 300.6 505.4 358.3 358.3	4 4 4	Acid (Organic) [Carboxylic Acid], Phenol Heterocyclic Compound Amidine Acid (Organic) [Carboxylic Acid], Ester Acid (Organic) [Carboxylic Acid],	Pesticide Disinfectant, Anti-Infective Chemical Intermediate	neat neat 20%	Solid Solid

Performance Standards and Proficiency Substances

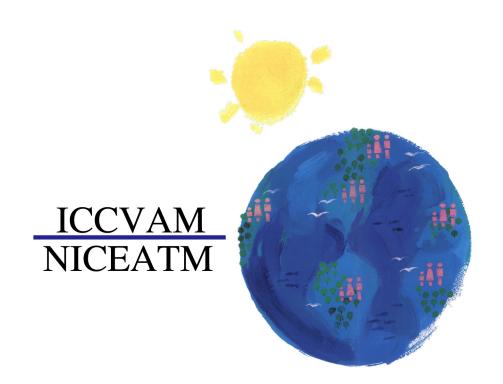
Following completion of validation studies, reference substances from this list can be selected for inclusion in performance standards and for proficiency testing. In this regard, subsets of substances from this list may be considered for:

- Optimization of a test method protocol
- Performance standard reference substances for use in the validation of test methods that are functionally and mechanistically similar to a validated ocular irritancy test method
- Proficiency testing to ensure the competency of a laboratory in performing a validated ocular irritancy test method

Substance	CAS RN	MW	Cat. 1 Subcat. ¹	Chemical Class	Product Class	Conc. Tested	Physic Form
Lauric acid	143-07-7	200.3	1	Acid (Organic) [Carboxylic Acid]	Surfactant, Anionic	neat	Solid
n-Acetyl-methionine	1115-47-5	191.3	4	Amide, Amino Acids	-	-	Solid
p-tert-Butylphenol	98-54-4	150.2	4	Phenol	-	undiluted	Solid
Promethazine hydrochloride	58-33-3	320.9	4	Amine, Amidine, Heterocyclic Compound, Sulfur Compound, Organic	Pharmaceutical	20%	Solid
Promethazine hydrochloride	58-33-3	320.9	3	Amine, Amidine, Heterocyclic Compound, Sulfur Compound, Organic	Pharmaceutical	neat	Solid
Propyl lactate	616-09-1	132.2	1	Ester, Alcohol	-	-	Solid
Quinacrine	69-05-6	472.9	1	Amine, Heterocyclic Compound, Polycyclic Compound	Pharmaceutical	20%	Solid
Quinacrine	69-05-6	472.9	3	Amine, Heterocyclic Compound, Polycyclic Compound	Pharmaceutical	neat	Solid
Sodium hydrogen sulfate	7681-38-1	120.1	4	Salt, Inorganic	-	-	Solid
Sodium oxalate	62-76-0	134.0	4	Salt, Organic	Industrial Chemical	neat	Solid
Sodium perborate tetrahydrate	10486-00-7	153.9	1	Salt, Inorganic, Boron Compound	Cleaner	neat	Solid
tetra-N-Octylammonium bromide	14866-33-2	546.8	0	Onium Compound	-	100%	Solid
Triton X-100	9002-93-1	250.4	2	Ether	Surfactant, Non-ionic	100%	Solid
Severe Human Eye Irritants	I				Fertilizer, Cleaning Agent,		
Ammonia	7664-41-7	17.0	-	Alkali	Refrigerant Anesthetic, Solvent, Chemical	-	Liquio
Chloroform	67-66-3	119.4	-	Hydrocarbon, Acyclic	Intermediate, Pharmaceutical Intermediate	-	Liqui
Nitric acid	7697-37-2	63.0	-	Acid (Inorganic)	Chemical Intermediate, Industrial Chemical, Laboratory Chemical	-	Liquio
Silver nitrate	7761-88-8	169.9	-	Nitrate, Salt, Inorganic	Industrial Chemical, Pharmaceutical, Chemical Intermediate	-	Liquio
Sodium hydrogen difluoride	1333-83-1	62.0	-	Salt, Inorganic	Cleaning Agent, Industrial Chemical, Preservative	-	Liquio
Sulfuric acid	7782-99-2	98.1	_	Acid (Inorganic), Sulfur	Industrial Chemical, Chemical Intermediate, Cleaning Agent,	-	Liqui
Lime	1305-78-8	56.1	_	Compound, Inorganic Salt, Inorganic	Battery Acid Building Material, Chemical	_	Solid
Magnesium hydroxide	12141-11-6		-	Salt, Inorganic Salt, Inorganic	Intermediate, Soil Amendment Pharmaceutical, Flame Retardant	_	Solid
Potassium hydroxide	1310-58-3	56.1	-	Alkali	Industrial Chemical, Veterinary	_	Solid
Zinc chloride	7646-85-7	136.3	_	Salt, Inorganic	Chemical, Cleaning Agent Pesticide, Herbicide,	_	Solid
	, 0.10-03-7	130.3		Sun, morganic	Anti-Microbial		50110
GHS Category 2A 2,6-Dichlorobenzoyl chloride	4659-45-4	209.5		Acyl halides	Anti-Infective, Anti-Fungal	undiluted	Liqui
2-Ethyl-1-hexanol	104-76-7	130.2		Alcohol	Solvent	undiluted	Liqui
4-Carboxybenzaldehyde	619-66-9	150.1	_	Acid (Organic) [Carboxylic Acid],	Industrial Chemical	undiluted	Liqui
Acetone	67-64-1	58.1	-	Aldehyde Ketone	Solvent	undiluted	Liqui
Benzotrichloride	98-07-7	195.5	-	Hydrocarbon, Cyclic	-	100%	Liqui
Cetylpyridinium bromide	140-72-7	384.4	-	Onium Compound, Heterocyclic Compound	Surfactant, Cationic	1%	Liqui
gamma-Butyrolactone	96-48-0	86.1	-	Heterocyclic Compound, Lactone	Solvent	undiluted	Liquio
iso-Butanol	78-83-1	74.1	-	Alcohol	Solvent	undiluted	Liqui
Methyl cyanoacetate	105-34-0	99.1	-	Ester, Nitrile	Chemical Intermediate	undiluted	Liqui
Methyl ethyl ketone	78-93-3	72.1	-	Ketone	Solvent	undiluted	Liqui
n-Butanol	71-36-3	74.1	-	Alcohol	Cosmetic Ingredient	undiluted	Liqui
n-Hexanol	111-27-3	102.2	-	Alcohol	Solvent	undiluted	Liqui
n-Octanol	111-87-5	130.2	-	Alcohol	Solvent	undiluted	Liqui
Triton X-100	9002-93-1	250.4	-	Ether	Surfactant, Non-ionic	10%	Liqui
Dibenzyl phosphate	1623-08-1	278.2	-	Ester, Organophosphorus Compound	Pesticide	neat	Solid
GHS Category 2B							
2-Methyl-1-pentanol	105-30-6	102.2	-	Alcohol	Solvent	undiluted	Liqui
3-Chloropropionitrile	542-76-7	89.5	-	Nitrile	-	undiluted	Liqui
Cyclopentanol	96-41-3	86.1	-	Alcohol	Pharmaceutical Intermediate	undiluted	Liquio
Ethyl-2-methyl acetoacetate	609-14-3	144.2	_	Ketone,	Chemical Intermediate	undiluted	Liqui
		216.3		Ester		12.5% in	
Hexyl cinnamic aldehyde	101-86-0		-	Aldehyde	-	alcohol	Liquid
Propasol Solvent P	1569-01-3	118.2	-	Alcohol	Surfactort Non ionia	100%	Liquid
Triton X-100	9002-93-1		-	Ether Acid (Organic) [Carboxylic Acid],	Surfactant, Non-ionic	5%	Liquid
3,3-Dithiopropionic acid	1119-62-6	210.3	-	Organosilicon Compound	-	neat	Solid
Ammonium nitrate	6484-52-2	80.0	-	Nitrate, Onium Compound, Salt, Organic	Industrial Chemical	neat	Solid
Maneb	12427-38-2	265.3	-	Amine, Salt, Organic, Urea Compound	Pesticide	neat	Solid
Nonirritant							
2,2-Dimethyl-3-pentanol	3970-62-5	116.2	-	Alcohol	Pharmaceutical	undiluted	Liqui
2,4-Difluoronitrobenzene	446-35-5	159.1	-	Hydrocarbons, Halogenated	-	undiluted	Liqui
2,4-Diffuoroiiitrobenzene		150.2	_	Ester, Ether	Dental Adhesive	undiluted	Liqui
2-Ethoxyethyl methacrylate	2370-63-0	158.2		,			Liqui
	2370-63-0 107-83-5	86.2	-	Hydrocarbon, Acyclic	Solvent	undiluted	Liqui
2-Ethoxyethyl methacrylate			-		Pharmaceutical Intermediate,	undiluted undiluted	
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone	107-83-5	86.2	-	Hydrocarbon, Acyclic Ketone		undiluted	Liqui
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate	107-83-5 108-83-8 29590-42-9	86.2 142.2 184.3	-	Hydrocarbon, Acyclic Ketone Ester	Pharmaceutical Intermediate, Solvent	undiluted undiluted	Liqui
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate iso-Octylthioglycolate	107-83-5 108-83-8 29590-42-9 25103-09-7	86.2 142.2 184.3 205.3	-	Hydrocarbon, Acyclic Ketone Ester Ester, Sulfur Compound, Organic	Pharmaceutical Intermediate, Solvent - Industrial Chemical	undiluted undiluted undiluted	Liquio Liquio Liquio
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate	107-83-5 108-83-8 29590-42-9	86.2 142.2 184.3	-	Hydrocarbon, Acyclic Ketone Ester Ester, Sulfur Compound, Organic Acid (Organic) [Carboxylic Acid], Salt, Organic	Pharmaceutical Intermediate, Solvent	undiluted undiluted	Liquio Liquio Liquio
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate iso-Octylthioglycolate	107-83-5 108-83-8 29590-42-9 25103-09-7	86.2 142.2 184.3 205.3		Hydrocarbon, Acyclic Ketone Ester Ester, Sulfur Compound, Organic Acid (Organic) [Carboxylic Acid],	Pharmaceutical Intermediate, Solvent - Industrial Chemical	undiluted undiluted undiluted	Liqui Liqui Liqui
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate iso-Octylthioglycolate Sodium lauryl sulfate	107-83-5 108-83-8 29590-42-9 25103-09-7 151-21-3	86.2 142.2 184.3 205.3 288.4		Hydrocarbon, Acyclic Ketone Ester Ester, Sulfur Compound, Organic Acid (Organic) [Carboxylic Acid], Salt, Organic Ester,	Pharmaceutical Intermediate, Solvent - Industrial Chemical Surfactant, Anionic	undiluted undiluted undiluted	Liqui Liqui Liqui Liqui
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate iso-Octylthioglycolate Sodium lauryl sulfate Tween 20	107-83-5 108-83-8 29590-42-9 25103-09-7 151-21-3 9005-64-5	86.2 142.2 184.3 205.3 288.4 1227.5	- - -	Hydrocarbon, Acyclic Ketone Ester Ester, Sulfur Compound, Organic Acid (Organic) [Carboxylic Acid], Salt, Organic Ester, Ether	Pharmaceutical Intermediate, Solvent - Industrial Chemical Surfactant, Anionic	undiluted undiluted undiluted 3% undiluted	Liquid Liquid Liquid Solid
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate iso-Octylthioglycolate Sodium lauryl sulfate Tween 20 2,4-Pentanediol	107-83-5 108-83-8 29590-42-9 25103-09-7 151-21-3 9005-64-5 625-69-4 123-86-4	86.2 142.2 184.3 205.3 288.4 1227.5 104.2	- - - -	Hydrocarbon, Acyclic Ketone Ester Ester, Sulfur Compound, Organic Acid (Organic) [Carboxylic Acid], Salt, Organic Ester, Ether Alcohol	Pharmaceutical Intermediate, Solvent - Industrial Chemical Surfactant, Anionic Surfactant, Non-ionic	undiluted undiluted 3% undiluted	Liquid Liquid Liquid Solid
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate iso-Octylthioglycolate Sodium lauryl sulfate Tween 20 2,4-Pentanediol Cellosolve acetate	107-83-5 108-83-8 29590-42-9 25103-09-7 151-21-3 9005-64-5 625-69-4 123-86-4	86.2 142.2 184.3 205.3 288.4 1227.5 104.2 305.2	- - - -	Hydrocarbon, Acyclic Ketone Ester Ester, Sulfur Compound, Organic Acid (Organic) [Carboxylic Acid], Salt, Organic Ester, Ether Alcohol Ether	Pharmaceutical Intermediate, Solvent - Industrial Chemical Surfactant, Anionic Surfactant, Non-ionic	undiluted undiluted 3% undiluted -	Liquid Liquid Liquid Solid Solid
2-Ethoxyethyl methacrylate 2-Methylpentane Di-iso-butyl ketone Iso-octyl acrylate iso-Octylthioglycolate Sodium lauryl sulfate Tween 20 2,4-Pentanediol Cellosolve acetate N,N-Dimethylguanidine sulfate	107-83-5 108-83-8 29590-42-9 25103-09-7 151-21-3 9005-64-5 625-69-4 123-86-4 598-65-2	86.2 142.2 184.3 205.3 288.4 1227.5 104.2 305.2 272.3 193.1	- - - - -	Hydrocarbon, Acyclic Ketone Ester Ester, Sulfur Compound, Organic Acid (Organic) [Carboxylic Acid], Salt, Organic Ester, Ether Alcohol Ether Amidine, Salt, Organic	Pharmaceutical Intermediate, Solvent - Industrial Chemical Surfactant, Anionic Surfactant, Non-ionic	undiluted undiluted 3% undiluted - neat	Liquid Liquid Liquid Liquid Solid Solid Solid Solid Solid

Cat. 1 Subcat. = GHS Category 1 subcategories = NICEATM-assigned subcategories for GHS Category 1 substances (ocular corrosives and evere irritants) were assigned based on the following: 1 = Positive response based on a persistent lesion involving the cornea, iris, and/or onjunctiva through to day 21 in at least one of three rabbits and not on severity; 2 = Positive response based on mean for first 3 days (CO > lays (CO > 3 and <4 or Iritis (IR) > 1.5) in at least two of three rabbits and a persistent (>21 days) lesion in at least one rabbit; 4 = CO score of 4 t any time in at least one of three rabbits; - indicates that the substance does not have a subcategory because it is not in GHS category 1. Chemical class in brackets provides additional information about chemical structure of the identified substance

More information on ICCVAM and NICEATM can be accessed at: http://iccvam.niehs.nih.gov/



Distribution of Substances in the *In Vivo* Rabbit Eye Test Database and Molecular **Weight Ranges of the Proposed** Reference Substances, by GHS¹ Ocular **Hazard Classification**

Classification (GHS)	Number of Entries in the <i>In Vivo</i> Rabbit Eye Test Database ² with a GHS Classification	Number of Candidate Substances (i.e., GHS- Classified Substances ³ Determined to be Commercially Available)	Number of Proposed Reference Substances	Additional Substances Identified as Causing Severe Ocular Damage in Humans	Final Number of Proposed Reference Substances	Molecular Weight Range for Proposed Reference Substances
Category 1	220	93	69	10	79	30.0 – 546.8
Category 2A	62	17	15	_5	15	58.1 – 384.4
Category 2B	51	23	13	-	13	80.0 – 265.3
Nonirritant	497	77	15	-	15	86.2 – 1227.5
Total	830	210	112	10	122	30.0 – 1227.5
² The complete data according to the Gl ³ "Substances" is do humans are substan 2A response in one substance. ⁴ The number of enthe persistence of a	HS ocular hazard classific efined as a unique entry (i. nees for which individual is study and a Category 2B atries decreased for some C	tries for some substances, as well ation system. e., a single substance tested at a stability eye test results were not located response in another study; for put GHS classification categories due reatment; (2) a reassessment of cut	single concentration). The cated. One substance (imposes of classification to (1) the reclassification	The substances identi Triton X-100), when in this table, Triton in	fied as causing severe tested at 5%, induced X-100 is classified as	ocular effects in a GHS Category a Category 2A irritants, based on

Chemical Classes and Properties of Interest Represented Among the Proposed Reference Substances, According to GHS¹ **Ocular Hazard Classification Category**

	Number of	Number of	GHS Ca	tegory 1 ²	GHS	GHS	
Chemical Class ¹	Candidate Substances	Proposed Reference Substances	Based on Human Data	Based on Rabbit Data	Category 2A	Category 2B	GHS NI ³
		Ch	emical Class	1,5			
Acid (inorganic)	2	2	2(2)	_6	-	-	-
Acid (organic)	20	17	-	13(14)	2(2)	1(3)	1(1)
Acyl Halide	3	3	1	2(2)	1(1)	-	_
Alcohol	30	22	-	11(17)	4(6)	4(4)	3(3)
Aldehyde	6	4	-	2(2)	1(1)	1(2)	0(1)
Alkali	3	3	2(2)	1(1)	-	-	-
Amide	2	2	1	1(1)	-	1(1)	_
Amidine	6	5	-	4(5)	-	-	1(1)
Amine	23	17	-	14(18)	-	2(2)	1(3)
Amino Acid	1	1	-	1(1)	-	-	-
Boron Compound	1	1	-	1(1)	-	-	-
Ester	32	15	-	8(9)	3(3)	2(5)	2(15)
Ether	26	11	-	8(12) ⁷	$1(1)^{7}$	$2(2)^{7}$	3(14)
Heterocyclic	19	13		9(13)8	2(2)8	2(2)	1(2)
Compound	19	13	-	9(13)	2(2)	2(2)	1(3)
Hydrocarbon (acyclic)	7	1	1(1)	-	-	-	0(6)
Hydrocarbon (cyclic)	11	2	-	-	1(1)	0(1)	1(9)
Hydrocarbon, Halogenated	13	2	-	-	-	-	2(13)
Isocyanate	2	2	_	2(2)	_	_	_
Ketone	8	5	_	-	2(2)	2(4)	1(2)
Lactone	1	1	_	_	1(1)	-	1(2)
Nitrate	2	2	1(1)	_	-	1(1)	_
Nitrile	3	3	-	1(1)	1(1)	1(1)	_
Nitro Compound	5	2	_	2(2)	-	-(2)	-(1)
Onium Compound	10	6	_	5(8)8	1(1)8	1(1)	-(1)
Organophosphorus Compound	3	2	-	1(1)	1(1)	-	0(1)
Organosilicon Compound	5	4	-	3(3)	-	1(1)	-(1)
Phenol	8	6		5(7)	_	_	1(1)
Polycyclic	4	3	-	2(3)	1(1)	-	-
Compound	1	1					
Quinone Salt (inorganic)	1 12	12	7(7)	1(1)	-	-	1(1)
	14	11	7(7)	4(4)	-	2(4)	1(1)
Salt (organic) Sulfur Compound	14	1	1(1)	6(7)	-	2(4)	3(3)
(inorganic) Sulfur Compound	16	9	_	7(8)	_	_	2(8)
(organic) Urea Compound	1	1	-	-	-	1(1)	-
Total ⁹	301	192	14(14)	116(143)	22(24)	24(37)	23(88
2 0 0001			erties of Inte		22(2-1)	21(37)	25(00
Liquid	163	79	6(6)	40(61)	14(16) ¹⁰	9(13)	10(67
Solid	53	43	4(4)	29(31)	1(1)	4(8)	5(9)
Chemical Class=Based o							

⁴Numbers in parenthesis indicate the number of candidate substances for that GHS category. Substances were assigned into one or more chemical classes (see Table 1

'-" indicates that there are no substances in this category. Triton X-100 classified as GHS Category 1, 2A/2B and NI

Cetylpyridinium bromide classified as GHS Category 1 and 2A. The total number is greater than the total number of proposed reference substances because some substances were assigned to ⁰Triton X-100, when tested at 5%, induced a GHS Category 2A response in one study and a Category 2B response in another study; for purposes of classification in this table, Triton X-100 is classified as a Category 2A substance.

References

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Table 4. Product Classes Represented Among the Proposed Reference Substances, According to GHS¹ Ocular Hazard **Classification Category**

		GHS Car	legory I	GHS	GHS	CIT
Product Class	Total ²	Based on Human Data	Based on Rabbit Data	Category ³ 2A	Category 2B	GH NI
Adjuvant, Solubilizer, Wetting Agent	1	_4	1	-	-	-
Anesthetic	2	1	1	-	-	-
Anti-Fungal	6	1	4	1	-	-
Anti-Infective	12	3	7 ³	3 ³	-	-
Battery Acid	1	1	-	-	-	-
Building Material	2	1	-	-	-	1
Caustic Agent	2	-	2	-	-	-
Chemical Intermediate	43	8	23	4	3	5
Cleaner or Cleaning Agent	15	6	6	1	1	1
Cosmetic Ingredients, & Perfumes	11	-	8	-	2	1
Fertilizers	4	4	-	-	-	-
Flame Retardant	3	2	1	-	-	-
Food Additives	9	2	4	1	1	1
Herbicides	5	2	2	1	-	-
Industrial Chemicals & Dyes	46	11	28	2	2	3
Laboratory Chemicals	28	3	16 ³	33	3	4
Pesticide & Pesticide Intermediates	17	1	11	1	1	3
Pharmaceuticals & Pharmaceutical Intermediates	29	5	15	1	4	4
Polish	1	-	1	-	-	-
Preservative	4	2	1	1	-	_
Refrigerant	1	1	-	-	-	_
Solvent	21	1	8	8	3	1
Surfactants:	7	-	5 ³	2^3	23	2^{3}
Anionic	3	-	1	-	1	1
Cationic	2	-	2^{3}	1^3	-	-
Nonionic	2	-	2 ³	13	13	13
Veterinary Agent	6	2	4	-	-	-

All substances were assigned into one or more product classes by referencing the National Library of Medicine Hazardous Substances Database, other information was obtained from Material Safety Data Sheets obtained from the commercial supplier; therefore, the total number is greater than the total number of proposed reference substances. ³Some substances, when tested at different concentrations, were assigned a different GHS ocular hazard classification. For this table, these substances (Triton X-100 and cetylpyridinium bromide) appear in more than one GHS category column; thus the total numbers in these columns do not add up to the numbers of substances in the total column. - " indicates that there are no substances in this category.

Table 5. NICEATM-Defined Subcategories for the Proposed GHS¹ Category 1 Reference

1 ccc or Pcc 2 >3 bu	Not Classifiable Positive response based on a persistent lesion involving the ornea, iris, and/or conjunctiva through to day 21 in at least	12
1 cc or Pc 2 >3 bu	1 1	_
2 >3 bu	ne of three rabbits and not on severity	9
D	Positive response based on mean for first 3 days (CO ³ score 3 and <4 or IR ⁴ score >1.5) in at least two of three rabbits at lesions do not persist through day 21	4
3 >3	Positive response based on mean for first 3 days (CO score 3 and <4 or IR score >1.5) in at least two of three rabbits and a persistent (>21 days) lesion in at least one rabbit	4
	CO score = 4 at any time in at least one of three rabbits	50

¹GHS = United Nations Globally Harmonized System (UN [2003]). ²Included are two GHS Category 1 substances that could not be subclassified because classification was based on an extreme response shortly after treatment in the only animal tested and 10 substances classified as GHS Category 1 irritants because they induced a severe ocular response in accidentally-exposed humans, and appropriate in vivo rabbit ocular irritancy test data was not located for these 10 substances $^{3}CO = corneal opacity$

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