Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

24-Jun-2013

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

STREAMLINED SUMMARY DOCUMENT SUPPORTING OECD TEST GUIDELINE 438 ON THE ISOLATED CHICKEN EYE FOR EYE IRRITATION/CORROSION

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JT03342323

ENV/JM/MONO(2013)12/PART2
Unclassified

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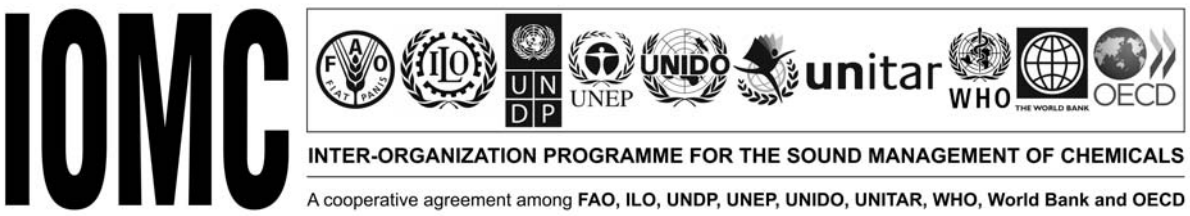

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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 2013

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**OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France**

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

FOREWORD

This streamlined summary document (SSD) was developed to provide summary information in support of OECD Test Guideline 438 on the Isolated Chicken Eye Test Method addressing the endpoint eye irritation/corrosion. This SSD was developed by a Secretariat consultant and submitted to the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) in March 2013, together with the updated version of TG 438 (originally adopted in 2009). The SSD provides useful and more detailed information than is otherwise available from the Test Guideline itself on: 1) the scientific basis of the test method, 2) the identified limitations, weaknesses and strengths, 3) the applicability domain, 4) the sensitivity, specificity and accuracy, and 5) the within-laboratory and between-laboratory reproducibility of the method.

The SSD was approved by the WNT with a few changes to paragraph 11, including additional references 22, 23, 24 and 25, on 30 April 2013.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 14 June, 2013.

This document is published under the responsibility of the Joint Meeting of the Chemicals committee and the Working Party on Chemicals, Pesticides and Biotechnology.

List of Appendixes

APPENDIX 1

List of Chemicals Tested with ICE and Comparison of *In Vivo* and *In Vitro* Classifications : Sorted by Chemical

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
1	Acetaldehyde	75-07-0	Aldehyde	Manufacture of acetic acid, perfumes & flavors; narcotic	liquid	99	undiluted	no raw data	n.a.	2A	2A	2	III	1.4	II	24	III	Prinsen and Koëter (1993)	-
2	Acetic acid	64-19-7	Carboxylic acid	Reagent; Indicator	liquid	99	10%	1	n.a.	1	1	3	IV	2.6	IV	31	III/IV	Prinsen and Koëter (1993)	*In vivo* data from Japanese study
3	Acetone	67-64-1	Ketone	Solvent; Antiseptic; Chemical intermediate; Raw material	liquid	99	undiluted	2A	n.a.	2A	2B	1.4	II	0.4	I	9.6	II	Balls et al. (1995)	-
											2A	1	II	1.7	III	49	IV		
											2B	1.83	III	1.17	II	7.64	II		
											2A	3	IV	1	II	13.8	II/III		
4	Ammonium nitrate	6484-52-2	Inorganic salt, Onium compound	Fertilizer; Chemical intermediate; Industrial explosive	solid	>99	undiluted	2A	n.a.	2B	2B	1.2	II	0.9	II	6.7	II	Balls et al. (1995)	-
											2A	2	III	1.3	II	42	IV		
											2B	1.33	II	1.5	II	12.33	II/III		
											2B	2	III	0.5	I	6	II		
5	L-Aspartic acid	70-47-3	Amino acid	Organic intermediate; Fungicides; Germicides	solid	100	neat	SCNM	2	2A	2B	1	II	0.7	II	3.2	I	Balls et al. (1995)	Uncertainity on identity of tested chemical (CAS n. not corresponding to chemical name)
											2A	2	III	2	III	56	IV		
											2A	1.83	III	1.67	III	14.67	II/III		
											2B	2	III	1	II	10	II		
6	Benzalkonium chloride (1%)	8001-54-5	Onium compound	Surfactant (cationic), Bactericide, Fungicide, Preservative	liquid	98	1%	1	n.a.	2A	2A/2B	1.8	III	0.6	II	18	II/III	Balls et al. (1995)	-
											2A	1.3	II	2.3	III	47	IV		
											2A	2.67	IV	1.5	II	12.66	II/III		
											2A	2	III	3	IV	8.8	II		
7	Benzalkonium chloride (5%)	8001-54-5	Onium compound	Surfactant (cationic), Bactericide, Fungicide, Preservative	liquid	98	5%	1	n.a.	1	1	3	IV	2.6	IV	36.3	IV	Balls et al. (1995)	-
											2A	1	II	2	III	42	IV		
											1	3	IV	2	III	33.77	IV		
											1	3	IV	3	IV	68.9	IV		
8	Benzalkonium chloride (10%)	8001-54-5	Onium compound	Surfactant (cationic), Bactericide, Fungicide, Preservative	liquid	98	10%	1	n.a.	1	1	3	IV	3	IV	37.7	IV	Balls et al. (1995)	-
											1	3	IV	2.3	III	95	IV		
											1	3	IV	2.33	III	40.72	IV		
											1	3	IV	2	III	41.1	IV		
9	Benzalkonium chloride (100%)	8001-54-5	Onium compound	Surfactant (cationic), Bactericide, Fungicide, Preservative	liquid	98	undiluted	1	n.a.	1	1	3	IV	3	IV	40	IV	Prinsen and Koëter (1993)	*In vivo* data from Japanese study
10	Brij 35	9002-92-0	Alcohol	Solvent; Excipient; Surfactant	liquid	n.p.	undiluted	no raw data	n.a.	NC	NC	0.9	II	0	I	5	I	Prinsen and Koëter (1993)	*In vivo* data available for 10% dilution. Dilution tested *in vitro* to be confirmed. Not considered in present calculations
11	1-Butanol	71-36-3	Alcohol	Ingredient of spray paint; Nail polish	liquid	99	undiluted	1/2A	n.a.	1	1	2.9	IV	2	III	54	IV	Prinsen and Koëter (1993)	*In vivo* GHS Cat 2A in ECETOC Database, and *in vivo* GHS Cat 1 in Zebet study
12	2-Butoxyethyl acetate	112-07-2	Alcohol	Cleaner; Polish; Sealant	liquid	99	undiluted	no raw data	n.a.	2B	2B	1	II	1	II	5	I	Prinsen and Koëter (1993)	The *in vitro* classification proposed by The Netherlands did not correspond their proposed criteria.
13	n-Butyl acetate	123-86-4	Ester	Solvent; Synthetic flavor ingredient	liquid	99	undiluted	NC	n.a.	2A	2A	1.8	III	1.8	III	13.9	II/III	Balls et al. (1995)	-
											1	0.5	I	2.7	IV	42	IV		
											2A/2B	1	II	2	III	14.67	II/III		
											2A	1	II	2	III	32.2	IV		

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
14	gamma-Butyrolactone	96-48-0	Heterocyclic, Lactone	Synthetic intermediate; Solvent	liquid	>99	undiluted	2A	n.a.	2A	2A	2.6	IV	1.4	II	15.8	II/III	Balls et al. (1995)	-
											2A	1.3	II	2	III	47	IV		
											2A/2B	1.67	III	1.5	II	13.1	II/III		
											2A/2B	1	II	2	III	13	II/III		
15	Captan 90 concentrate	133-06-2	Imide, Organic sulfur compound	Pesticide	solid	90	neat	1	n.a.	2B	NC	0	I	0.4	I	1.7	I	Balls et al. (1995)	-
											2A/2B	0.2	I	1	II	27	III/IV		
											2B	0	I	1.33	II	19.17	III		
											2B	1	II	1	II	20	III		
16	4-Carboxybenzaldehyde	619-66-9	Carboxylic acid, Aldehyde	Manufacturing impurity (polyester); Developer intermediate	solid	95	neat	2A	n.a.	2A	2B	1	II	0.5	I	5.4	II	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by ICCVAM and The Netherlands were unclear
											1	1.3	II	3	IV	89	IV		
											NC	0.67	II	0.5	I	-1.4	I		
											2A/2B	2	III	1	II	12.7	II/III		
17	Cetylpyridinium bromide (0.1%)	140-72-7	Heterocyclic, Onium compound	Surfactant (cationic), Germicide, Laboratory reagent	liquid	98	0.1%	NC	n.a.	2B	NC	1	II	0	I	2.2	I	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seemed based on results of one lab only
											2B	0.7	II	0	I	21	III		
											2B	0.67	II	1	II	10.29	II		
											2B	1	II	1	II	14.6	II/III		
18	Cetylpyridinium bromide (6%)	140-72-7	Heterocyclic, Onium compound	Surfactant (cationic), Germicide, Laboratory reagent	liquid	98	6%	1	n.a.	2A	2A	2	III	1.2	II	27.2	III/IV	Balls et al. (1995)	-
											2A	2	III	0.5	I	49	IV		
											2A	3	IV	1.83	III	24.55	III		
											2A	2.7	IV	1.7	III	13.5	II/III		
19	Cetylpyridinium bromide (6%)	140-72-7	Heterocyclic, Onium compound	Surfactant (cationic), Germicide, Laboratory reagent	liquid	n.p.	undiluted	1	n.a.	2A	2A	2	III	2	III	22	III	Balls et al. (1995)	The *in vitro* classification proposed by ICCVAM did not correspond to their proposed classification criteria (GD 160). The rationale for the *in vitro* classification proposed by The Netherlands was also unclear.
											2A	1.8	III	1.7	III	21	III		
											2A	2	III	2	III	21	III		
											2A	1.7	III	1.7	III	18	II/III		
20	Cetylpyridinium bromide (10%)	140-72-7	Heterocyclic, Onium compound	Surfactant (cationic), Germicide, Laboratory reagent	liquid	98	10%	1	n.a.	2A	2A	2.6	IV	1	II	25.8	III	Balls et al. (1995)	-
											2A	1.7	III	2	III	41	IV		
											2A	2	III	1.67	III	27.2	III/IV		
											1	3	IV	3	IV	17.8	II/III		
21	Chlorhexidine	55-56-1	Amine/Amidine	Disinfectant; Mouthwash; Anti-infective agent	solid	n.p.	neat	1	n.a.	1	1	3	IV	4	IV	32	III/IV	Balls et al. (1995)	-
											1	3	IV	4	IV	150	IV		
											1	3	IV	3	IV	53.13	IV		
											1	3	IV	4	IV	n.p.	n.p.		
22	Chloroform	67-66-3	Hydrocarbon (halogenated)	Solvent; Cleaner	liquid	99.8	undiluted	no raw data	n.a.	2A	2A	2.5	III	1	II	21	III	Prinsen and Koëter (1993)	-
23	Cyclohexanol	108-93-0	Alcohol	Solvent; Chemical intermediate	liquid	97	undiluted	1	n.a.	1	2A	2.2	III	2.2	III	24.7	III	Balls et al. (1995)	-
											1	3	IV	2	III	103	IV		
											1	3	IV	2.5	III	35.7	IV		
											1	3	IV	2.5	III	45.3	IV		
24	Cyclohexylamino-functional PMS	n.p.	Organosilicon compound	_	liquid	n.p.	undiluted	no raw data	n.a.	2A	2A	1.8	III	2.5	III	14	II/III	Prinsen (2000)	-
											2A	1.7	III	2	III	13	II/III		
											2A	2	III	2.3	III	17	II/III		
											2A	2	III	2.3	III	14	II/III		
											2A	2	III	2	III	13	II/III		

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
25	Decamethylcyclopentasiloxane	n.p.	Organosilicon compound	Ingredient of hair conditioner; Diaper rash ointment; Car wax	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.3	I	0.3	I	1	I	Prinsen (2000)	*In vivo* data shared by TNO
											NC	0.3	I	0.3	I	1	I		
											NC	0	I	0.5	I	2	I		
											NC	0	I	0	I	0	I		
											NC	0	I	0	I	2	I		
26	Dibenzoyl-L-tartaric acid	2743-38-6	Carboxylic acid, Ester	Optical resolution agent	solid	98	neat	1	n.a.	1	1	2.8	IV	3	IV	12.8	II/III	Balls et al. (1995)	-
											1	1	II	2.7	IV	75	IV		
											2B	2	III	1.5	II	6.36	II		
											2B	1	II	2	III	6.7	II		
27	Dibenzyl phosphate	1623-08-1	Ester, Organophosphor us compound	Not classified	solid	99	neat	2A	n.a.	2A	2A	2.6	IV	2	III	12.2	II/III	Balls et al. (1995)	-
											2B	1	II	0	I	22	III		
											2A/2B	2	III	1.5	II	17.07	II/III		
											2A	2	III	2	III	40.9	IV		
28	Dibutyltin dichloride	683-18-1	Organometallic compound	Industrial chemical; Immunosuppress ive agent	solid	97	undiluted	no raw data	n.a.	1	1	3	IV	2.5	III	34	IV	Prinsen and Koëter (1993)	-
29	2,6-Dichlorobenzoyl chloride	4659-45-4	Acyl halide	Anti-infective; Anti-fungal; Preservative	liquid	99	undiluted	2A	n.a.	2A	2A/2B	2.3	III	0.8	II	12.7	II/III	Balls et al. (1995)	-
											2A	2	III	1.3	II	26	III		
											2A	1.83	III	1.67	III	17.15	II/III		
											2A/2B	1.8	III	0.8	II	16.8	II/III		
30	2,2-Dimethylbutanoic acid	595-37-9	Carboxylic acid	Pharmaceutical metabolite	liquid	96	undiluted	SCNM	1	1	1	3	IV	2.4	III	43.8	IV	Balls et al. (1995)	-
											1	3	IV	2.7	IV	74	IV		
											1	3	IV	2.5	III	35.9	IV		
											1	3	IV	3	IV	62.7	IV		
31	2,5-Dimethylohexanediol	110-03-2	Alcohol	Intermediate for pharmaceuticals; Pesticides; perfumes	solid	99.5	neat	1	n.a.	2B	2B	2	III	1	II	11.9	II	Balls et al. (1995)	-
											1	2	III	3	IV	64	IV		
											2B	1.33	II	1.67	III	11.57	II		
											2B	2	III	1	II	6.7	II		
32	Dimethyl sulfoxide	67-68-5	Organic sulfur compound	Solvent; Cryoprotective agent	liquid	99.9	undiluted	NC	n.a.	NC	NC	1	II	0.5	I	4	I	Prinsen and Koëter (1993)	*In vivo* classification derived from Gautheron et al. (1994). *In vivo* classification proposed by ICCVAM was unclear.
33	Ethanol	64-17-5	Alcohol	Solvent; Beverages; Antifreeze agent	liquid	n.p.	undiluted	2A	n.a.	1	1	2.8	IV	2.8	IV	30.7	III/IV	Balls et al. (1995)	Cat 2A also in study of SC Johnson according to GHS (Cat 1 according to CLP)
											1	2	III	3	IV	74	IV		
											2A	2.5	III	2.33	III	35.88	IV		
											2A	2	III	2.3	III	34.6	IV		
34	Ethyl acetate	141-78-6	Ester	Solvent; Synthetic flavoring	liquid	99	undiluted	NC	n.a.	2A	2A	2	III	2	III	22	III	Balls et al. (1995)	-
											2A	1.7	III	2.3	III	76	IV		
											2A	2	III	2	III	25.08	III		
											2A	3	IV	2	III	23	III		
35	2-Ethyl-1-hexanol	104-76-7	Alcohol	Solvent; Plasticizer	liquid	99	undiluted	2A	n.a.	2A	2A	2	III	2.2	III	43	IV	Balls et al. (1995)	-
											2A	1	II	2.3	III	62	IV		
											2A	3	IV	1.5	II	13.31	II/III		
											2A	1	II	2	III	52.4	IV		

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
36	Ethyl-2-methylacetoacetate	609-14-3	Ketone, Ester	Not classified	liquid	97	undiluted	2B	n.a.	2B	NC	0.4	I	0.3	I	-2.8	I	Balls et al. (1995)	The *in vitro* classification proposed by The Netherlands did not correspond to their proposed criteria
											2B	1	II	0	I	7	II		
											2B	0.67	II	1	II	11.52	II		
											NC	1	II	0.5	I	4.5	I		
37	Ethyl trimethyl acetate	3938-95-2	Ester	Solvent	liquid	99	undiluted	NC	n.a.	2B	2B	1.2	II	0.4	I	7.2	II	Balls et al. (1995)	-
											2A	2	III	2	III	31	III/IV		
											NC	0	I	0	I	1.44	I		
											2B	1	II	0.5	I	6.7	II		
38	Fomesafen	72128-02-0	Imide, Ether, Nitro compound	Pesticide	solid	97.5	neat	SCNM	NC	2B	2B	0.9	II	1.2	II	5.3	II	Balls et al. (1995)	The *in vitro* classification proposed by The Netherlands did not correspond to their proposed criteria. Chemical considered GHS NC by both ICCVAM and The Netherlands, but not considered in the present calculations as SCNM
											NC	0	I	0.2	I	11	II		
											NC	1	II	0.5	I	2.82	I		
											2B	1	II	1	II	4.3	I		
39	Glycerol	56-81-5	Alcohol	Solvent; Plasticizer; Lubricant; Emollient; Drug vehicle	liquid	>99.5	undiluted	NC	n.a.	2B	2B	1.2	II	1	II	5	I	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by The Netherlands was unclear
											NC	0	I	0	I	11	II		
											2B	1.17	II	1	II	8.3	II		
											2A	2	III	0.5	I	29.4	III/IV		
40	Glycerol	56-81-5	Alcohol	Solvent; Plasticizer; Lubri-cant; Emollient; Drug vehicle	liquid	99	undiluted	NC	n.a.	NC	NC	0.5	I	0.4	I	4	I	Prinsen and Koëter (1993)	-
41	n-Hexane	110-54-3	Hydrocarbon (acyclic)	Solvent; Adhesive; Gasoline additive	liquid	99	undiluted	NC	n.a.	NC	NC	0.5	I	0	I	1	I	Prinsen and Koëter (1993)	-
42	n-Hexanol	111-27-3	Alcohol	Solvent; Chemical intermediate; Synthetic flavor ingredient	liquid	98	undiluted	2A	n.a.	1	2A	2.8	IV	1.6	III	17.4	II/III	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seemed based on results of one lab only
											2A	0.2	I	1.7	III	82	IV		
											1	3	IV	2.83	IV	28.89	III/IV		
											1	3	IV	3	IV	58.9	IV		
43	Imidazole	288-32-4	Heterocyclic	Anti-fungal; Enzyme inhibitor	solid	99	neat	1	n.a.	1	1	3	IV	4	IV	40.3	IV	Balls et al. (1995)	-
											1	3	IV	3	IV	224	IV		
											1	3	IV	2.5	III	36.96	IV		
											1	3	IV	3	IV	97.8	IV		
44	Isobutanol	78-83-1	Alcohol	Solvent; Chemical intermediate; Flavor ingredient	liquid	99.9	undiluted	2A	n.a.	1	1	2.8	IV	2.5	III	46.4	IV	Balls et al. (1995)	-
											1	3	IV	2.7	IV	93	IV		
											1	3	IV	2.5	III	37.06	IV		
											1	3	IV	2	III	69.2	IV		
45	Isopropanol	67-63-0	Alcohol	Solvent; Aerosol formulations (ingredient)	liquid	99.9	undiluted	2A	n.a.	1	2A	2	III	1.6	III	23.3	III	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seems based on results of one lab only
											1	0.7	II	2.7	IV	72	IV		
											1	3	IV	2.5	III	37.84	IV		
											2B	2.3	III	0.5	I	8.9	II		
46	Maneb	12427-38-2	Amine/Amidine, Organic salt, Urea compound	Pesticide	solid	90	neat	SCNM	2A	2B	NC	0	I	0.5	I	2.8	I	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by ICCVAM and by The Netherlands were unclear
											2A	1	II	2	III	33	IV		
											NC	0	I	0.5	I	8.03	II		
											2B	1	II	1	II	6.7	II		
47	Mercury (II) chloride	7487-94-7	Inorganic chloride	Antiseptic; Disinfectant	solid	99.5	undiluted	no raw data	n.a.	1	1	2	III	3.1	IV	55	IV	Prinsen and Koëter (1993)	-
48	2-Methoxyethanol	109-86-4	Alcohol	Solvent	liquid	99.9	undiluted	NC	n.a.	2A	2A	2	III	2	III	18	II/III	Prinsen and Koëter (1993)	*In vivo* classification derived from Gautheron et al. (1994)

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
49	Methyl acetate	79-20-9	Ester	Solvent; Chemical intermediate; Synthetic flavor ingredient	liquid	98	undiluted	2A	n.a.	1	2A	1.4	II	2.4	III	20.3	III	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seems based on results of one lab only
											1	1	II	2.7	IV	93	IV		
											2A	2	III	2	III	22.5	III		
											1	3	IV	3	IV	17.5	II/III		
50	Methyl cyanoacetate	105-34-0	Ester, Nitrile compound	Adhesive; Pharmaceutical intermediate	liquid	99	undiluted	2A	n.a.	2B	NC	0.4	I	0.3	I	4.5	I	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by ICCVAM and by The Netherlands were unclear
											2A	0.5	I	0.7	II	44	IV		
											NC	0.17	I	0.5	I	4.93	I		
											2B	1	II	1	II	10.7	II		
51	Methylcyclopentane	96-37-7	Hydrocarbon (cyclic)	Solvent	liquid	>99	undiluted	NC	n.a.	NC	NC	0.4	I	0.5	I	2.3	I	Balls et al. (1995)	-
											2B	1	II	0	I	22	III		
											NC	0	I	0.5	I	5.83	II		
											NC	1	II	0.5	I	0	I		
52	Methyl ethyl ketone	78-93-3	Ketone	Solvent; Manufacture of lacquers, varnishes, cosmetics, pharmaceuticals	liquid	99	undiluted	2A	n.a.	1	2A	2	III	2.2	III	23.1	III	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seemed based on results of one lab only
											1	2.7	IV	3	IV	99	IV		
											1	3	IV	2.33	III	34.88	IV		
											2A	3	IV	2	III	12.6	II/III		
53	Methyl isobutyl ketone	108-10-1	Ketone	Solvent; Synthetic flavor; Drycleaning	liquid	98	undiluted	NC	n.a.	2A	1/2A	2.6	IV	2	III	26.5	III/IV	Balls et al. (1995)	-
											1	3	IV	3	IV	64	IV		
											2A	2	III	2.17	III	21.69	III		
											2A	2	III	2	III	12.3	II/III		
54	1-Napthaleneacetic acid	86-87-3	Carboxylic acid, Polycyclic compound	Pesticide	solid	96	neat	1	n.a.	2B	2B	1	II	0.9	II	5.6	II	Balls et al. (1995)	-
											2B	1	II	1	II	24	III		
											2B	2	III	1	II	8.86	II		
											2A	1	II	1	II	46.7	IV		
55	1-Napthaleneacetic acid, sodium salt	61-31-4	Carboxylic acid (salt), Polycyclic compound	Pesticide	solid	95	neat	1	n.a.	1	1	3	IV	3	IV	46.6	IV	Balls et al. (1995)	-
											1	3	IV	2.7	IV	122	IV		
											1	3	IV	2.5	III	44.19	IV		
											1	3	IV	3	IV	64.1	IV		
56	n-Octanol	111-87-5	Alcohol	Solvent; Fragrance	liquid	>99	undiluted	2A/2B	n.a.	2A	2A	2	III	2.4	III	36.5	IV	Balls et al. (1995)	-
											2A	1.3	II	2	III	108	IV		
											2B	1.17	II	1.5	II	10.18	II		
											2A	2	III	1	II	25.7	III		
57	Parafluoraniline	371-40-4	Amine/Amidine	Intermediate for herbicides; Dyes	liquid	99	undiluted	SCNM	1/2	1	1	3	IV	2.2	III	35.3	IV	Balls et al. (1995)	-
											1	3	IV	2	III	79	IV		
											1	3	IV	2	III	33.44	IV		
											1	3	IV	2	III	38.5	IV		
58	Polyethylene glycol 400	25322-68-3	Alcohol, Polyether	Surfactant (nonionic), Lubricant, Plasticizer, Solvent	liquid	n.p.	undiluted	NC	n.a.	2B	2B	1.4	II	1	II	9.8	II	Balls et al. (1995) / Prinsen (2000)	*In vitro* classification proposed by The Netherlands seemed based on results of one lab (repeated twice, but in one experiment EU DSD R36 class. used)
											2B	0.2	I	0	I	26	III		
											2B	2	III	1	II	5.88	II		
											2B	1	II	0.5	I	14.8	II/III		

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
59	Potassium cyanate	590-28-3	Inorganic salt	Herbicide; Pharmaceutical intermdiate	solid	97	neat	SCNM	1/2	2B	2B	1	II	0.7	II	8	II	Balls et al. (1995)	-
											2B	0.2	I	0	I	25	III		
											2B	1.67	III	1	II	10.45	II		
											2A	1.3	II	1.7	III	25.3	III		
60	Promethazine HCl	58-33-3	Amine/Amidine, Heterocyclic, Organic sulfur compound	Antihistamine; Anti-nausea drug	solid	98	neat	1	n.a.	1	1	2.6	IV	1.6	III	33	IV	Balls et al. (1995)	-
											1	3	IV	3	IV	143	IV		
											2A	3	IV	2	III	23.02	III		
											1/2A	2	III	3	IV	28.6	III/IV		
61	Pyridine	110-86-1	Heterocyclic	Solvent; Intermediate for pharmaceuticals, dyes, pesticides	liquid	>99	undiluted	1/2A	n.a.	1	1	3	IV	2	III	32.7	IV	Balls et al. (1995)	-
											1	3	IV	3	IV	95	IV		
											1	3	IV	2.5	III	37.47	IV		
											1	3	IV	3	IV	78.6	IV		
62	Quinacrine	69-05-6	Amine/Amidine, Heterocyclic, Polycyclic compound	Anti-infective (anti-helmentic)	solid	n.p.	neat	1	n.a.	2B	2B	1.2	II	0.6	II	4.1	I	Balls et al. (1995)	The *in vitro* classification proposed by The Netherlands did not correspond to their proposed criteria
											NC	0.2	I	0.2	I	12	II		
											2A	2	III	2	III	11.49	II		
											2B	1	II	0.5	I	6.8	II		
63	Silver (I) nitrate	7761-88-8	Inorganic silver/nitrogen compound	Aniti-infective; Diagnostic agent	solid	99.5	3%	no raw data	n.a.	2B	2B	1	II	1	II	12	II	Prinsen and Koëter (1993)	-
64	Sodium dodecyl sulfate	151-21-3	Carboxylic acid (salt)	Surfactant (anionic), Detergent	solid	70	undiluted	no raw data	n.a.	1	2B	0.8	II	1	II	22	III	Prinsen and Koëter (1993)	The SSD reports 'loosening of corneal epithelium' in the ICE test (p.77), which justifies an *in vitro* cat 1 classification
65	Sodium fluorescein	518-47-8	Polycyclic	Stain; Dye	liquid	70	20%	no raw data	n.a.	NC	NC	0.1	I	0	I	0	I	Prinsen and Koëter (1993)	-
66	Sodium hydroxide	1310-73-2	Alkali	Caustic agent	liquid	97	10% (*)	1	n.a.	1	1	3	IV	3	IV	60	IV	Prinsen and Koëter (1993)	Mistake inICCVAM BRD reported concentration tested in Prinsen & Koeter (10% is the correct concentration & not 1% as stated in ICCVAM BRD)
67	Sodium hydroxide (1%)	1310-73-2	Alkali	Caustic agent	liquid	RG	1%	2B	n.a.	2A	2B	1	II	0.6	II	14.1	II/III	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seemed based on results of one lab only
											2A	0.7	II	2.3	III	55	IV		
											2A	2.33	III	2.5	III	30.31	III/IV		
											2A	2	III	2	III	33.3	IV		
68	Sodium hydroxide (10%)	1310-73-2	Alkali	Caustic agent	liquid	RG	10%	1	n.a.	1	1	3	IV	4	IV	32	III/IV	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seemed based on results of one lab only
											1	3	IV	3.3	IV	194	IV		
											1	3	IV	3.17	IV	68.86	IV		
											1	3	IV	4	IV	151.7	IV		
69	Sodium lauryl sulfate (3%)	151-21-3	Carboxylic acid (salt)	Surfactant (anionic), Detergent	liquid	98	3%	NC	n.a.	2B	NC	1	II	0.2	I	3.9	I	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seemed based on results of one lab only
											2A	0	I	0	I	39	IV		
											NC	1	II	0	I	2.75	I		
											2B	1	II	1	II	15.9	II/III		
70	Sodium lauryl sulfate (15%)	151-21-3	Carboxylic acid (salt)	Surfactant (anionic), Detergent	liquid	98	15%	1	n.a.	2B	2B	0.6	II	0.4	I	7	II	Balls et al. (1995)	-
											2A	1	II	0.2	I	33	IV		
											2B	1.67	III	1	II	9.56	II		
											2B	1	II	1	II	12.2	II/III		

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
71	Sodium oxalate	62-76-0	Carboxylic acid (salt)	Textile finishing; Pyrotechnic, Industrial byproduct	solid	>99	neat	1	n.a.	2B	2B	0.7	II	0.7	II	6.3	II	Balls et al. (1995)	-
											2B	0.2	I	0	I	24	III		
											NC	0.5	I	0.5	I	2.62	I		
											NC	1	II	0	I	2.4	I		
72	Sodium perborate, $4H_2O$	10486-00-7	Inorganic salt, Boron compound	Household cleaner; Detergent	solid	98.6	neat	1	n.a.	2B	NC	0.6	II	0.5	I	3.1	I	Balls et al. (1995)	-
											2B	0.2	I	0.7	II	23	III		
											2B	1.33	II	1	II	7.54	II		
											2B	1	II	0.5	I	14.6	II/III		
73	Tetraaminopyrimidine sulfate	5392-28-9	Amine/Amidine, Heterocyclic	Developer	solid	97	neat	NC	n.a.	2B	2B	1.3	II	1	II	7.5	II	Balls et al. (1995)	*In vivo* GHS NC in the ECETOC report, and *in vivo* GHS Cat 2A in the study from Gautheron et al. (1994). If data on the two sudies are combined, that would result on a GHS NC
											2A	1	II	2	III	31	III/IV		
											2B	1.5	II	1.5	II	7.3	II		
											2B	1	II	1	II	8.9	II		
74	TNO-01 (Formulation-1)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	0	I	Prinsen (1996)	-
75	TNO-02 (Formulation-2)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	2A	n.a.	2A	2A	2.7	IV	2	III	24	III	Prinsen (1996)	-
76	TNO-03 (Pesticide-1)	n.p.	Not classified	Pesticide	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.6	II	0.3	I	3	I	Prinsen (1996)	-
77	TNO-04 (Detergent-1)	n.p.	Not classified	Soaps; Surfactants	liquid	n.p.	undiluted	2A	n.a.	2B	2B	1.5	II	1.5	II	9	II	Prinsen (1996)	-
78	TNO-05 (Silicone powder-1)	n.p.	Not classified	Not classified	solid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	0	I	Prinsen (1996)	-
79	TNO-06 (Lubricant)	n.p.	Not classified	Not classified	gel	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	1	I	Prinsen (1996)	-
80	TNO-07 (Ink-1)	n.p.	Not classified	Dyes	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.8	II	0	I	2	I	Prinsen (1996)	-
81	TNO-08 (Ink-2)	n.p.	Not classified	Dyes	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.1	I	0	I	3	I	Prinsen (1996)	-
82	TNO-09 (Paint)	n.p.	Not classified	Paint	liquid	n.p.	undiluted	NC	n.a.	NC	NC	1.3	II	0.5	I	5	I	Prinsen (1996)	-
83	TNO-10 (Silicone powder-2)	n.p.	Not classified	Not classified	solid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	1	I	Prinsen (1996)	-
84	TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	Hydrocarbon; Acid	Industrial chemical	solid	n.p.	undiluted	2A	n.a.	2A	2A	2	III	1.3	II	19	III	Prinsen (1996)	-
85	TNO-12 (Formulation-3)	n.p.	Not classified	Not classified	paste	n.p.	undiluted	SCNM	1/2	2A	2A	2.5	III	2	III	35	IV	Prinsen (1996)	-
86	TNO-13 (Pesticide-2)	n.p.	Not classified	Pesticide	solid	n.p.	undiluted	NC	n.a.	NC	NC	0.7	II	0	I	1	I	Prinsen (1996)	-
87	TNO-14 (Polydisaccharide)	n.p.	Carbohydrate	Not classified	liquid	n.p.	14.5%	NC	n.a.	NC	NC	0.3	I	0	I	2	I	Prinsen (1996)	-
88	TNO-15 (Polydisaccharide)	n.p.	Carbohydrate	Not classified	liquid	n.p.	50%	NC	n.a.	NC	NC	0	I	0	I	2	I	Prinsen (1996)	-
89	TNO-16 (Liquid nylon product)	n.p.	Not classified	Industrial formulation	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	1	I	Prinsen (1996)	-
90	TNO-17 (Solvent-1)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.3	I	0	I	0	I	Prinsen (1996)	-
91	TNO-18 (Solvent-2)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	0	I	Prinsen (1996)	-
92	TNO-19 (Solvent-3)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	0	I	Prinsen (1996)	-
93	TNO-20 (Solvent-4)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.5	I	0.3	I	3	I	Prinsen (1996)	-
94	TNO-21 (Solvent-5)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.3	I	0.3	I	0	I	Prinsen (1996)	-
95	TNO-22 (Solvent-6)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.2	I	0.3	I	0	I	Prinsen (1996)	-
96	TNO-23 (Solvent-7)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.2	I	0	I	2	I	Prinsen (1996)	-
97	TNO-24 (Solvent-8)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.2	I	0	I	3	I	Prinsen (1996)	-
98	TNO-25 (Solvent-9)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	1	I	Prinsen (1996)	-
99	TNO-26 (Ink-3)	n.p.	Not classified	Dyes	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.1	I	0	I	0	I	Prinsen (1996)	-

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100	TNO-27 (Thermal paper coating-1)	n.p.	Not classified	Industrial chemical	liquid	n.p.	undiluted	2B	n.a.	2B	2B	1	II	0.6	II	9	II	Prinsen (1996)	-
101	TNO-28 (Toilet cleaner-1)	n.p.	Not classified	Household cleaner	liquid	n.p.	undiluted	1/2A	n.a.	2B	2B	1.4	II	0.8	II	12	II	Prinsen (1996)	-
102	TNO-29 (Toilet cleaner-2)	n.p.	Not classified	Household cleaner	liquid	n.p.	undiluted	2A	n.a.	2B	2B	1.3	II	1	II	11	II	Prinsen (1996)	-
103	TNO-30 (Pesticide-3)	n.p.	Not classified	Pesticide	solid	n.p.	undiluted	NC	n.a.	2B	2B	1.5	II	1	II	7	II	Prinsen (1996)	-
104	TNO-31 (Sulfur)	7704-34-9	Inorganic chemical	Industrial chemical	solid	n.p.	undiluted	NC	n.a.	NC	NC	0.2	I	0	I	1	I	Prinsen (1996)	-
105	TNO-32 (Ink-4)	n.p.	Not classified	Dyes	liquid	n.p.	undiluted	NC	n.a.	2B	2B	1	II	0.5	I	7	II	Prinsen (1996)	The *in vitro* classification proposed by The Netherlands did not correspond to their proposed criteria
106	TNO-33 (Thermal paper coating-2)	n.p.	Not classified	Industrial chemical	liquid	n.p.	undiluted	NC	n.a.	2B	2B	2	III	0.5	I	5	I	Prinsen (1996)	The *in vitro* classification proposed by The Netherlands did not correspond to their proposed criteria
107	TNO-34 (Detergent-2)	n.p.	Not classified	Soaps; Surfactants	liquid	n.p.	undiluted	SCNM	1/2	2B	2B	1	II	1	II	25	III	Prinsen (1996)	The rationale for the *in vitro* classification proposed by ICCVAM and by The Netherlands were unclear
108	TNO-35 (Propyl-lactate)	616-09-1	Acid	Food additive; Solvent	liquid	n.p.	undiluted	1	n.a.	1	1	3	IV	3	IV	45	IV	Prinsen (1996)	-
109	TNO-36 (Ethylhexyl lactate)	6283-86-9	Acid; Ester	Solvent; Wetting agent	liquid	n.p.	undiluted	SCNM	2	2A	2A	2	III	2	III	18	II/III	Prinsen (1996)	-
110	TNO-37 (Pesticide-4)	n.p.	Not classified	Pesticide	solid	n.p.	undiluted	2B	n.a.	2B	2B	1.5	II	1	II	15	II/III	Prinsen (1996)	-
111	TNO-38 (Solvent-10)	n.p.	Not classified	Solvent	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	3	I	Prinsen (1996)	-
112	TNO-39 (Detergent-3)	n.p.	Not classified	Soaps; Surfactants	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.5	I	0.5	I	4	I	Prinsen (1996)	-
113	TNO-40 (Glycolbromoacetate form.)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	no raw data	n.a.	1	1	2.6	IV	1.9	III	41	IV	Prinsen (1996)	-
114	TNO-41 (Amidosulfonic acid)	5329-14-6	Acid	Herbicide; Flame retardant; Metal cleaning; Acid dye	solid	n.p.	undiluted	no raw data	n.a.	1	1	2.7	IV	4	IV	46	IV	Prinsen (1996)	-
115	TNO-42 (Glycolbromoacetate)	3785-34-0	Acetate	Not classified	liquid	n.p.	85%	no raw data	n.a.	1	1	3	IV	3	IV	36	IV	Prinsen (1996)	-
116	TNO-43 (Monobromoacetic acid)	79-08-3	Acid	Chlorination byproduct	solid	n.p.	undiluted	no raw data	n.a.	1	1	3	IV	4	IV	80	IV	Prinsen (1996)	-
117	TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	Not classified	Household cleaner (disinfectant)	liquid	n.p.	23%	n.a.	n.a.	1	1	3	IV	3.5	IV	39	IV	Prinsen (1996)	*In vivo* Eye Cat 1 classification derived from skin corrosion test
118	TNO-45 (Aqueous framing solution)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	NC	n.a.	NC	NC	1	II	0.5	I	5	I	Prinsen (2005)	-
119	TNO-46 (Raw material powder)	n.p.	Not classified	Raw material	solid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	1	I	Prinsen (2005)	-
120	TNO-47 (Ferro powder)	n.p.	Not classified	Not classified	solid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	1	I	Prinsen (2005)	-
121	TNO-48 (Corrosion inhibitor liquid)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	n.a.	n.a.	2A	2A	3	IV	1	II	25	III	Prinsen (2005)	*In vivo* Eye Cat 1 classification derived from skin corrosion test
122	TNO-49 (Wood impregnator liquid)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	n.a.	n.a.	1	1	3	IV	4	IV	n.p.	n.p.	Prinsen (2005)	*In vivo* classification derived from skin corrosion test
123	TNO-50 (Sodium hypochlorite-containing formulation)	n.p.	Not classified	Disinfectant	n.p.	n.p.	undiluted	n.a.	n.a.	1	1	3	IV	3	IV	41.1	IV	Prinsen (2005)	*In vivo* Eye Cat 1 classification derived from skin corrosion test
124	TNO-51 (Disinfectant)	n.p.	Not classified	Disinfectant	n.p.	n.p.	undiluted	n.a.	n.a.	1	1	3	IV	3	IV	33.9	IV	Prinsen (2005)	*In vivo* Eye Cat 1 classification derived from skin corrosion test
125	TNO-52 (Pesticide liquid)	n.p.	Not classified	Pesticide	liquid	n.p.	undiluted	2A	n.a.	2B	2B	1.7	III	1	II	5	I	Prinsen (2005)	-
126	TNO-53 (Ink formulation)	n.p.	Not classified	Dyes	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.5	I	0.2	I	3	I	Prinsen (2005)	-

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
127	TNO-54 (Raw material powder)	n.p.	Not classified	Raw material	solid	n.p.	undiluted	2B	n.a.	2B	2B	1	II	1	II	9	II	Prinsen (2005)	-
128	TNO-55 (Elastomer liquid)	n.p.	Not classified	Elastomer	liquid	n.p.	undiluted	2A	n.a.	2B	2B	1.7	III	1.3	II	10	II	Prinsen (2005)	-
129	TNO-56 (Elastomer liquid)	n.p.	Not classified	Elastomer	liquid	n.p.	undiluted	2B	n.a.	2B	2B	2	III	1.3	II	10	II	Prinsen (2005)	-
130	TNO-57 (Epoxy resin liquid)	n.p.	Not classified	Resin	liquid	n.p.	undiluted	NC	n.a.	2B	2B	1.5	II	1.3	II	12	II	Prinsen (2005)	-
131	TNO-58 (Styrene resin powder)	n.p.	Not classified	Resin	solid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	-1	I	Prinsen (2005)	-
132	TNO-59 (Ferro powder)	n.p.	Not classified	Not classified	solid	n.p.	undiluted	NC	n.a.	NC	NC	0.2	I	0	I	-2	I	Prinsen (2005)	-
133	TNO-60 (Fungicide paint)	n.p.	Not classified	Paint	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.2	I	0.5	I	1	I	Prinsen (2005)	-
134	TNO-61 (Silver thiosulfate liquid)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	1	I	Prinsen (2005)	-
135	TNO-62 (Lactate liquid)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	NC	n.a.	2B	2B	2	III	1	II	12	II	Prinsen (2005)	-
136	TNO-63 (Copolymer powder)	n.p.	Not classified	Copolymer	solid	n.p.	undiluted	NC	n.a.	NC	NC	0.3	I	0.5	I	3	I	Prinsen (2005)	-
137	TNO-64 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	emulsion	n.p.	undiluted	NC	n.a.	2B	2B	1	II	1	II	5	I	Prinsen (2005)	-
138	TNO-65 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	emulsion	n.p.	undiluted	NC	n.a.	NC	NC	0.7	II	0.5	I	4	I	Prinsen (2005)	-
139	TNO-66 (Raw material powder)	n.p.	Not classified	Raw material	solid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	0	I	Prinsen (2005)	-
140	TNO-67 (Ink formulation)	n.p.	Not classified	Dyes	liquid	n.p.	undiluted	NC	n.a.	2B	2B	1	II	1	II	6	II	Prinsen (2005)	-
141	TNO-68 (Cleaning product)	n.p.	Not classified	Cleaner	liquid	n.p.	undiluted	2A	n.a.	2B	2B	1/2	II/III	1	II	8	II	Prinsen (2005)	The rationale for the *in vitro* classification proposed by ICCVAM and by The Netherlands were unclear
142	TNO-69 (Cleaning product)	n.p.	Not classified	Not classified	liquid	n.p.	50%	NC	n.a.	NC	NC	1	II	0	I	0	I	Prinsen (2005)	-
143	TNO-70 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	emulsion	n.p.	undiluted	2A	n.a.	2A	2A	2	III	1	II	20	III	Prinsen (2005)	-
144	TNO-71 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	emulsion	n.p.	undiluted	NC	n.a.	2B	2B	1	II	0.5	I	13	II/III	Prinsen (2005)	-
145	TNO-72 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	emulsion	n.p.	undiluted	NC	n.a.	NC	NC	1.5	II	0.5	I	5	I	Prinsen (2005)	-
146	TNO-73 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	emulsion	n.p.	undiluted	2A	1	1	1	2.7	IV	2	III	18	II/III	Prinsen (2005)	The ICE test showed 'loosening of corneal epithelium' which justifies an *in vitro* Cat. 1 classification
147	TNO-74 (Raw material powder)	n.p.	Not classified	Raw material	solid	n.p.	undiluted	NC	n.a.	NC	NC	0.5	I	0	I	0	I	Prinsen (2005)	-
148	TNO-75 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	2	I	Prinsen (2005)	-
149	TNO-76 (Ferro powder)	n.p.	Not classified	Not classified	solid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	2	I	Prinsen (2005)	-
150	TNO-77 (Raw material liquid)	n.p.	Not classified	Raw material	liquid	n.p.	undiluted	NC	n.a.	2B	2B	1	II	0.5	I	7	II	Prinsen (2005)	-
151	TNO-78 (Raw material liquid)	n.p.	Not classified	Raw material	liquid	n.p.	undiluted	2B	n.a.	2B	2B	1.3	II	1	II	15	II/III	Prinsen (2005)	-
152	TNO-79 (Silicon resin powder)	n.p.	Not classified	Silicone resin	solid	n.p.	undiluted	NC	n.a.	2B	2B	1	II	1	II	10	II	Prinsen (2005)	-
153	TNO-80 (Raw material powder)	n.p.	Not classified	Raw material	solid	n.p.	undiluted	NC	n.a.	NC	NC	0.3	I	0	I	-1	I	Prinsen (2005)	-
154	TNO-81 (Surfactant liquid)	n.p.	Not classified	Soaps; Surfactants	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	1	I	Prinsen (2005)	-
155	TNO-82 (Surfactant liquid)	n.p.	Not classified	Soaps; Surfactants	n.p.	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	-2	I	Prinsen (2005)	-
156	TNO-83 (Surfactant liquid)	n.p.	Not classified	Soaps; Surfactants	n.p.	n.p.	undiluted	2B	n.a.	2B	2B	0.8	II	0.7	II	10	II	Prinsen (2005)	-

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
157	TNO-84 (Surfactant liquid)	n.p.	Not classified	Soaps; Surfactants	n.p.	n.p.	undiluted	NC	n.a.	2B	2B	0.7	II	0.7	II	2	I	Prinsen (2005)	-
158	TNO-85 (Surfactant liquid)	n.p.	Not classified	Soaps; Surfactants	n.p.	n.p.	undiluted	1	n.a.	2A/2B	2A/2B	2	III	1.3	II	14	II/III	Prinsen (2005)	-
159	TNO-86 (Surfactant liquid)	n.p.	Not classified	Soaps; Surfactants	n.p.	n.p.	undiluted	SCNM	NC	2B	2B	1	II	1	II	7	II	Prinsen (2005)	Chemical considered GHS NC by both ICCVAM and The Netherlands, but not considered in the present calculations as SCNM
160	TNO-87 (Enzyme liquid)	n.p.	Not classified	Enzyme solution	liquid	n.p.	undiluted	NC	n.a.	2B	2B	0.7	II	1	II	1	I	Prinsen (2005)	-
161	TNO-88 (Miscellaneous liquid)	n.p.	Not classified	Not classified	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0.3	I	0.7	II	3	I	Prinsen (2005)	-
162	TNO-89 (Ferro powder)	n.p.	Not classified	Not classified	solid	n.p.	undiluted	NC	n.a.	NC	NC	0.2	I	0.7	II	1	I	Prinsen (2005)	-
163	TNO-90 (Enzyme solution)	n.p.	Not classified	Enzyme solution	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0	I	2	I	Prinsen (2005)	-
164	TNO-91 (Enzyme solution)	n.p.	Not classified	Enzyme solution	liquid	n.p.	undiluted	NC	n.a.	NC	NC	0	I	0.2	I	1	I	Prinsen (2005)	-
165	TNO-92 (raw material solid)	n.p.	Not classified	Raw material	solid	n.p.	undiluted	SCNM	1	2A/2B	2A/2B	0.8	II	1.7	III	16	II/III	Prinsen (2005)	-
166	TNO-93 (Antifouling paint)	n.p.	Not classified	Paint	emulsion	n.p.	undiluted	SCNM	1	2A	2A	3	IV	2	III	17	II/III	Prinsen (2005)	-
167	TNO-94 (anti-fouling paint)	n.p.	Not classified	Paint	liquid	n.p.	undiluted	1	n.a.	NC	NC	1	II	0.5	I	2	I	Prinsen (2005)	-
168	Toluene	108-88-3	Hydrocarbon (cyclic)	Solvent; Gasoline additive; Manufacture of benzene derivatives, medicines, dyes, perfumes	liquid	99	undiluted	NC	n.a.	2A	2B	1.4	II	1	II	5.2	II	Balls et al. (1995)	*In vivo* GHS Cat 2 in study from TSCA database, but when combined to EC/HO study: GHS NC . The *in vitro* classification proposed by The Netherlands seemed based on results of one lab only
											2A	2	III	1.3	II	29	III/IV		
											2A/2B	1.33	II	2	III	13.87	II/III		
											2A	1	II	2	III	58.2	IV		
169	Toluene	108-88-3	Hydrocarbon (cyclic)	Solvent; Gasoline additive; Manufacture of benzene derivatives, medicines, dyes, perfumes	liquid	99.9	undiluted	NC	n.a.	2B	2B	1.1	II	1.4	II	4	I	Prinsen and Koëter (1993)	*In vivo* GHS Cat 2 in study from TSCA database, but when combined to EC/HO study: GHS NC
170	Triacetin	102-76-1	Lipid	Anti-fungal	liquid	99	undiluted	NC	n.a.	NC	NC	0.5	I	0.4	I	4	I	Prinsen and Koëter (1993)	-
171	Tributyltin chloride	1461-22-9	Organometallic compound, Heavy metal	Pesticide; Preservative	liquid	96	undiluted	no raw data	n.a.	1	1	3	IV	2.5	III	48	IV	Prinsen and Koëter (1993)	-
172	Trichloroacetic acid (3%)	76-03-9	Carboxylic acid	Caustic agent; Fixative; Herbicide	liquid	RG	3%	NC	n.a.	2A	2A/2B	2.4	III	1.2	II	13.2	II/III	Balls et al. (1995)	*The in vitro* classification proposed by The Netherlands seemed based on results of one lab only
											2A	2.3	III	2	III	38	IV		
											2A	1.5	II	2.5	III	27.88	III/IV		
											2A	1.7	III	2	III	26.4	III/IV		
173	Trichloroacetic acid (30%)	76-03-9	Carboxylic acid	Caustic agent; Fixative; Herbicide	liquid	RG	30%	1	n.a.	1	1	3	IV	4	IV	32	III/IV	Balls et al. (1995)	-
											1	3	IV	4	IV	153	IV		
											(b)	(b)	(b)	4	IV	(b)	(b)		
											1	3	IV	4	IV	(b)	(b)		
174	Triethanolamine	102-71-6	Amine/Amidine, Alcohol	Cleaner; Cosmetic ingredient; Intermediate for waxes, cutting oils	liquid	99	undiluted	NC	n.a.	2B	2B	0.9	II	0.7	II	4	I	Prinsen and Koëter (1993)	The *in vitro* classification proposed by The Netherlands did not correspond to their proposed criteria
175	Triton X-100 (5%)	9002-93-1	Polyether	Surfactant (nonionic), Detergent, Emulsifier	liquid	98	5%	2A	n.a.	2A	2B	1	II	0.6	II	9.8	II	Balls et al. (1995)	-
											2A	1.3	II	0	I	38	IV		
											2B	2	III	0	I	3.97	I		
											2A	1	II	2	III	39.6	IV		

No	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	*In Vivo* GHS Cat. based on expert judgement	Overall *In Vitro* ICE (GHS)	*In Vitro* ICE (GHS)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
176	Triton X-100 (10%)	9002-93-1	Polyether	Surfactant (nonionic), Detergent, Emulsifier	liquid	98	10%	1	n.a.	2B	2B	1.4	II	0.1	I	9.9	II	Balls et al. (1995)	-
											2A/2B	1	II	0.8	II	29	III/IV		
											2A	2.67	IV	1.17	II	20.2	III		
											2B	1.7	III	1	II	11.2	II		
177	Triton X-500 (5%)	n.p.	Polyether	Surfactant (nonionic), Detergent, Emulsifier	liquid	n.p.	undiluted	2A	n.a.	2B	2B	1	II	0.7	II	14	II/III	Prinsen (2000)	*In vivo* classification proposed by ICCVAM was unclear.
											2B	1	II	0.7	II	14	II/III		
											2B	1	II	0.7	II	13	II/III		
											2B	1	II	0.8	II	8	II		
											2B	1	II	0.7	II	11	II		
178	Tween 20	9005-64-5	Ester, Polyether	Surfactant (nonionic), Detergent	liquid	98	undiluted	NC	n.a.	2B	2B	1	II	1	II	3.6	I	Balls et al. (1995)	-
											2B	0.2	I	0	I	31	III/IV		
											2B	2.5	III	1	II	5.63	II		
											2B	1	II	0.5	I	6.7	II		

SCNM - Study criteria not met
SC - Classification assigned on the basis of skin corrosion assay
n.a.= not applicable
n.p.= not provided
RG = reagent Grade

(a) combination of effects not defined by ICCVAM and OECD GD 160 proposed criteria for classification
(b) solubility uncertain
RG = reagent Grade

APPENDIX 2

Examples of chemicals from the original validation dataset found to have discrepancies in *In Vitro* Classifications

Extract from Appendix C2 of ICE BRD from March 2006
http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd_ice.htm

In vitro classification per laboratory (GHS) and overall *in vitro* classification (GHS)

4- Carboxybenzaldehyde (*in vivo* GHS Cat 2A)

NI	NI
1	
NI	
2A/2B	

Ethyl-2-methylacetoacetate (*in vivo* GHS Cat 2B)

NI	2B
2B	
2B	
NI	

Fomesafen (*in vivo* SCNM)

2B	2B
NI	
NI	
2B	

Maneb (*in vivo* GHS Cat 2A based on expert judgement)

NI	NI
2A	
NI	
2B	

Methyl cyanoacetate (*in vivo* GHS Cat 2A)

NI	NI
2A	
NI	
2B	

Sodim lauryl sulfate (3%) (*in vivo* GHS NC)

NI	2B
2B	
NI	
2B	

Sodium oxalate (*in vivo* GHS Cat 1)

2B	2B
2B	
NI	
NI	

APPENDIX 3

ISSUE PAPER

Use of the Isolated Chicken Eye (ICE) Test Method (OECD TG 438) to Identify Substances Not Requiring Classification for Serious Eye Damage / Eye Irritation According to UN GHS

Prepared by:

Chantra Eskes
Services & Consultation on Alternative Methods (SeCAM)

For the:

Organisation for Economic Co-operation and Development (OECD)

16 November 2012 – updated on 28 March 2013

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A. Background

1. Comments received from The Netherlands regarding the update of TG 437 (Document ENV/JM/TG/RD(2012)2 from 15 May 2012[1]) proposed to include the ICE test method for the same purposes as the BCOP (i.e., identification of chemicals not requiring classification), on basis of the information from the expert Panel and added information provided by the developer.

2. A Streamlined Summary Document (SSD) dated of 7 March 2012 (Document n. ENV/JM/TG(2012)20) was submitted supporting revisions of TG 438, and in particular regarding the enlargement of the applicability domain of the ICE test method to identify chemicals not requiring a classification for serious eye damage and eye irritation under the UN GHS classification system.

3. The SSD provides a revised evaluation of the *in vivo* and *in vitro* data as presented in the ICCVAM Retrospective evaluation carried out from 2003 to 2006 for the identification of substances causing serious eye damage and from 2006 to 2009 for the identification of other ranges of ocular toxicity. The re-evaluation of the data was made with the focus on filling in the *in vivo* data gaps and to discuss the relevance of certain *in vivo* classifications of substances on the basis of their physico-chemical properties (solids) and on the basis of the individual *in vivo* reactions.

4. Based on the SSD, a revised TG 438 was submitted proposing the enlargement of the applicability domain of the ICE test method to identify chemicals not requiring a classification

[1] Document ENV/JM/TG/RD(2012)2 from 15 May 2012 named "Comments received on documents already posted on the meeting site regarding the 24th Meeting of the Working Group of National Coordinators of the Test Guidelines Programme (WNT)" held on 24-27 April 2012 at the OECD Headquarters, Paris, France.

for serious eye damage and eye irritation under the UN GHS classification system. Comments received on the revised TG 438 were also taken into consideration when drafting the present issue paper.

B. Initial Considerations

5. An independent evaluation was carried out to review and assess the proposal to include in the TG 438 the use of the ICE test method to identify chemicals not requiring a classification for serious eye damage and eye irritation under the UN GHS classification system.

6. In view of the recent updates of TG 430, 431 and 439 to mention only to the GHS Classification System, the present evaluation focused on the evaluation of the ICE test according to the Globally Harmonized System (GHS) of Classification as recommended by the United Nations. Furthermore, it focused on the performances of the ICE test method to identify chemicals not requiring a classification for eye irritation, i.e., to be used as a initial step of a bottom-up approach according to the strategies proposed by Scott and co-workers[2].

7. The following documents were considered and reviewed in the present evaluation:
- The *in vivo* and *in vitro* classifications presented in the SSD dated of 7 March 2012 (Document n. ENV/JM/TG(2012)20)
- The *in vitro* data presented in Appendix B2 of the ICCVAM Background Review Document on the ICE test method from March/April 2009[3].
- The *in vivo* data presented in Appendix C2 of the ICCVAM Background Review Document on the ICE test method from March/April 2009[4].

C. ICE Data Interpretation Procedures

8. Before initiating the present evaluation, a review was carried out on the Data Interpretation Procedures (DIP) recommended for the ICE test method to derive GHS *in vitro* classifications from the individual *in vitro* data. For this purpose, the following documents were considered: OECD TG 438, OECD GD 160, revised OECD TG 438 from 14.09.2012, ICCVAM BRDs and Invittox protocol n. 80 on the ICE test method.

9. In general, the criteria used to apply ICE classes (I to IV) based on mean corneal swelling, mean opacity and mean fluorescein retention appeared to be the same across different documents evaluated. These are reported in Annex 1 as extracted from the OECD TG 438 adopted on 7 September 2009.

10. In contrast, the overall ICE Classification criteria used to apply classification categories, appeared to differ, in particularly in what regards the identification of GHS non-classified materials and GHS Cat. 2 materials. Table 1 summarizes the main criteria recommended

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[2] Scott L, Eskes C, Hoffman S, Adriaens E, Alepee N, Bufo M, Clothier R, Facchini D, Faller C, Guest R, Hamernik K, Harbell J, Hartung T, Kamp H, Le Varlet B, Meloni M, Mcnamee P, Osborn R, Pape W, Pfannenbecker U, Prinsen M, Seaman C, Spielmann H, Stokes W, Trouba K, Vassallo M, Van den Berghe C, Van Goethem F, Vinardell P, Zuang V (2010). A proposed Eye Irritation Testing Strategy to Reduce and Replace in vivo Studies Using Bottom-up and Top-down Approaches. *Toxicology In Vitro* **24**, 1-9.

[3] ICCVAM – NICEATM (2009). Draft Background Review Document - Current Status of In vitro Test Methods for Identifying Mild/Moderate Ocular Irritants: The Isolated Chicken Eye (ICE) Test Method. National Institute of Environmental Health Sciences, Resesarch Triangle Park, North Carolina, USA.

[4] ICCVAM – NICEATM (2009). Draft Background Review Document - Current Status of In vitro Test Methods for Identifying Mild/Moderate Ocular Irritants: The Isolated Chicken Eye (ICE) Test Method. National Institute of Environmental Health Sciences, Resesarch Triangle Park, North Carolina, USA.

depending upon the document considered, where the main differences observed are highlighted in red. Regarding the Invittox protocol 80, no classification criteria were found for the GHS classification, but only for the EU DSD classification as well as for the general irritancy effects (see annex 2).

11. Due to the fact that such differences in Data Interpretation Procedures may have an impact on the final *in vitro* classifications and ensuing predictive capacity of the ICE test method, the three DIPs as shown in Table 1 were considered in the present evaluation.

Table 1: Overview of the criteria proposed for *in vitro* GHS classifications. Highlighted in red are the main differences observed.

GHS classification	OECD GD 160 & ICCVAM BRD from 1.04.2009	Revised OECD TG 438 from 14.09.2012	Suggested
NC	3xI 2xI, 1xII	3xI 2xI, 1xII 2xII, 1xI	3xI 2xI, 1xII
Cat. 2B	3xII 2xII, IxI 2xII, 1xIII 2xI, 1xIV 1xI, 1xII, 1xIII	Not specified, but assumed as all other categories not stated in the NC and Cat 1 classifications	3xII 2xII, IxI 2xII, 1xIII 2xI, 1xIII* 1xI, 1xII, 1xIII
Cat. 2A	3xIII 2xIII, 1xII 2xIII, 1xIV 2xIII, 1xI 2xII, 1xIV 1xII, 1xIII, 1xIV		3xIII 2xIII, 1xII 2xIII, 1xIV 2xIII, 1xI 2xII, 1xIV 1xII, 1xIII, 1xIV 2xI, 1xIV* 1xI, 1xII, 1xIV* 1xI, 1xIII, 1xIV*
Cat. 1	3xIV 2xIV, 1XIII 2xIV, 1XII 2xIV, 1XI	3xIV 2xIV, 1XIII 2xIV, 1XII 2xIV, 1XI CO ≥ 3 at 30 min (in at least 2 eyes) CO = 4 at any time point (in at least 2 eyes) Severe loosening of the epithelium (in at least 1 eye)	3xIV 2xIV, 1XIII 2xIV, 1XII 2xIV, 1XI CO ≥ 3 at 30 min (in at least 2 eyes) CO = 4 at any time point (in at least 2 eyes) Severe loosening of the epithelium (in at least 1 eye)

* Criteria not comprised in any evaluated document, perhaps due to the fact that these are combinations less likely to occur. However, as in the present evaluation such combinations did occur, it was proposed to allocate them to specific GHS classification categories.

D. *In vitro* ICE data and derived GHS classification

12. The detailed individual *in vitro* data as reported in appendix B2 from the ICCVAM BRD from March/April 2009 were compiled and scores assigned based on the criteria described in annex 1 of the present document (see Table in annex 3 – columns 17 to 22).

13. The ensuing GHS classification derived from the *in vitro* scores were then derived for individual laboratories as described below (see table in annex 3 - columns 11 to 13).

- Table in annex 3 column "*In vitro* ICE (ICCVAM BRD – criteria OECD GD160)": reports the *in vitro* GHS classifications as reported in appendix B2 of the ICCVAM BRD. In case of discordant *in vitro* GHS classifications to the criteria suggested in the OECD GD 160 and in the ICCVAM BRD (see table 1 column "OECD GD 160 & ICCVAM BRD"), cells were highlighted in orange and an explanatory text added in the last column 'Notes';
- Table in annex 3 column "*In Vitro* ICE (revised criteria on TG 438)": reports the *in vitro* GHS classifications derived from the criteria proposed in the revised OECD TG 438 from Sept 2012 (table 1 column "Revised OECD TG 438");
- Table in annex 3 column "*In Vitro* ICE (suggested)": reports the *in vitro* GHS classifications derived from the suggested criteria (table 1 column "Suggested").

14. For those chemicals tested in more than one laboratory, the final GHS classification for each chemical was proposed based on the classifications obtained in the various laboratories (see table in annex 3 columns 14-16).

- Table in annex 3 column "Overall *In Vitro* ICE (reported in ICCVAM BRD)": reports the GHS classification proposed for each chemical in appendix B2 of the ICCVAM BRD. In case these were found to be discordant to the findings retrieved by the various laboratories, cells were highlighted in orange and an explanatory text added in the last column 'Notes';
- Table in annex 3 column "Overall *In Vitro* ICE (reported in SSD)": reports the GHS classification proposed for each chemical in annex 1 of the Draft SSD from 7 March 2012 (Document ENV/JM/TG(2012)20). In case these were found to be discordant to the findings retrieved by the various laboratories, or different to the criteria proposed in the revised TG 438, cells were highlighted in orange and an explanatory text added in the last column 'Notes';
- Table in annex 3 column "Overall *In Vitro* ICE (suggested)": reports the GHS classification suggested for each chemical based on the results from the various laboratories using the criteria for classification as described in table 1 – column "suggested".

E. *In vivo* GHS classifications

15. While the current *in vivo* Draize eye irritation test method has been widely used, the test has often been reason for criticism. Besides the fact that the Draize test can be very painful to the rabbits, some of the drawbacks referred in literature include (Eskes, 2010[5]; see annex 4 for details):

- The ill-defined duration of exposure to the test material;
- The limited reproducibility within and between the laboratories that may be due to the ill-defined duration of exposure, but also to the subjectivity in the allocation of the respective scores and to the rabbits individual variability;
- The type of exposure which does not reflect a potential human accidental exposure;

[5] Eskes, C., 2010. Guidance Document on the Application of Alternative methods in the Regulatory Assessment of Chemical Safety Related to Human Eye Irritation and Severe Irritation: Current Status and Future Prospects. FOPH, 65 pp. Available at: http://www.bag.admin.ch/themen/chemikalien/00253/03225/12694/index.html?lang=fr. Accessed on 13.11.2010.

- The differences in physiology and sensitivity to tested substances between rabbit and human eyes;
- Ethical issues.

16. The proposal by the Netherlands to revise the *in vivo* GHS classifications obtained from the Draize rabbit eye test could allow to take into considerations such limitations and drawbacks of the Draize rabbit eye test. However, for doing so it would be helpful to establish agreed criteria on how to perform such an evaluation. Furthermore, such evaluation would benefit of being carried out on an independent manner without knowledge of the *in vitro* responses.

17.The present evaluation reported nevertheless the *in vivo* GHS classifications proposed both in the ICCVAM BRD and in the SSD as follows (see table in annex 3 - columns 7 & 8):

- Table in annex 3 column "*In Vivo* Draize GHS (reported by ICCVAM)": reports the *in vivo* GHS classifications for each chemical as described in appendix C2 of the ICCVAM BRD from 2009;
- Table in annex 3 column "*In Vivo* Draize GHS (reported in SSD)": reports the *in vivo* GHS classifications for each chemical as described in the Draft SSD from 7 March 2012 (Document ENV/JM/TG(2012)20).

18. In addition, a review of the *in vivo* GHS classifications was carried out based on the raw *in vivo* data published in the ECETOC report[6], on raw data shared by TNO and ICCVAM on the TNO chemicals, and on raw data shared by Philippe Van Parys and João Barroso on chemicals used within the study from Gautheron and co-workers[7]. Based on this review the following was reported (see table in annex 3 - column 9):

- Table in annex 3 column "*In Vivo* Draize GHS (suggested)": provides with a suggested *in vivo* Draize GHS classification based on the review of the raw *in vivo* data.

19. For those cases where Study Criteria were Not Met (SCNM) to be able to assign an *in vivo* GHS classification (e.g., incomplete dataset to assess reversibility / irreversibility of effects at day 21), but from which it was possible to estimate a classification with certain confidence (e.g., in case of a material that was found to be at least an Eye Cat 2 but no complete data was available on day 21 to assess reversibility / persistency of effects, a GHS Cat. 1/2 would be suggested), it was proposed to report it separately from the main *in vivo* classification as *In Vivo Classification based on Expert Judgement* (EJ) (see table in annex 3 - column 10):

- Table in annex 3 column "*In Vivo* Draize GHS (suggested based on expert judgment)": provides with a suggested *in vivo* Draize GHS classification based on expert judgment when applicable.

F. Predictive Capacities of the ICE test method for the identification of chemicals not requiring GHS classification

20. Based on the compiled dataset as shown in the table presented in annex 3 and taking into account the above considerations, the predictive capacities of the ICE test method to identify chemicals not requiring a classification for serious eye damage and eye irritation (i.e.,

[6] ECETOC, 1995. Technical Report No. 66. Skin Irritation and corrosion chemicals data bank. European Centre for Ecotoxicology and Toxicology of chemicals, 247 pp.

[7] Gautheron P, Giroux J, Cottin M, Audegond L, Morilla A, Mayordomo-Blanco L, Tortajada A, Haynes G, Vericat JA, Pirovano R, Tos EG, Hagemann C, Vanparys P, Deknudt G, Jacobs G, Prinsen M, Kalweit S, Spielmann H. (1994). Interlaboratory assessment of the bovine corneal opacity and permeability (BCOP) assay. Toxicol In Vitro 8(3):381-392.

to be used as a initial step of a bottom-up approach) were calculated and reported in table 2. The following data interpretations were considered:

- ICCVAM: based on the *in vitro* and *in vivo* data reported in the ICCVAM BRD from March/April 2009 (independent from any discordant results).
- ICCVAM-reviewed: as above but corrected in case of discordant *in vitro* GHS classifications and individual *in vitro* data (i.e., corrections were applied to chemicals n. 16, 19, 46, 50, 64, 107, 141). In case of corrections, the suggested *in vitro* GHS classifications as shown in column 13 from the table in annex 3 were used.
- SSD: based on the *in vitro* and *in vivo* data reported in the Draft SSD from 7 March 2012 (Document ENV/JM/TG(2012)20) (independent from any discordant results).
- SSD-reviewed: as above but corrected in case of: i) discordant *in vitro* GHS classifications and individual *in vitro* data using the criteria proposed in the revised OECD TG 438 from 14.9.2012 (i.e., corrections applied to chemicals n. 12, 36, 38, 62, 105, 106, 174); ii) proposed *in vitro* GHS classification based on results of one laboratory only (i.e., corrections applied to chemicals n. 17, 42, 45, 49, 52, 58, 67, 69, 168, 172); and iii) in case of unclear rationale for the derived *in vitro* classifications (i.e., corrections applied to chemicals n. 16, 19, 39, 46, 50, 107, 141). In case of corrections for i) the classifications based on the criteria proposed in the revised OECD TG 438 from 14.9.2012 were used, and for ii) and iii) the suggested *in vitro* GHS classifications as shown in column 13 from the table in annex 3 were used.
- Suggested-1: based on the *in vitro* and *in vivo* data suggested in the present evaluation following review of individual *in vitro* data and raw *in vivo* data. This evaluation does not comprehend materials for which Study Criteria were Not Met (SCNM) to assign an *in vivo* classification.
- Suggested-2: as Suggested-1 with 9 additional chemicals for which no raw data was available but the ICCVAM and SSD *in vivo* GHS classifications were identical (i.e., inclusion of chemicals n. 2, 9, 25, 66, 117, 121, 122, 123, 124).
- Suggested + Expert Judgment: as Suggested-2 with in addition all materials that had SCNM for *in vivo* GHS classification, but for which it was possible to estimate an *in vivo* GHS classification with certain confidence (i.e., chemicals n. 5, 30, 38, 46, 57, 59, 85, 107, 109, 135, 159, 165, 166).

Table 2: Predictive capacities of the ICE test methods *to identify chemicals not requiring a GHS classification for eye irritation* (i.e., to be used as a initial step of a bottom-up approach) according to different data interpretations

	Concordance	**Sensitivity**	**Specificity**	**False negatives**	**False positives**
ICCVAM	77.9% (116/149)	92.6% (63/68)	65.4% (53/81)	7.4% (5/68)	34.6% (28/81)
ICCVAM-reviewed	80.5% (120/149)	98.5% (66/67)	65.9% (54/82)	1.5% (1/67)	34.1% (28/82)
SSD	86.0% (153/178)	95.7% (88/92)	75.6% (65/86)	4.3% (4/92)	24.4% (21/86)
SSD-reviewed	86.0% (153/178)	96.7% (89/92)	74.4% (64/86)	3.3% (3/92)	25.6% (22/86)
Suggested-1	81.6% (115/141)	98.4% (61/62)	68.4% (54/79)	1.6% (1/62)	31.6% (25/79)
Suggested-2	82.7% (124/150)	98.6% (69/70)	68.7% (55/80)	1.4% (1/70)	31.3% (25/80)
Suggested + EJ	82.2% (134/163)	98.7% (79/80)	66.3% (55/83)	1.3% (1/80)	33.7% (28/83)

EJ=Expert Judgment

G. Misclassified substances

21. The under-predicted substances (identified as false negatives) are shown in table 3 according to the different data evaluation. Table 4 provides with the identity and overall *in vitro* and *in vivo* GHS classifications for each of these under-predicted substance, and table 5 shows the detailed *in vitro* data and ensuing *in vitro* GHS classifications.

Table 3: Substances identified as false negative the ICE test method depending upon the data evaluation carried out.

	Under-predicted substances (chemical n. referring to table in annex 3)
ICCVAM	16, 32, 46, 50, 167*
ICCVAM-reviewed	167*
SSD	16, 46, 50, 167*
SSD-reviewed	36, 62*, 167*
Suggested-1	167*
Suggested-2	167*
Suggested + EJ	167*

* *In vivo* GHS Cat. 1 materials.

22. Regarding the ICCVAM evaluation: substances 16, 46 and 50 were found to have discordant *in vitro* GHS classification as compared to the results obtained in the four laboratories. For example, for substance 16, the results from the 4 laboratories were: NI, 1, NI, 2A/2B, whereas ICCVAM reported an *in vitro* classification is 'NI'. It is believed that for such compound an *in vitro* classification of 2A/2B would be more appropriate. A similar reasoning can be made for compounds 46 and 50. Furthermore, regarding substance 32, according to the raw data from the study of Gautheron et al. (1994) (see annex 5), this substance appears to be an *in vivo* GHS non-classified substances. If corrected *in vitro* GHS classifications are applied, only one substances remains under-classified, i.e., substance 167.

23. Regarding the SSD evaluation: similar to the ICCVAM evaluation substances 16, 46 and 50 were found to have discordant *in vitro* GHS classification as compared to the results obtained in the four laboratories. Regarding substances 36 and 62, these are substances that would be *in vitro* NC if the criteria proposed in the revised OECD TG 438 is applied. This is of concern especially because substance 62 is an *in vivo* GHS Cat. 1.

24. Finally if the suggested classification is applied only one compound is found to be misclassified, which is compound 167, equivalent to TNO-94, a anti-fouling paint. According to comments received from The Netherlands regarding the update of TG 437[1] it is stated that: "*TNO-94 was an anti-fouling paint for the shipping industry, a specific type of product, which produced irritating but reversible eye effects in two out of three rabbits. In the third rabbit an unusual effect had occurred, i.e. adherence of the paint to the cornea which was reason to humanely sacrifice the animal on day 1. Anti-fouling paints are designed to be very durable and thus may explain this phenomenon observed in this rabbit. Whether or not this particular effect is relevant for humans, excluding (anti-fouling) paints from ICE testing would have no major consequences for the applicability of the method for screening of non-irritants in general*."

Table 4. *In vivo* and *in vitro* GHS classification for substances found to be under-classified in one or more data evaluation procedures. Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concen-tration Tested	*In Vivo* Draize GHS (reported by ICCVAM)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Revised ICCVAM	*In Vivo* Draize GHS (reported in SSD)	Overall *In Vitro* ICE (reported in SSD)	Revised SSD	*In Vivo* Draize GHS (suggested)	*In Vivo* Draize GHS (suggested based on expert judgement)	Overall *In Vitro* ICE (suggested)	Reference	Notes
16	4-Carboxybenzaldehyde	619-66-9	solid	95	neat	2A	NI	2A/2B	2A	NI	2	2A	n.a.	2A/2B	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by ICCVAM and in the SSD appears unclear
32	Dimethyl sulfoxide	67-68-5	liquid	99.9	undiluted	2B/n.p.	NI	n.a.	NI (2B)	NI	n.a.	NC	n.a.	NC	Prinsen and Koëter (1993)	Suggested *in vivo* classification derived from the study of Gautheron et al. (1994)
36	Ethyl-2-methylacetoacetate	609-14-3	liquid	97	undiluted	2B	2B	n.a.	2B	2B	NC	2B	n.a.	2B	Balls et al. (1995)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438
46	Maneb	12427-38-2	solid	90	neat	SCNM/2B	NI	2B	2B/2A (SCNM)	NI	2	SCNM	2A	2B	Balls et al. (1995)	The rationale for the in vitro classification proposed by ICCVAM and in the SSD appears unclear
50	Methyl cyanoacetate	105-34-0	liquid	99	undiluted	2A	NI	2B	2A	NI	2	2A	n.a.	2B	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by ICCVAM and in the SSD appears unclear
62	Quinacrine	69-05-6	solid	n.p.	neat	1	2B	n.a.	1	2B	NC	1	n.a.	2B	Balls et al. (1995)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438
167	TNO-94 (anti-fouling paint)	n.p.	liquid	n.p.	undiluted	1/n.p.	NI	n.a.	1	NI	n.a.	1	n.a.	NC	Prinsen (2005)	-

n.a.= not applicable; NC = not classified; NI = non-irritating; n.p.= not provided; SCNM - Study criteria not met.

Table 5. Detailed *in vitro* data for substances found to be under-classified in one or more data evaluation procedures. Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration Tested	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (suggested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference
16	4-Carboxybenzaldehyde	619-66-9	solid	95	neat	NI	NC	NC	1	I	0.5	I	5.4	II	Balls et al. (1995)
						1	1	1	1.3	II	3	IV	89	IV	Balls et al. (1995)
						NI	NC	NC	0.67	II	0.5	I	-1.4	I	Balls et al. (1995)
						2A/2B	2	2A/2B	2	III	1	II	12.7	II/III	Balls et al. (1995)
32	Dimethyl sulfoxide	67-68-5	liquid	99.9	undiluted	NI	NC	NC	1	II	0.5	I	4	I	Prinsen and Koëter (1993)
36	Ethyl-2-methylacetoacetate	609-14-3	liquid	97	undiluted	NI	NC	NC	0.4	I	0.3	I	-2.8	I	Balls et al. (1995)
						2B	NC	2B	1	II	0	I	7	II	Balls et al. (1995)
						2B	2	2B	0.67	II	1	II	11.52	II	Balls et al. (1995)
						NI	NC	NC	1	II	0.5	I	4.5	I	Balls et al. (1995)
46	Maneb	12427-38-2	solid	90	neat	NI	NC	NC	0	I	0.5	I	2.8	I	Balls et al. (1995)
						2A	2	2A	1	II	2	III	33	IV	Balls et al. (1995)
						NI	NC	NC	0	I	0.5	I	8.03	II	Balls et al. (1995)
						2B	2	2B	1	II	1	II	6.7	II	Balls et al. (1995)
50	Methyl cyanoacetate	105-34-0	liquid	99	undiluted	NI	NC	NC	0.4	I	0.3	I	4.5	I	Balls et al. (1995)
						2A [(a)]	2	2A	0.5	I	0.7	II	44	IV	Balls et al. (1995)
						NI	NC	NC	0.17	I	0.5	I	4.93	I	Balls et al. (1995)
						2B	2	2B	1	II	1	II	10.7	II	Balls et al. (1995)
62	Quinacrine	69-05-6	solid	n.p.	neat	2B	NC	2B	1.2	II	0.6	II	4.1	I	Balls et al. (1995)
						NI	NC	NC	0.2	I	0.2	I	12	II	Balls et al. (1995)
						2A	2	2A	2	III	2	III	11.49	II	Balls et al. (1995)
						2B	NC	2B	1	II	0.5	I	6.8	II	Balls et al. (1995)
167	TNO-94 (anti-fouling paint)	n.p.	liquid	n.p.	undiluted	NI	NC	NC	1	II	0.5	I	2	I	Prinsen (2005)

n.a.= not applicable; NC = not classified; NI = non-irritating; n.p.= not provided; SCNM - Study criteria not met.
(a) combination of effects not defined by ICCVAM and OECD GD 160 proposed criteria for classification.

H. Restriction of the applicability domain of the ICE test method

25. Suggestions received from The Netherlands regarding the update of TG 437[1] state in annex 9 that: "*As a concession, to avoid false negatives as much as possible (true or not by the so-called Golden Standard), the ICE criteria for classification as a non irritant could be set at a lower combination of effects (threshold), i.e. no corneal effect with two out of three parameters measured and, at maximum, a slight corneal effect with the third parameter, and 2) solids and paints could be excluded or tested additionally with a longer exposure period, although we keep strong reservations with the idea that the ICE should try to match the unrealistic exposure regimen of the Draize Eye test.*"

26. It is believed that solids do not present a major role in the under-classification of the ICE test method. However the exclusion of 'paints' from the applicability domain of the ICE test method for the identification of non-classified substances should be considered, until an optimized protocol for that purpose is developed. By 'paints' the test developer means: "anti-fouling sticky paints that contain organic solvents and biocides". Paints on a water basis do not fall within this limitation and may be tested in the ICE.

27. Based on such definition, if 'paints' are excluded from ICE's applicability domain, the following chemicals would be excluded from the evaluation: chemicals n. 82, 166 and 167 as indicated in the table presented annex 3. In this case, improved sensitivity and concordance of the ICE test method would be achieved as shown in table 6.

Table 6: Predictive capacity of the ICE test methods *to identify chemicals not requiring a GHS classification for eye irritation* – excluding 'paints'

	Concordance	**Sensitivity**	**Specificity**	**False negative rate**	**False positive rate**
Suggested-1 without paints	82.0% (114/139)	100% (61/61)	67.9% (53/78)	0% (0/61)	32.1% (25/78)
Suggested-2 without paints	83.1% (123/148)	100% (69/69)	68.4% (54/79)	0% (0/69)	31.6% (25/79)
Suggested + EJ without paints	82.5% (132/160)	100% (78/78)	65.9% (54/82)	0% (0/78)	34.1% (28/82)

I. Summary & recommendations from 16 November 2012

28. The present evaluation reviewed the assessed the *in vitro* and *in vivo* data reported in the ICCVAM BRD from March/April 2009 and in the SSD from 7 March 2012 (Document ENV/JM/TG(2012)20). Furthermore, it proposed with "suggested" *in vitro* and *in vivo* GHS classifications and calculated the ensuing predictive capacities of the ICE test method.

29. Differences were found in the Data Interpretation Procedures (and in particular in the criteria used to derive ICE GHS classification) proposed in the OECD GD 160 and in the proposed TG 438 from 14.9.2012. The use of the criteria proposed in the revised OECD TG 438 from September 2012 has the potential to lead to additional under-predictions of *in vivo* ocular irritants (e.g., substance n. 62, Quinacrine). In order to avoid unnecessary false negative materials, it is suggested to maintain the criteria as suggested in the OECD GD 160 for the identification of chemicals not requiring GHS classification, and to supplement the Cat. 2 criteria with those not yet covered by such criteria. As such the proposed criteria for ICE GHS classification is the one reported in Table 1 under the column 'Suggested'.

30. A number of discrepancies of *in vitro* GHS classifications were found in both the ICCVAM BRD and SSD documents. In particular in the SSD document the criteria proposed in the revised TG 438 TG for the identification of chemicals not requiring GHS classification does not seem to have been taken into account for all substances.

31. If the 'suggested' *in vitro* and *in vivo* GHS classification is used, only 1 false negative is found out of 62 to 80 materials (depending on considering or not materials classified based on expert judgment and with no raw data available but concordant evaluations between ICCVAM and SSD). This is substance n. 167, TNO-94 which is an anti-fouling paint classified as GHS Eye Cat.1 i*n vivo*.

32. It is believed that the suggestion from the Netherlands to exclude 'paints' from the applicability domain of the ICE test method should be considered until an optimized protocol for that purpose is developed.

33. With the exclusion of 'paints' the predictive capacity of the ICE test method to identify chemicals not requiring GHS classification would be :

- 0% false negatives (0 out of 61 to 78 depending on the quality of *in vivo* data considered);
- 32.1% to 34.1% false positives (25/78 to 28/82). Such amount is lower than other methods considered valid for the identification of GHS non-classified materials (i.e., 50 to 68% false positives for the Cytosensor Microphysiometer as reported in the Draft TG from 20.12.2010; and 53 to 69% for the BCOP as reported in the revised OECD TG 437 from 14.09.2012), and
- 82.0% to 83.1% of concordance (114/139 to 123/148).

34. With the above-mentioned predictive capacity, it is believed that the use of the ICE test method to identify chemicals not requiring GHS classification for serious eye damage and eye irritation can be included in the revised TG 438, with the exclusion of paints.

35. It is further suggested that a small group including the authors of the ICCVAM BRD, the SSD and the present document, discuss about the differences in data interpretation as reported in the present evaluation in order to find agreements or compromises on the final *in vitro* and *in vivo* GHS classifications to be used for reporting the predictive capacity of the ICE test method for identifying chemicals not requiring GHS classification.

36. Such set of agreed substances will then be used to define proficiency chemicals and evaluate the chemical classes covered by the ICE test method for the identification of GHS NC substances. In that regard, it is also advised that the Expert Group finds agreement on how to perform the chemical categorisation. For instance, it would be helpful if the expert group agrees on which tool(s) should be used to assign chemical classes / functions to the substances from the ICE agreed dataset: i) the National Library of Medicine Medical Subject Headings (MeSH) as used in the ICCVAM BRD; ii) the OECD QSAR Toolbox, or iii) other tools.

J. Conclusions based on input from the OECD Expert Meeting on Eye Irritation (March 2013)

37. Following reviews and comments provided by FDA and TNO representatives during a Teleconference with the OECD Secretariat on 3 December 2012, an Addendum was added to the Issue Paper to include additional analyses of predictive capacity (see Addendum to Issue Paper from 4 December 2012).

38. Additional comments were then received by NIEHS and further suggestions discussed during the OECD Expert Meeting that took place in 6-7 December 2012. Based on such suggestions, a further analyses of predictive capacity was carried out and presented during the meeting itself.

39. Based on the new analyses and corrected predictions of the ICE Test Method, the Expert Panel recommended to include in the revised TG 438 the use of the ICE to also identify non-classified chemicals and as such be used as e.g., an initial step in a Bottom-Up approach as described by Scott and co-authors[8].

40. The expert group further recommended that for the purposes of the Test Guideline, the best approach to report the predictive capacity of the ICE test method would be to:

- Make use of the 'Suggested' Decision Criteria as shown in Table 1 of the Issue Paper and in the revised Table 6 of the Draft TG 438
- Apply the analyses of predictive capacity based on the outcome of individual substances (and not on individual laboratory outcome), to be in alignment with previous ICCVAM evaluations and with the BCOP Test Guideline.
- Make use of the 'Suggested' *in vitro* and *in vivo* classifications, considering all substances for which raw *in vivo* data was available and could be reviewed.
- For the Bottom-Up approach analyses, include also those materials for which Study Criteria were not Met (SCNM) to allocate an *in vivo* classification but for which sufficient information was available to determine if the material would be classified (UN GHS Category 1 or 2). However, it was discussed to exclude those materials which had SCNM and were estimated to be Non-Classified in order to follow the precautionary principle. In addition, it was discussed not to include, for the purposes of the Test Guideline, those materials classified as Eye GHS Category 1 based on skin corrosivity effects, in order to consider only materials for which high quality *in vivo* data was available.

41. As discrepancies were found in some *in vitro* and *in vivo* classifications from previous analyses (i.e., ICCVAM and SSD), the predictive capacity of the ICE was also re-calculated to identify UN GHS Category to e.g., be used e.g., in a Top-Down testing strategy approach as suggested by Scott and co-authors[1]. In this case, test substances that had SCNM for the *in vivo* classification were not considered due to the impossibility and/or high uncertainty to attribute a Category 1 versus Category 2 classification according to UN GHS. In addition, similar to the Bottom-up approach, those materials classified as Eye GHS Category 1 based on skin corrosivity effects were not included in the analyses.

42. Based on the criteria described above, a total of 152 chemicals, representing 72 substances and 80 mixtures, were included in the analyses of predictive capacity of the ICE test method. The predictive capacity values obtained for both i) identification of GHS

[8] Scott L, Eskes C, Hoffman S, Adriaens E, Alepee N, Bufo M, Clothier R, Facchini D, Faller C, Guest R, Hamernik K, Harbell J, Hartung T, Kamp H, Le Varlet B, Meloni M, Mcnamee P, Osborn R, Pape W, Pfannenbecker U, Prinsen M, Seaman C, Spielmann H, Stokes W, Trouba K, Vassallo M, Van den Berghe C, Van Goethem F, Vinardell P, Zuang V (2010). A proposed Eye Irritation Testing Strategy to Reduce and Replace in vivo Studies Using Bottom-up and Top-down Approaches. Toxicology In Vitro 24, 1-9.

Category 1 chemicals (Top-Down approach) and ii) identification of non-classified chemicals (Bottom-up approach) are shown in table 1.1.

Table 1.1. Predictive capacities of the ICE test methods according to the recommended data interpretation

	Overall accuracy	Sensitivity	Specificity	False Negatives	False Positives
Category 1 versus the rest (Top-Down approach)	86% (120/140)	52% (14/27)	94% (106/113)	48% (13/27)	6% (7/113)
Not Classified versus the rest (Bottom-Up approach)	82% (125/152)	99% (72/73)	67% (53/79)	1% (1/73)	33% (26/79)

43. For the Top-Down approach, alcohols were found to risk over-prediction (4 alcohols out of 10 non-Category 1 were over-predicted as Category 1) whereas solids and surfactants were found to risk under-prediction (6 out of 11 Category 1 solids were found to be under-predicted, and 6 out of 9 Category 1 surfactants were found to be under-predicted). If alcohols, solids and surfactants are not considered, the predictive capacity values as shown in table 1.2 are obtained for the ICE test method.

Table 1.2. ICE Predictive capacity for the Top-Down approach excluding alcohols, solids and surfactants

without alcohols, solids and surfactants	Overall accuracy	Sensitivity	Specificity	False Negatives	False Positives
Category 1 versus the rest (Top-Down approach)	94% (77/82)	71% (5/7)	96% (72/75)	29% (2/7)	4% (3/75)

44. For the Bottom-up approach, anti-fouling organic solvent containing paint were found to risk under-prediction (1 out of 2 classified anti-fouling solvent containing paint was found to be under-predicted as non-classified). Due to the precautionary principle, if such product category is not considered, the predictive capacity values as shown in table 1.3 are obtained for the ICE test method.

Table 1.3. ICE Predictive capacity for the Bottom-Up approach excluding anti-fouling organic-solvent containing paints

Without anti-fouling organic-solvent containing paints	Overall accuracy	Sensitivity	Specificity	False Negatives	False Positives
Not Classified versus the rest (Bottom-Up approach)	83% (123/149)	100% (71/71)	67% (52/78)	0% (0/71)	33% (26/78)

45. Regarding the false positive rate of ICE to identify non-classified chemicals, it was found to be much lower than other test methods currently accepted for that purpose (e.g., 33% for ICE versus 69% and 68% for BCOP and CM false positive rates respectively). In addition, surfactants may risk over-prediction (5 out of 8 non-classified surfactants were over-classified).

ANNEX 1

ICE classification criteria for corneal swelling, opacity and fluorescein retention

Extracted from OECD TG 438 (p.7) adopted on 7 September 2009

Table 3. ICE classification criteria for corneal thickness.

Mean Corneal Swelling (%)*	ICE Class
0 to 5	I
>5 to 12	II
>12 to 18 (>75 min after treatment)	II
>12 to 18 (≤75 min after treatment)	III
>18 to 26	III
>26 to 32 (>75 min after treatment)	III
>26 to 32 (≤75 min after treatment)	IV
>32	IV

*Corneal swelling scores only applicable if thickness is measured with a Haag-Streit BP900 slit-lamp microscope with depth-measuring device no. I and slit-width setting at 9⅓, equaling 0.095 mm. Users should be aware that slit-lamp microscopes could yield different corneal thickness measurements if the slit-width setting is different.

Table 4. ICE classification criteria for opacity.

Mean Maximum Opacity Score*	ICE Class
0.0-0.5	I
0.6-1.5	II
1.6-2.5	III
2.6-4.0	IV

*See Table 1.

Table 5. ICE classification criteria for mean fluorescein retention.

Mean Fluorescein Retention Score at 30 minutes post-treatment*	ICE Class
0.0-0.5	I
0.6-1.5	II
1.6-2.5	III
2.6-3.0	IV

*See Table 2.

ANNEX 2

Invittox / DB-ALM Protocol n. 80 on the Chicken Enucleated Eye Test (CEET) / Isolated Chicken Eye (ICE) Test - Tables extracted from p. 9 & 10

Assessment of the ex vivo eye irritancy classification

Classification	***Combination of the three categories***
A. Not irritating	3xI 2xI, 1xII
B. Slightly irritating	3xII 2xII, 1xI 2xII, 1xIII 2xI, 1xIV[1] 1xI, 1xII, 1xIII
C. Moderately irritating	3xIII 2xIII, 1xII 2xIII, 1xIV[2] 2xIII, 1xI[1] 2xII, 1xIV[1] 1xII, 1xIII, 1xIV[1]
D. Severely irritating	3xIV 2xIV, 1xIII 2xIV, 1xII[1] 2xIV, 1xI[1] immediate corneal opacity score 3 corneal opacity score 4 severe loosening of epithelium

[1] Combinations of categories less likely to occur
[2] Combination can be considered a borderline case between moderately and severely irritating

EC-Classification of eye irritants and extrapolation from *ex vivo* results to EC-classification

Classification	***Combination of the three categories***
NI = not irritating (combination of A and B)	3xI 3xII 2xI, 1xII 2xII, 1xI 1xI, 1xII, 1xIII[1]
R36 = irritating (combination of B and C)	3xIII 2xII, 1xIII 2xIII, 1xII 2xIII, 1xIV 2xI, 1xIV[1] 2xII, 1xIV[1] 2xIII, 1xI[1] 1xII, 1xIII, 1xIV[1]
R41 = severely irritating	3xIV 2xIV, 1xIII 2xIV, 1xII[1] 2xIV, 1xI[1] immediate corneal opacity score 3 corneal opacity score 4 severe loosening of epithelium

[1] Combinations of categories less likely to occur
The combination 3xII can be considered as a borderline case between non-irritating and irritating
The combinations 2x III and 1xIV can be considered as a borderline case between irritating and severely irritating

ANNEX 3 - Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
1	Acetaldehyde	75-07-0	liquid	99	undiluted	n.p.	n.p. (2A)	no raw data	n.a.	2A	2	2A	2A	2A	2A	2	III	1.4	II	24	III	Prinsen and Koëter (1993)	-
2	Acetic acid	64-19-7	liquid	99	10%	1	1	no raw data	n.a.	1	1	1	1	1	1	3	IV	2.6	IV	31	III/IV	Prinsen and Koëter (1993)	-
3	Acetone	67-64-1	liquid	99	undiluted	2A	2A	2A	n.a.	2B	NC	2B	2A	2A	2A	1.4	II	0.4	I	9.6	II	Balls et al. (1995)	-
3	Acetone	67-64-1	liquid	99	undiluted					2A	2	2A				1	II	1.7	III	49	IV	Balls et al. (1995)	-
3	Acetone	67-64-1	liquid	99	undiluted					2B	2	2B				1.83	III	1.17	II	7.64	II	Balls et al. (1995)	-
3	Acetone	67-64-1	liquid	99	undiluted					2A	2	2A				3	IV	1	II	13.8	II/III	Balls et al. (1995)	-
4	Ammonium nitrate	6484-52-2	solid	>99	undiluted	2B	2B	2A	n.a.	2B	2	2B	2B	2B	2B	1.2	II	0.9	II	6.7	II	Balls et al. (1995)	-
4	Ammonium nitrate	6484-52-2	solid	>99	undiluted					2A	2	2A				2	III	1.3	II	42	IV	Balls et al. (1995)	-
4	Ammonium nitrate	6484-52-2	solid	>99	undiluted					2B	2	2B				1.33	II	1.5	II	12.33	II/III	Balls et al. (1995)	-
4	Ammonium nitrate	6484-52-2	solid	>99	undiluted					2B	2	2B				2	III	0.5	I	6	II	Balls et al. (1995)	-
5	L-Aspartic acid	70-47-3	solid	100	neat	SCNM	2A (SCNM)	SCNM	2	2B	NC	2B	2A	2A	2A	1	II	0.7	II	3.2	I	Balls et al. (1995)	Uncertainity on identity of tested chemical (CAS n. not corresponding to chemical name)
5	L-Aspartic acid	70-47-3	solid	100	neat					2A	2	2A				2	III	2	III	56	IV	Balls et al. (1995)	
5	L-Aspartic acid	70-47-3	solid	100	neat					2A	2	2A				1.83	III	1.67	III	14.67	II/III	Balls et al. (1995)	
5	L-Aspartic acid	70-47-3	solid	100	neat					2B	2	2B				2	III	1	II	10	II	Balls et al. (1995)	
6	Benzalkonium chloride (1%)	8001-54-5	liquid	98	1%	1	2A (1)	1	n.a.	2A/2B	2	2A/2B	2A	2A	2A	1.8	III	0.6	II	18	II/III	Balls et al. (1995)	-
6	Benzalkonium chloride (1%)	8001-54-5	liquid	98	1%					2A	2	2A				1.3	II	2.3	III	47	IV	Balls et al. (1995)	-
6	Benzalkonium chloride (1%)	8001-54-5	liquid	98	1%					2A	2	2A				2.67	IV	1.5	II	12.66	II/III	Balls et al. (1995)	-
6	Benzalkonium chloride (1%)	8001-54-5	liquid	98	1%					2A	2	2A				2	III	3	IV	8.8	II	Balls et al. (1995)	-
7	Benzalkonium chloride (5%)	8001-54-5	liquid	98	5%	1	1	1	n.a.	1	1	1	1	1	1	3	IV	2.6	IV	36.3	IV	Balls et al. (1995)	-
7	Benzalkonium chloride (5%)	8001-54-5	liquid	98	5%					2A	2	2A				1	II	2	III	42	IV	Balls et al. (1995)	-
7	Benzalkonium chloride (5%)	8001-54-5	liquid	98	5%					1	1	1				3	IV	2	III	33.77	IV	Balls et al. (1995)	-
7	Benzalkonium chloride (5%)	8001-54-5	liquid	98	5%					1	1	1				3	IV	3	IV	68.9	IV	Balls et al. (1995)	-
8	Benzalkonium chloride (10%)	8001-54-5	liquid	98	10%	1	1	1	n.a.	1	1	1	1	1	1	3	IV	3	IV	37.7	IV	Balls et al. (1995)	-
8	Benzalkonium chloride (10%)	8001-54-5	liquid	98	10%					1	1	1				3	IV	2.3	III	95	IV	Balls et al. (1995)	-
8	Benzalkonium chloride (10%)	8001-54-5	liquid	98	10%					1	1	1				3	IV	2.33	III	40.72	IV	Balls et al. (1995)	-
8	Benzalkonium chloride (10%)	8001-54-5	liquid	98	10%					1	1	1				3	IV	2	III	41.1	IV	Balls et al. (1995)	-
9	Benzalkonium chloride (100%)	8001-54-5	liquid	98	undiluted	1	1	no raw data	n.a.	1	1	1	1	1	1	3	IV	3	IV	40	IV	Prinsen and Koëter (1993)	-
10	Brij 35	9002-92-0	liquid	n.p.	undiluted	n.p.	NI (n.p.)	NC	n.a.	NI	NC	NC	NI	NI	NC	0.9	II	0	I	5	I	Prinsen and Koëter (1993)	-
11	1-Butanol	71-36-3	liquid	99	undiluted	2A	1 (2A)	1/2A	n.a.	1	1	1	1	1	1	2.9	IV	2	III	54	IV	Prinsen and Koëter (1993)	*In vivo* GHS Cat 2A in ECETOC Database, and *in vivo* GHS Cat 1 in Zebet study
12	2-Butoxyethyl acetate	112-07-2	liquid	99	undiluted	n.p.	n.p. (NI)	no raw data	n.a.	2B	NC	2B	2B	2B	2B	1	II	1	II	5	I	Prinsen and Koëter (1993)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438

ANNEX 3 (cont' 2) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
13	n-Butyl acetate	123-86-4	liquid	99	undiluted	NI	NI	NC	n.a.	2A	2	2A	2A	2A	2A	1.8	III	1.8	III	13.9	II/III	Balls et al. (1995)	-
13	n-Butyl acetate	123-86-4	liquid	99	undiluted					1	1	1				0.5	I	2.7	IV	42	IV	Balls et al. (1995)	-
13	n-Butyl acetate	123-86-4	liquid	99	undiluted					2A/2B	2	2A/2B				1	II	2	III	14.67	II/III	Balls et al. (1995)	-
13	n-Butyl acetate	123-86-4	liquid	99	undiluted					2A	2	2A				1	II	2	III	32.2	IV	Balls et al. (1995)	-
14	gamma-Butyrolactone	96-48-0	liquid	>99	undiluted	2A/2B	2A	2A	n.a.	2A	2	2A	2A	2A	2A	2.6	IV	1.4	II	15.8	II/III	Balls et al. (1995)	-
14	gamma-Butyrolactone	96-48-0	liquid	>99	undiluted					2A	2	2A				1.3	II	2	III	47	IV	Balls et al. (1995)	-
14	gamma-Butyrolactone	96-48-0	liquid	>99	undiluted					2A/2B	2	2A/2B				1.67	III	1.5	II	13.1	II/III	Balls et al. (1995)	-
14	gamma-Butyrolactone	96-48-0	liquid	>99	undiluted					2A/2B	2	2A/2B				1	II	2	III	13	II/III	Balls et al. (1995)	-
15	Captan 90 concentrate	133-06-2	solid	90	neat	1	1	1	n.a.	NI	NC	NC	2B	2B	2B	0	I	0.4	I	1.7	I	Balls et al. (1995)	-
15	Captan 90 concentrate	133-06-2	solid	90	neat					2B [(a)]	2	2A/2B				0.2	I	1	II	27	III/IV	Balls et al. (1995)	-
15	Captan 90 concentrate	133-06-2	solid	90	neat					2B	2	2B				0	I	1.33	II	19.17	III	Balls et al. (1995)	-
15	Captan 90 concentrate	133-06-2	solid	90	neat					2B	2	2B				1	II	1	II	20	III	Balls et al. (1995)	-
16	4-Carboxybenzaldehyde	619-66-9	solid	95	neat	2A	2A	2A	n.a.	NI	NC	NC	NI	NI	2A/2B	1	I	0.5	I	5.4	II	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by ICCVAM and in the SSD appears unclear
16	4-Carboxybenzaldehyde	619-66-9	solid	95	neat					1	1	1				1.3	II	3	IV	89	IV	Balls et al. (1995)	
16	4-Carboxybenzaldehyde	619-66-9	solid	95	neat					NI	NC	NC				0.67	II	0.5	I	-1.4	I	Balls et al. (1995)	
16	4-Carboxybenzaldehyde	619-66-9	solid	95	neat					2A/2B	2	2A/2B				2	III	1	II	12.7	II/III	Balls et al. (1995)	
17	Cetylpyridinium bromide (0.1%)	140-72-7	liquid	98	0.1%	NI	NI	NC	n.a.	NI	NC	NC	2B	NI (2B)	2B	1	II	0	I	2.2	I	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
17	Cetylpyridinium bromide (0.1%)	140-72-7	liquid	98	0.1%					2B	2	2B				0.7	II	0	I	21	III	Balls et al. (1995)	
17	Cetylpyridinium bromide (0.1%)	140-72-7	liquid	98	0.1%					2B	2	2B				0.67	II	1	II	10.29	II	Balls et al. (1995)	
17	Cetylpyridinium bromide (0.1%)	140-72-7	liquid	98	0.1%					2B	2	2B				1	II	1	II	14.6	II/III	Balls et al. (1995)	
18	Cetylpyridinium bromide (6%)	140-72-7	liquid	98	6%	1	n.p.	1	n.a.	2A	2	2A	2A	2A	2A	2	III	1.2	II	27.2	III/IV	Balls et al. (1995)	-
18	Cetylpyridinium bromide (6%)	140-72-7	liquid	98	6%					2A [(a)]	2	2A				2	III	0.5	I	49	IV	Balls et al. (1995)	-
18	Cetylpyridinium bromide (6%)	140-72-7	liquid	98	6%					2A	2	2A				3	IV	1.83	III	24.55	III	Balls et al. (1995)	-
18	Cetylpyridinium bromide (6%)	140-72-7	liquid	98	6%					2A	2	2A				2.7	IV	1.7	III	13.5	II/III	Balls et al. (1995)	-
19	Cetylpyridinium bromide (6%)	140-72-7	liquid	n.p.	undiluted	1	1	1	n.a.	1	2	2A	1	1	2A	2	III	2	III	22	III	Prinsen (2000)	The *in vitro* classification proposed by ICCVAM does not correspond to the criteria for classification proposed in the BRD and in GD 160. The rationale for the *in vitro* classification proposed in the SSD appears unclear
19	Cetylpyridinium bromide (6%)	140-72-7	liquid	n.p.	undiluted					1	2	2A				1.8	III	1.7	III	21	III	Prinsen (2000)	
19	Cetylpyridinium bromide (6%)	140-72-7	liquid	n.p.	undiluted					1	2	2A				2	III	2	III	21	III	Prinsen (2000)	
19	Cetylpyridinium bromide (6%)	140-72-7	liquid	n.p.	undiluted					1	2	2A				1.7	III	1.7	III	18	II/III	Prinsen (2000)	
20	Cetylpyridinium bromide (10%)	140-72-7	liquid	98	10%	1	1	1	n.a.	2A	2	2A	2A	2A	2A	2.6	IV	1	II	25.8	III	Balls et al. (1995)	-
20	Cetylpyridinium bromide (10%)	140-72-7	liquid	98	10%					2A	2	2A				1.7	III	2	III	41	IV	Balls et al. (1995)	-
20	Cetylpyridinium bromide (10%)	140-72-7	liquid	98	10%					2A	2	2A				2	III	1.67	III	27.2	III/IV	Balls et al. (1995)	-
20	Cetylpyridinium bromide (10%)	140-72-7	liquid	98	10%					1	1	1				3	IV	3	IV	17.8	II/III	Balls et al. (1995)	-

ANNEX 3 (cont' 3) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
21	Chlorhexidine	55-56-1	solid	n.p.	neat					1	1	1				3	IV	4	IV	32	III/IV	Balls et al. (1995)	-
21	Chlorhexidine	55-56-1	solid	n.p.	neat	1	1	1	n.a.	1	1	1	1	1	1	3	IV	4	IV	150	IV	Balls et al. (1995)	-
21	Chlorhexidine	55-56-1	solid	n.p.	neat					1	1	1				3	IV	3	IV	53.13	IV	Balls et al. (1995)	-
21	Chlorhexidine	55-56-1	solid	n.p.	neat					1	1	1				3	IV	4	IV	n.p.	n.p.	Balls et al. (1995)	-
22	Chloroform	67-66-3	liquid	99.8	undiluted	n.p.	n.p. (2A)	no raw data	n.a.	2A	2	2A	2A	2A	2A	2.5	III	1	II	21	III	Prinsen and Koëter (1993)	-
23	Cyclohexanol	108-93-0	liquid	97	undiluted					2A	2	2A				2.2	III	2.2	III	24.7	III	Balls et al. (1995)	-
23	Cyclohexanol	108-93-0	liquid	97	undiluted	1	1	1	n.a.	1	1	1	1	1	1	3	IV	2	III	103	IV	Balls et al. (1995)	-
23	Cyclohexanol	108-93-0	liquid	97	undiluted					1	1	1				3	IV	2.5	III	35.7	IV	Balls et al. (1995)	-
23	Cyclohexanol	108-93-0	liquid	97	undiluted					1	1	1				3	IV	2.5	III	45.3	IV	Balls et al. (1995)	-
24	Cyclohexylamino-functional PMS	n.p.	liquid	n.p.	undiluted					2A	2	2A				1.8	III	2.5	III	14	II/III	Prinsen (2000)	-
24	Cyclohexylamino-functional PMS	n.p.	liquid	n.p.	undiluted					2A	2	2A				1.7	III	2	III	13	II/III	Prinsen (2000)	-
24	Cyclohexylamino-functional PMS	n.p.	liquid	n.p.	undiluted	n.p.	n.p. (2A)	no raw data	n.a.	2A	2	2A	2A	2A	2A	2	III	2.3	III	17	II/III	Prinsen (2000)	-
24	Cyclohexylamino-functional PMS	n.p.	liquid	n.p.	undiluted					2A	2	2A				2	III	2.3	III	14	II/III	Prinsen (2000)	-
24	Cyclohexylamino-functional PMS	n.p.	liquid	n.p.	undiluted					2A	2	2A				2	III	2	III	13	II/III	Prinsen (2000)	-
25	Decamethylcyclopentasiloxane	n.p.	liquid	n.p.	undiluted					NI	NC	NC				0.3	I	0.3	I	1	I	Prinsen (2000)	-
25	Decamethylcyclopentasiloxane	n.p.	liquid	n.p.	undiluted					NI	NC	NC				0.3	I	0.3	I	1	I	Prinsen (2000)	-
25	Decamethylcyclopentasiloxane	n.p.	liquid	n.p.	undiluted	n.p./NI	n.p. (NI)	no raw data	n.a.	NI	NC	NC	NI	NI	NC	0	I	0.5	I	2	I	Prinsen (2000)	-
25	Decamethylcyclopentasiloxane	n.p.	liquid	n.p.	undiluted					NI	NC	NC				0	I	0	I	0	I	Prinsen (2000)	-
25	Decamethylcyclopentasiloxane	n.p.	liquid	n.p.	undiluted					NI	NC	NC				0	I	0	I	2	I	Prinsen (2000)	-
26	Dibenzoyl-L-tartaric acid	2743-38-6	solid	98	neat					1	1	1				2.8	IV	3	IV	12.8	II/III	Balls et al. (1995)	-
26	Dibenzoyl-L-tartaric acid	2743-38-6	solid	98	neat	1	1	1	n.a.	1	1	1	1	1	1	1	II	2.7	IV	75	IV	Balls et al. (1995)	-
26	Dibenzoyl-L-tartaric acid	2743-38-6	solid	98	neat					2B	2	2B				2	III	1.5	II	6.36	II	Balls et al. (1995)	-
26	Dibenzoyl-L-tartaric acid	2743-38-6	solid	98	neat					2B	2	2B				1	II	2	III	6.7	II	Balls et al. (1995)	-
27	Dibenzyl phosphate	1623-08-1	solid	99	neat					2A	2	2A				2.6	IV	2	III	12.2	II/III	Balls et al. (1995)	-
27	Dibenzyl phosphate	1623-08-1	solid	99	neat	2A	2A	2A	n.a.	2B	2	2B	2A/2B	2A/2B	2A	1	II	0	I	22	III	Balls et al. (1995)	-
27	Dibenzyl phosphate	1623-08-1	solid	99	neat					2A/2B	2	2A/2B				2	III	1.5	II	17.07	II/III	Balls et al. (1995)	-
27	Dibenzyl phosphate	1623-08-1	solid	99	neat					1	2	2A				2	III	2	III	40.9	IV	Balls et al. (1995)	-
28	Dibutyltin dichloride	683-18-1	solid	97	undiluted	n.p.	n.p. (1)	no raw data	n.a.	1	1	1	1	1	1	3	IV	2.5	III	34	IV	Prinsen and Koëter (1993)	-
29	2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	99	undiluted					2A/2B	2	2A/2B				2.3	III	0.8	II	12.7	II/III	Balls et al. (1995)	-
29	2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	99	undiluted	2A	2A	2A	n.a.	2A	2	2A	2A	2A	2A	2	III	1.3	II	26	III	Balls et al. (1995)	-
29	2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	99	undiluted					2A	2	2A				1.83	III	1.67	III	17.15	II/III	Balls et al. (1995)	-
29	2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	99	undiluted					2A/2B	2	2A/2B				1.8	III	0.8	II	16.8	II/III	Balls et al. (1995)	-
30	2,2-Dimethylbutanoic acid	595-37-9	liquid	96	undiluted					1	1	1				3	IV	2.4	III	43.8	IV	Balls et al. (1995)	-
30	2,2-Dimethylbutanoic acid	595-37-9	liquid	96	undiluted	SCNM/1	1 (SCNM)	SCNM	1	1	1	1	1	1	1	3	IV	2.7	IV	74	IV	Balls et al. (1995)	-
30	2,2-Dimethylbutanoic acid	595-37-9	liquid	96	undiluted					1	1	1				3	IV	2.5	III	35.9	IV	Balls et al. (1995)	-
30	2,2-Dimethylbutanoic acid	595-37-9	liquid	96	undiluted					1	1	1				3	IV	3	IV	62.7	IV	Balls et al. (1995)	-

ANNEX 3 (cont' 4) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
31	2,5-Dimethylohexanediol	110-03-2	solid	99.5	neat	1	2B/2A (1)	1	n.a.	2B	2	2B	2B	2B	2B	2	III	1	II	11.9	II	Balls et al. (1995)	-
31	2,5-Dimethylohexanediol	110-03-2	solid	99.5	neat					1	1	1				2	III	3	IV	64	IV	Balls et al. (1995)	-
31	2,5-Dimethylohexanediol	110-03-2	solid	99.5	neat					2B	2	2B				1.33	II	1.67	III	11.57	II	Balls et al. (1995)	-
31	2,5-Dimethylohexanediol	110-03-2	solid	99.5	neat					2B	2	2B				2	III	1	II	6.7	II	Balls et al. (1995)	-
32	Dimethyl sulfoxide	67-68-5	liquid	99.9	undiluted	2B/n.p.	NI (2B)	NC	n.a.	NI	NC	NC	NI	NI	NC	1	II	0.5	I	4	I	Prinsen and Koëter (1993)	Suggested *in vivo* classification derived from the study of Gautheron et al. (1994)
33	Ethanol	64-17-5	liquid	n.p.	undiluted	2A/2B	2A	2A	n.a.	1	1	1	1	1	1	2.8	IV	2.8	IV	30.7	III/IV	Balls et al. (1995)	-
33	Ethanol	64-17-5	liquid	n.p.	undiluted					1	1	1				2	III	3	IV	74	IV	Balls et al. (1995)	-
33	Ethanol	64-17-5	liquid	n.p.	undiluted					2A	2	2A				2.5	III	2.33	III	35.88	IV	Balls et al. (1995)	-
33	Ethanol	64-17-5	liquid	n.p.	undiluted					2A	2	2A				2	III	2.3	III	34.6	IV	Balls et al. (1995)	-
34	Ethyl acetate	141-78-6	liquid	99	undiluted	NI	NI	NC	n.a.	2A	2	2A	2A	2A	2A	2	III	2	III	22	III	Balls et al. (1995)	-
34	Ethyl acetate	141-78-6	liquid	99	undiluted					2A	2	2A				1.7	III	2.3	III	76	IV	Balls et al. (1995)	-
34	Ethyl acetate	141-78-6	liquid	99	undiluted					2A	2	2A				2	III	2	III	25.08	III	Balls et al. (1995)	-
34	Ethyl acetate	141-78-6	liquid	99	undiluted					2A	2	2A				3	IV	2	III	23	III	Balls et al. (1995)	-
35	2-Ethyl-1-hexanol	104-76-7	liquid	99	undiluted	2A/2B	2A	2A	n.a.	2A	2	2A	2A	2A	2A	2	III	2.2	III	43	IV	Balls et al. (1995)	-
35	2-Ethyl-1-hexanol	104-76-7	liquid	99	undiluted					2A	2	2A				1	II	2.3	III	62	IV	Balls et al. (1995)	-
35	2-Ethyl-1-hexanol	104-76-7	liquid	99	undiluted					2A	2	2A				3	IV	1.5	II	13.31	II/III	Balls et al. (1995)	-
35	2-Ethyl-1-hexanol	104-76-7	liquid	99	undiluted					2A	2	2A				1	II	2	III	52.4	IV	Balls et al. (1995)	-
36	Ethyl-2-methylacetoacetate	609-14-3	liquid	97	undiluted	2B	2B	2B	n.a.	NI	NC	NC	2B	2B	2B	0.4	I	0.3	I	-2.8	I	Balls et al. (1995)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438
36	Ethyl-2-methylacetoacetate	609-14-3	liquid	97	undiluted					2B	NC	2B				1	II	0	I	7	II	Balls et al. (1995)	
36	Ethyl-2-methylacetoacetate	609-14-3	liquid	97	undiluted					2B	2	2B				0.67	II	1	II	11.52	II	Balls et al. (1995)	
36	Ethyl-2-methylacetoacetate	609-14-3	liquid	97	undiluted					NI	NC	NC				1	II	0.5	I	4.5	I	Balls et al. (1995)	
37	Ethyl trimethyl acetate	3938-95-2	liquid	99	undiluted	NI	NI	NC	n.a.	2B	NC	2B	2B	2B	2B	1.2	II	0.4	I	7.2	II	Balls et al. (1995)	-
37	Ethyl trimethyl acetate	3938-95-2	liquid	99	undiluted					2A	2	2A				2	III	2	III	31	III/IV	Balls et al. (1995)	-
37	Ethyl trimethyl acetate	3938-95-2	liquid	99	undiluted					NI	NC	NC				0	I	0	I	1.44	I	Balls et al. (1995)	-
37	Ethyl trimethyl acetate	3938-95-2	liquid	99	undiluted					2B	NC	2B				1	II	0.5	I	6.7	II	Balls et al. (1995)	-
38	Fomesafen	72128-02-0	solid	97.5	neat	NI	NI	SCNM	NC	2B	2	2B	2B	2B	2B	0.9	II	1.2	II	5.3	II	Balls et al. (1995)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438
38	Fomesafen	72128-02-0	solid	97.5	neat					NI	NC	NC				0	I	0.2	I	11	II	Balls et al. (1995)	
38	Fomesafen	72128-02-0	solid	97.5	neat					NI	NC	NC				1	II	0.5	I	2.82	I	Balls et al. (1995)	
38	Fomesafen	72128-02-0	solid	97.5	neat					2B	NC	2B				1	II	1	II	4.3	I	Balls et al. (1995)	
39	Glycerol	56-81-5	liquid	>99.5	undiluted	n.p.	NI	NC	n.a.	2B	NC	2B	2B	NI	2B	1.2	II	1	II	5	I	Balls et al. (1995)	The rationale for the *in vitro* classification proposed in the SSD appears unclear
39	Glycerol	56-81-5	liquid	>99.5	undiluted					NI	NC	NC				0	I	0	I	11	II	Balls et al. (1995)	
39	Glycerol	56-81-5	liquid	>99.5	undiluted					2B	2	2B				1.17	II	1	II	8.3	II	Balls et al. (1995)	
39	Glycerol	56-81-5	liquid	>99.5	undiluted					2A [(a)]	2	2A				2	III	0.5	I	29.4	III/IV	Balls et al. (1995)	
40	Glycerol	56-81-5	liquid	99	undiluted	NI/n.p.?	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.5	I	0.4	I	4	I	Prinsen and Koëter (1993)	-
41	n-Hexane	110-54-3	liquid	99	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.5	I	0	I	1	I	Prinsen and Koëter (1993)	-

ANNEX 3 (cont' 5) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
42	n-Hexanol	111-27-3	liquid	98	undiluted	2A	2A	2A	n.a.	2A	2	2A	1	2A (1)	1	2.8	IV	1.6	III	17.4	II/III	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
42	n-Hexanol	111-27-3	liquid	98	undiluted					2A [a]	2	2A				0.2	I	1.7	III	82	IV	Balls et al. (1995)	
42	n-Hexanol	111-27-3	liquid	98	undiluted					1	1	1				3	IV	2.83	IV	28.89	III/IV	Balls et al. (1995)	
42	n-Hexanol	111-27-3	liquid	98	undiluted					1	1	1				3	IV	3	IV	58.9	IV	Balls et al. (1995)	
43	Imidazole	288-32-4	solid	99	neat	1	1	1	n.a.	1	1	1	1	1	1	3	IV	4	IV	40.3	IV	Balls et al. (1995)	-
43	Imidazole	288-32-4	solid	99	neat					1	1	1				3	IV	3	IV	224	IV	Balls et al. (1995)	-
43	Imidazole	288-32-4	solid	99	neat					1	1	1				3	IV	2.5	III	36.96	IV	Balls et al. (1995)	-
43	Imidazole	288-32-4	solid	99	neat					1	1	1				3	IV	3	IV	97.8	IV	Balls et al. (1995)	-
44	Isobutanol	78-83-1	liquid	99.9	undiluted	2A/2B	2A	2A	n.a.	1	1	1	1	1	1	2.8	IV	2.5	III	46.4	IV	Balls et al. (1995)	-
44	Isobutanol	78-83-1	liquid	99.9	undiluted					1	1	1				3	IV	2.7	IV	93	IV	Balls et al. (1995)	-
44	Isobutanol	78-83-1	liquid	99.9	undiluted					1	1	1				3	IV	2.5	III	37.06	IV	Balls et al. (1995)	-
44	Isobutanol	78-83-1	liquid	99.9	undiluted					1	1	1				3	IV	2	III	69.2	IV	Balls et al. (1995)	-
45	Isopropanol	67-63-0	liquid	99.9	undiluted	2A/2B	2A	2A	n.a.	2A	2	2A	1	2A (1)	1	2	III	1.6	III	23.3	III	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
45	Isopropanol	67-63-0	liquid	99.9	undiluted					1	1	1				0.7	II	2.7	IV	72	IV	Balls et al. (1995)	
45	Isopropanol	67-63-0	liquid	99.9	undiluted					1	1	1				3	IV	2.5	III	37.84	IV	Balls et al. (1995)	
45	Isopropanol	67-63-0	liquid	99.9	undiluted					2B	2	2B				2.3	III	0.5	I	8.9	II	Balls et al. (1995)	
46	Maneb	12427-38-2	solid	90	neat	SCNM/2B	2B/2A (SCNM)	SCNM	2A	NI	NC	NC	NI	NI	2B	0	I	0.5	I	2.8	I	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by ICCVAM and in the SSD appears unclear
46	Maneb	12427-38-2	solid	90	neat					2A	2	2A				1	II	2	III	33	IV	Balls et al. (1995)	
46	Maneb	12427-38-2	solid	90	neat					NI	NC	NC				0	I	0.5	I	8.03	II	Balls et al. (1995)	
46	Maneb	12427-38-2	solid	90	neat					2B	2	2B				1	II	1	II	6.7	II	Balls et al. (1995)	
47	Mercury (II) chloride	7487-94-7	solid	99.5	undiluted	n.p.	n.p. (1)	no raw data	n.a.	1	1	1	1	1	1	2	III	3.1	IV	55	IV	Prinsen and Koëter (1993)	-
48	2-Methoxyethanol	109-86-4	liquid	99.9	undiluted	n.p.	n.p. (2A)	no raw data	n.a.	2A	2	2A	2A	2A	2A	2	III	2	III	18	II/III	Prinsen and Koëter (1993)	-
49	Methyl acetate	79-20-9	liquid	98	undiluted	2A/2B	2A	2A	n.a.	2A	2	2A	1	2A (1)	1	1.4	II	2.4	III	20.3	III	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
49	Methyl acetate	79-20-9	liquid	98	undiluted					1	1	1				1	II	2.7	IV	93	IV	Balls et al. (1995)	
49	Methyl acetate	79-20-9	liquid	98	undiluted					2A	2	2A				2	III	2	III	22.5	III	Balls et al. (1995)	
49	Methyl acetate	79-20-9	liquid	98	undiluted					1	1	1				3	IV	3	IV	17.5	II/III	Balls et al. (1995)	
50	Methyl cyanoacetate	105-34-0	liquid	99	undiluted	2A	2A	2A	n.a.	NI	NC	NC	NI	NI	2B	0.4	I	0.3	I	4.5	I	Balls et al. (1995)	The rationale for the *in vitro* classification proposed by ICCVAM and in the SSD appears unclear
50	Methyl cyanoacetate	105-34-0	liquid	99	undiluted					2A [a]	2	2A				0.5	I	0.7	II	44	IV	Balls et al. (1995)	
50	Methyl cyanoacetate	105-34-0	liquid	99	undiluted					NI	NC	NC				0.17	I	0.5	I	4.93	I	Balls et al. (1995)	
50	Methyl cyanoacetate	105-34-0	liquid	99	undiluted					2B	2	2B				1	II	1	II	10.7	II	Balls et al. (1995)	
51	Methylcyclopentane	96-37-7	liquid	>99	undiluted	NI	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.4	I	0.5	I	2.3	I	Balls et al. (1995)	-
51	Methylcyclopentane	96-37-7	liquid	>99	undiluted					2B	2	2B				1	II	0	I	22	III	Balls et al. (1995)	-
51	Methylcyclopentane	96-37-7	liquid	>99	undiluted					NI	NC	NC				0	I	0.5	I	5.83	II	Balls et al. (1995)	-
51	Methylcyclopentane	96-37-7	liquid	>99	undiluted					NI	NC	NC				1	II	0.5	I	0	I	Balls et al. (1995)	-

ANNEX 3 (cont' 6) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
52	Methyl ethyl ketone	78-93-3	liquid	99	undiluted	2A/2B	2A	2A	n.a.	2A	2	2A	1	2A (1)	1	2	III	2.2	III	23.1	III	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
52	Methyl ethyl ketone	78-93-3	liquid	99	undiluted					1	1	1				2.7	IV	3	IV	99	IV	Balls et al. (1995)	
52	Methyl ethyl ketone	78-93-3	liquid	99	undiluted					1	1	1				3	IV	2.33	III	34.88	IV	Balls et al. (1995)	
52	Methyl ethyl ketone	78-93-3	liquid	99	undiluted					2A	2	2A				3	IV	2	III	12.6	II/III	Balls et al. (1995)	
53	Methyl isobutyl ketone	108-10-1	liquid	98	undiluted	NI	NI	NC	n.a.	2A	1/2	1/2A	2A	2A	2A	2.6	IV	2	III	26.5	III/IV	Balls et al. (1995)	-
53	Methyl isobutyl ketone	108-10-1	liquid	98	undiluted					1	1	1				3	IV	3	IV	64	IV	Balls et al. (1995)	-
53	Methyl isobutyl ketone	108-10-1	liquid	98	undiluted					2A	2	2A				2	III	2.17	III	21.69	III	Balls et al. (1995)	-
53	Methyl isobutyl ketone	108-10-1	liquid	98	undiluted					2A	2	2A				2	III	2	III	12.3	II/III	Balls et al. (1995)	-
54	1-Napthaleneacetic acid	86-87-3	solid	96	neat	1	2A (1)	1	n.a.	2B	2	2B	2B	2B/2A (2B)	2B	1	II	0.9	II	5.6	II	Balls et al. (1995)	-
54	1-Napthaleneacetic acid	86-87-3	solid	96	neat					2B	2	2B				1	II	1	II	24	III	Balls et al. (1995)	-
54	1-Napthaleneacetic acid	86-87-3	solid	96	neat					2B	2	2B				2	III	1	II	8.86	II	Balls et al. (1995)	-
54	1-Napthaleneacetic acid	86-87-3	solid	96	neat					2A	2	2A				1	II	1	II	46.7	IV	Balls et al. (1995)	-
55	1-Napthaleneacetic acid, sodium salt	61-31-4	solid	95	neat	1	1	1	n.a.	1	1	1	1	1	1	3	IV	3	IV	46.6	IV	Balls et al. (1995)	-
55	1-Napthaleneacetic acid, sodium salt	61-31-4	solid	95	neat					1	1	1				3	IV	2.7	IV	122	IV	Balls et al. (1995)	-
55	1-Napthaleneacetic acid, sodium salt	61-31-4	solid	95	neat					1	1	1				3	IV	2.5	III	44.19	IV	Balls et al. (1995)	-
55	1-Napthaleneacetic acid, sodium salt	61-31-4	solid	95	neat					1	1	1				3	IV	3	IV	64.1	IV	Balls et al. (1995)	-
56	n-Octanol	111-87-5	liquid	>99	undiluted	2B	2B	2A	n.a.	2A	2	2A	2A	2A	2A	2	III	2.4	III	36.5	IV	Balls et al. (1995)	-
56	n-Octanol	111-87-5	liquid	>99	undiluted					2A	2	2A				1.3	II	2	III	108	IV	Balls et al. (1995)	-
56	n-Octanol	111-87-5	liquid	>99	undiluted					2B	2	2B				1.17	II	1.5	II	10.18	II	Balls et al. (1995)	-
56	n-Octanol	111-87-5	liquid	>99	undiluted					2A	2	2A				2	III	1	II	25.7	III	Balls et al. (1995)	-
57	Parafluoraniline	371-40-4	liquid	99	undiluted	SCNM	1 (SCNM)	SCNM	1/2	1	1	1	1	1	1	3	IV	2.2	III	35.3	IV	Balls et al. (1995)	-
57	Parafluoraniline	371-40-4	liquid	99	undiluted					1	1	1				3	IV	2	III	79	IV	Balls et al. (1995)	-
57	Parafluoraniline	371-40-4	liquid	99	undiluted					1	1	1				3	IV	2	III	33.44	IV	Balls et al. (1995)	-
57	Parafluoraniline	371-40-4	liquid	99	undiluted					1	1	1				3	IV	2	III	38.5	IV	Balls et al. (1995)	-
58	Polyethylene glycol 400	25322-68-3	liquid	n.p.	undiluted	NI	NI	NC	n.a.	2B	2	2B	2B	NI (2B)	2B	1.4	II	1	II	9.8	II	Balls et al. (1995) / Prinsen (2000)	*In vitro* classification proposed in SSD based on results of one lab (repeated twice, but in one experiment EU DSD R36 class. used)
58	Polyethylene glycol 400	25322-68-3	liquid	n.p.	undiluted					NI [a]	2	2B				0.2	I	0	I	26	III	Balls et al. (1995) / Prinsen (2000)	
58	Polyethylene glycol 400	25322-68-3	liquid	n.p.	undiluted					2B	2	2B				2	III	1	II	5.88	II	Balls et al. (1995) / Prinsen (2000)	
58	Polyethylene glycol 400	25322-68-3	liquid	n.p.	undiluted					2B	2/NC	2B				1	II	0.5	I	14.8	II/III	Balls et al. (1995) / Prinsen (2000)	
59	Potassium cyanate	590-28-3	solid	97	neat	SCNM	2A/2B (SCNM)	SCNM	1/2	2B	2	2B	2B	2B	2B	1	II	0.7	II	8	II	Balls et al. (1995)	-
59	Potassium cyanate	590-28-3	solid	97	neat					2B [a]	2	2B				0.2	I	0	I	25	III	Balls et al. (1995)	-
59	Potassium cyanate	590-28-3	solid	97	neat					2B	2	2B				1.67	III	1	II	10.45	II	Balls et al. (1995)	-
59	Potassium cyanate	590-28-3	solid	97	neat					2A	2	2A				1.3	II	1.7	III	25.3	III	Balls et al. (1995)	-
60	Promethazine HCl	58-33-3	solid	98	neat	1	1	1	n.a.	1	1	1	1	1	1	2.6	IV	1.6	III	33	IV	Balls et al. (1995)	-
60	Promethazine HCl	58-33-3	solid	98	neat					1	1	1				3	IV	3	IV	143	IV	Balls et al. (1995)	-
60	Promethazine HCl	58-33-3	solid	98	neat					2A	2	2A				3	IV	2	III	23.02	III	Balls et al. (1995)	-
60	Promethazine HCl	58-33-3	solid	98	neat					1/2A	1/2	1/2A				2	III	3	IV	28.6	III/IV	Balls et al. (1995)	-

ANNEX 3 (cont' 7) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
61	Pyridine	110-86-1	liquid	>99	undiluted	1	1	1/2A	n.a.	1	1	1	1	1	1	3	IV	2	III	32.7	IV	Balls et al. (1995)	-
61	Pyridine	110-86-1	liquid	>99	undiluted					1	1	1				3	IV	3	IV	95	IV	Balls et al. (1995)	-
61	Pyridine	110-86-1	liquid	>99	undiluted					1	1	1				3	IV	2.5	III	37.47	IV	Balls et al. (1995)	-
61	Pyridine	110-86-1	liquid	>99	undiluted					1	1	1				3	IV	3	IV	78.6	IV	Balls et al. (1995)	-
62	Quinacrine	69-05-6	solid	n.p.	neat	1	1	1	n.a.	2B	NC	2B	2B	2B	2B	1.2	II	0.6	II	4.1	I	Balls et al. (1995)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438
62	Quinacrine	69-05-6	solid	n.p.	neat					NI	NC	NC				0.2	I	0.2	I	12	II	Balls et al. (1995)	
62	Quinacrine	69-05-6	solid	n.p.	neat					2A	2	2A				2	III	2	III	11.49	II	Balls et al. (1995)	
62	Quinacrine	69-05-6	solid	n.p.	neat					2B	NC	2B				1	II	0.5	I	6.8	II	Balls et al. (1995)	
63	Silver (I) nitrate	7761-88-8	solid	99.5	3%	n.p.	n.p. (NI)	no raw data	n.a.	2B	2	2B	2B	2B	2B	1	II	1	II	12	II	Prinsen and Koëter (1993)	-
64	Sodium dodecyl sulfate	151-21-3	solid	70	undiluted	n.p.	n.p. (1)	no raw data	n.a.	2B	2	2B	2B	1 (2B)	1	0.8	II	1	II	22	III	Prinsen and Koëter (1993)	The SSD reports 'loosening of corneal epithelium' in the ICE test (p.77), which justifies an *in vitro* cat 1 classification
65	Sodium fluorescein	518-47-8	liquid	70	20%	n.p.	n.p. (NI)	no raw data	n.a.	NI	NC	NC	NI	NI	NC	0.1	I	0	I	0	I	Prinsen and Koëter (1993)	-
66	Sodium hydroxide	1310-73-2	liquid	97	1%	1	1	See notes	n.a.	1	1	1	1	1	1	3	IV	3	IV	60	IV	Prinsen and Koëter (1993)	*In vivo* classification proposed by ICCVAM and in SSD does not correspond to correct in vivo class (see chemical 66). Mistake confirmed in reporting concentration tested (it was 10% instead of 1%)
67	Sodium hydroxide (1%)	1310-73-2	liquid	RG	1%	2B	2B	2B	n.a.	2B	2	2B	2A	2B (2A)	2A	1	II	0.6	II	14.1	II/III	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
67	Sodium hydroxide (1%)	1310-73-2	liquid	RG	1%					2A	2	2A				0.7	II	2.3	III	55	IV	Balls et al. (1995)	
67	Sodium hydroxide (1%)	1310-73-2	liquid	RG	1%					2A	2	2A				2.33	III	2.5	III	30.31	III/IV	Balls et al. (1995)	
67	Sodium hydroxide (1%)	1310-73-2	liquid	RG	1%					2A	2	2A				2	III	2	III	33.3	IV	Balls et al. (1995)	
68	Sodium hydroxide (10%)	1310-73-2	liquid	RG	10%	1	1	1	n.a.	1	1	1	1	1	1	3	IV	4	IV	32	III/IV	Balls et al. (1995)	-
68	Sodium hydroxide (10%)	1310-73-2	liquid	RG	10%					1	1	1				3	IV	3.3	IV	194	IV	Balls et al. (1995)	-
68	Sodium hydroxide (10%)	1310-73-2	liquid	RG	10%					1	1	1				3	IV	3.17	IV	68.86	IV	Balls et al. (1995)	-
68	Sodium hydroxide (10%)	1310-73-2	liquid	RG	10%					1	1	1				3	IV	4	IV	151.7	IV	Balls et al. (1995)	-
69	Sodium lauryl sulfate (3%)	151-21-3	liquid	98	3%	NI	NI	NC	n.a.	NI	NC	NC	2B	NI (2B)	2B	1	II	0.2	I	3.9	I	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
69	Sodium lauryl sulfate (3%)	151-21-3	liquid	98	3%					2B	2	2A				0	I	0	I	39	IV	Balls et al. (1995)	
69	Sodium lauryl sulfate (3%)	151-21-3	liquid	98	3%					NI	NC	NC				1	II	0	I	2.75	I	Balls et al. (1995)	
69	Sodium lauryl sulfate (3%)	151-21-3	liquid	98	3%					2B	2	2B				1	II	1	II	15.9	II/III	Balls et al. (1995)	
70	Sodium lauryl sulfate (15%)	151-21-3	liquid	98	15%	1	2A (1)	1	n.a.	2B	NC	2B	2B	2B	2B	0.6	II	0.4	I	7	II	Balls et al. (1995)	-
70	Sodium lauryl sulfate (15%)	151-21-3	liquid	98	15%					2A [(a)]	2	2A				1	II	0.2	I	33	IV	Balls et al. (1995)	-
70	Sodium lauryl sulfate (15%)	151-21-3	liquid	98	15%					2B	2	2B				1.67	III	1	II	9.56	II	Balls et al. (1995)	-
70	Sodium lauryl sulfate (15%)	151-21-3	liquid	98	15%					2B	2	2B				1	II	1	II	12.2	II/III	Balls et al. (1995)	-
71	Sodium oxalate	62-76-0	solid	>99	neat	1	1	1	n.a.	2B	2	2B	2B	2B	2B	0.7	II	0.7	II	6.3	II	Balls et al. (1995)	-
71	Sodium oxalate	62-76-0	solid	>99	neat					2B [(a)]	NC	2B				0.2	I	0	I	24	III	Balls et al. (1995)	-
71	Sodium oxalate	62-76-0	solid	>99	neat					NI	NC	NC				0.5	I	0.5	I	2.62	I	Balls et al. (1995)	-
71	Sodium oxalate	62-76-0	solid	>99	neat					NI	NC	NC				1	II	0	I	2.4	I	Balls et al. (1995)	-

ANNEX 3 (cont' 8) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
72	Sodium perborate, $4H_2O$	10486-00-7	solid	98.6	neat	1	1	1	n.a.	NI	NC	NC	2B	2B	2B	0.6	II	0.5	I	3.1	I	Balls et al. (1995)	-
72	Sodium perborate, $4H_2O$	10486-00-7	solid	98.6	neat					2B	2	2B				0.2	I	0.7	II	23	III	Balls et al. (1995)	-
72	Sodium perborate, $4H_2O$	10486-00-7	solid	98.6	neat					2B	2	2B				1.33	II	1	II	7.54	II	Balls et al. (1995)	-
72	Sodium perborate, $4H_2O$	10486-00-7	solid	98.6	neat					2B	2/NC	2B				1	II	0.5	I	14.6	II/III	Balls et al. (1995)	-
73	Tetraaminopyrimidine sulfate	5392-28-9	solid	97	neat	NI	NI	NC	n.a.	2B	2	2B	2B	2B	2B	1.3	II	1	II	7.5	II	Balls et al. (1995)	*In vivo* GHS NC in the ECETOC report, and *in vivo* GHS Cat 2A in the study from Gautheron et al. (1994). If data on the two sudies are combined, that would result on a GHS NC
73	Tetraaminopyrimidine sulfate	5392-28-9	solid	97	neat					2A	2	2A				1	II	2	III	31	III/IV	Balls et al. (1995)	
73	Tetraaminopyrimidine sulfate	5392-28-9	solid	97	neat					2B	2	2B				1.5	II	1.5	II	7.3	II	Balls et al. (1995)	
73	Tetraaminopyrimidine sulfate	5392-28-9	solid	97	neat					2B	2	2B				1	II	1	II	8.9	II	Balls et al. (1995)	
74	TNO-01 (Formulation-1)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	0	I	Prinsen (1996)	-
75	TNO-02 (Formulation-2)	n.p.	liquid	n.p.	undiluted	2A/n.p.	2A	2A	n.a.	2A	2	2A	2A	2A	2A	2.7	IV	2	III	24	III	Prinsen (1996)	-
76	TNO-03 (Pesticide-1)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.6	II	0.3	I	3	I	Prinsen (1996)	-
77	TNO-04 (Detergent-1)	n.p.	liquid	n.p.	undiluted	2A/n.p.	2A	2A	n.a.	2B	2	2B	2B	2B	2B	1.5	II	1.5	II	9	II	Prinsen (1996)	-
78	TNO-05 (Silicone powder-1)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	0	I	Prinsen (1996)	-
79	TNO-06 (Lubricant)	n.p.	gel	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	1	I	Prinsen (1996)	-
80	TNO-07 (Ink-1)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.8	II	0	I	2	I	Prinsen (1996)	-
81	TNO-08 (Ink-2)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.1	I	0	I	3	I	Prinsen (1996)	-
82	TNO-09 (Paint)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	1.3	II	0.5	I	5	I	Prinsen (1996)	-
83	TNO-10 (Silicone powder-2)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	1	I	Prinsen (1996)	-
84	TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	solid	n.p.	undiluted	SCNM/n.p.	2A (SCNM)	2A	n.a.	2A	2	2A	2A	2A	2A	2	III	1.3	II	19	III	Prinsen (1996)	-
85	TNO-12 (Formulation-3)	n.p.	paste	n.p.	undiluted	NI/n.p.	1 (NI)	SCNM	1/2	2A	2	2A	2A	2A/1 (2A)	2A	2.5	III	2	III	35	IV	Prinsen (1996)	-
86	TNO-13 (Pesticide-2)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.7	II	0	I	1	I	Prinsen (1996)	-
87	TNO-14 (Polydisaccharide)	n.p.	liquid	n.p.	14.5%	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.3	I	0	I	2	I	Prinsen (1996)	-
88	TNO-15 (Polydisaccharide)	n.p.	liquid	n.p.	50%	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	2	I	Prinsen (1996)	-
89	TNO-16 (Liquid nylon product)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	1	I	Prinsen (1996)	-
90	TNO-17 (Solvent-1)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.3	I	0	I	0	I	Prinsen (1996)	-
91	TNO-18 (Solvent-2)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	0	I	Prinsen (1996)	-
92	TNO-19 (Solvent-3)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	0	I	Prinsen (1996)	-
93	TNO-20 (Solvent-4)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.5	I	0.3	I	3	I	Prinsen (1996)	-
94	TNO-21 (Solvent-5)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.3	I	0.3	I	0	I	Prinsen (1996)	-
95	TNO-22 (Solvent-6)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.2	I	0.3	I	0	I	Prinsen (1996)	-
96	TNO-23 (Solvent-7)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.2	I	0	I	2	I	Prinsen (1996)	-
97	TNO-24 (Solvent-8)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.2	I	0	I	3	I	Prinsen (1996)	-
98	TNO-25 (Solvent-9)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	1	I	Prinsen (1996)	-
99	TNO-26 (Ink-3)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.1	I	0	I	0	I	Prinsen (1996)	-

ANNEX 3 (cont' 9) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
100	TNO-27 (Thermal paper coating-1)	n.p.	liquid	n.p.	undiluted	2B/n.p.	2B	2B	n.a.	2B	2	2B	2B	2B	2B	1	II	0.6	II	9	II	Prinsen (1996)	-
101	TNO-28 (Toilet cleaner-1)	n.p.	liquid	n.p.	undiluted	1/n.p.	2B (1)	1/2A	n.a.	2B	2	2B	2B	2B	2B	1.4	II	0.8	II	12	II	Prinsen (1996)	-
102	TNO-29 (Toilet cleaner-2)	n.p.	liquid	n.p.	undiluted	2A/n.p.	2B/2A (2A)	2A	n.a.	2B	2	2B	2B	2B	2B	1.3	II	1	II	11	II	Prinsen (1996)	-
103	TNO-30 (Pesticide-3)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	2	2B	2B	2B	2B	1.5	II	1	II	7	II	Prinsen (1996)	-
104	TNO-31 (Sulfur)	7704-34-9	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.2	I	0	I	1	I	Prinsen (1996)	-
105	TNO-32 (Ink-4)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	NC	2B	2B	2B	2B	1	II	0.5	I	7	II	Prinsen (1996)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438
106	TNO-33 (Thermal paper coating-2)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B [a]	2	2B	2B	NI (2B)	2B	2	III	0.5	I	5	I	Prinsen (1996)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438
107	TNO-34 (Detergent-2)	n.p.	liquid	n.p.	undiluted	SCNM/n.p.	1 (SCNM)	SCNM	1/2	1	2	2B	1	1	2B	1	II	1	II	25	III	Prinsen (1996)	The rationale for the *in vitro* classification proposed by ICCVAM and in the SSD appears unclear
108	TNO-35 (Propyl-lactate)	616-09-1	liquid	n.p.	undiluted	1/n.p.	1	1	n.a.	1	1	1	1	1	1	3	IV	3	IV	45	IV	Prinsen (1996)	-
109	TNO-36 (Ethylhexyl lactate)	6283-86-9	liquid	n.p.	undiluted	SCNM/n.p.	2A (SCNM)	SCNM	2	2A	2	2A	2A	2A	2A	2	III	2	III	18	II/III	Prinsen (1996)	-
110	TNO-37 (Pesticide-4)	n.p.	solid	n.p.	undiluted	2B/n.p.	2B	2B	n.a.	2B	2	2B	2B	2B	2B	1.5	II	1	II	15	II/III	Prinsen (1996)	-
111	TNO-38 (Solvent-10)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	3	I	Prinsen (1996)	-
112	TNO-39 (Detergent-3)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.5	I	0.5	I	4	I	Prinsen (1996)	-
113	TNO-40 (Glycolbromoacetate form.)	n.p.	liquid	n.p.	undiluted	n.p.	1 (n.p.)	no raw data	n.a.	1	1	1	1	1	1	2.6	IV	1.9	III	41	IV	Prinsen (1996)	-
114	TNO-41 (Amidosulfonic acid)	5329-14-6	solid	n.p.	undiluted	n.p.	1 (n.p.)	no raw data	n.a.	1	1	1	1	1	1	2.7	IV	4	IV	46	IV	Prinsen (1996)	-
115	TNO-42 (Glycolbromoacetate)	3785-34-0	liquid	n.p.	85%	n.p.	1 (n.p.)	no raw data	n.a.	1	1	1	1	1	1	3	IV	3	IV	36	IV	Prinsen (1996)	-
116	TNO-43 (Monobromoacetic acid)	79-08-3	solid	n.p.	undiluted	n.p.	1 (n.p.)	no raw data	n.a.	1	1	1	1	1	1	3	IV	4	IV	80	IV	Prinsen (1996)	-
117	TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	liquid	n.p.	23%	n.p.	1 (SC) (n.p.)	n.a.	n.a.	1	1	1	1	1	1	3	IV	3.5	IV	39	IV	Prinsen (1996)	*In vivo* classification derived from skin corrosion test
118	TNO-45 (Aqueous framing solution)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	1	I	0.5	I	5	I	Prinsen (2005)	-
119	TNO-46 (Raw material powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	1	I	Prinsen (2005)	-
120	TNO-47 (Ferro powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	1	I	Prinsen (2005)	-
121	TNO-48 (Corrosion inhibitor liquid)	n.p.	liquid	n.p.	undiluted	n.p.	1(SC) (n.p.)	n.a.	n.a.	2A	2	2A	2A	2A	2A	3	IV	1	II	25	III	Prinsen (2005)	*In vivo* classification derived from skin corrosion test
122	TNO-49 (Wood impregnator liquid)	n.p.	liquid	n.p.	undiluted	n.p.	1(SC) (n.p.)	n.a.	n.a.	1	1	1	1	1	1	3	IV	4	IV	n.p.	n.p.	Prinsen (2005)	*In vivo* classification derived from skin corrosion test
123	TNO-50 (Sodium hypochlorite-containing formulation)	n.p.	n.p.	n.p.	undiluted	n.p.	1(SC) (n.p.)	n.a.	n.a.	1	1	1	1	1	1	3	IV	3	IV	41.1	IV	Prinsen (2005)	*In vivo* classification derived from skin corrosion test
124	TNO-51 (Disinfectant)	n.p.	n.p.	n.p.	undiluted	n.p.	1(SC) (n.p.)	n.a.	n.a.	1	1	1	1	1	1	3	IV	3	IV	33.9	IV	Prinsen (2005)	*In vivo* classification derived from skin corrosion test
125	TNO-52 (Pesticide liquid)	n.p.	liquid	n.p.	undiluted	2A/n.p.	2A	2A	n.a.	2B	2	2B	2B	2B	2B	1.7	III	1	II	5	I	Prinsen (2005)	-
126	TNO-53 (Ink formulation)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.5	I	0.2	I	3	I	Prinsen (2005)	-
127	TNO-54 (Raw material powder)	n.p.	solid	n.p.	undiluted	2B/n.p.	2B	2B	n.a.	2B	2	2B	2B	2B	2B	1	II	1	II	9	II	Prinsen (2005)	-
128	TNO-55 (Elastomer liquid)	n.p.	liquid	n.p.	undiluted	2A/n.p.	2A	2A	n.a.	2B	2	2B	2B	2B	2B	1.7	III	1.3	II	10	II	Prinsen (2005)	-
129	TNO-56 (Elastomer liquid)	n.p.	liquid	n.p.	undiluted	2B/n.p.	2B	2B	n.a.	2B	2	2B	2B	2B	2B	2	III	1.3	II	10	II	Prinsen (2005)	-
130	TNO-57 (Epoxy resin liquid)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	2	2B	2B	2B	2B	1.5	II	1.3	II	12	II	Prinsen (2005)	-
131	TNO-58 (Styrene resin powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	-1	I	Prinsen (2005)	-
132	TNO-59 (Ferro powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.2	I	0	I	-2	I	Prinsen (2005)	-
133	TNO-60 (Fungicide paint)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.2	I	0.5	I	1	I	Prinsen (2005)	-
134	TNO-61 (Silver thiosulfate liquid)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	1	I	Prinsen (2005)	-

ANNEX 3 (cont' 10) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concentration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
135	TNO-62 (Lactate liquid)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	SCNM	NC	2B	2	2B	2B	2B	2B	2	III	1	II	12	II	Prinsen (2005)	-
136	TNO-63 (Copolymer powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.3	I	0.5	I	3	I	Prinsen (2005)	-
137	TNO-64 (Fluoroallyl acrylate copolymer)	n.p.	emulsion	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	NC	2B	2B	NI	2B	1	II	1	II	5	I	Prinsen (2005)	-
138	TNO-65 (Fluoroallyl acrylate copolymer)	n.p.	emulsion	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.7	II	0.5	I	4	I	Prinsen (2005)	-
139	TNO-66 (Raw material powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	0	I	Prinsen (2005)	-
140	TNO-67 (Ink formulation)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	2	2B	2B	2B	2B	1	II	1	II	6	II	Prinsen (2005)	-
141	TNO-68 (Cleaning product)	n.p.	liquid	n.p.	undiluted	2A/n.p.	2A	2A	n.a.	2A	2	2B	2A	2A	2B	1/2	II/III	1	II	8	II	Prinsen (2005)	The rationale for the *in vitro* classification proposed by ICCVAM and in the SSD appears unclear
142	TNO-69 (Cleaning product)	n.p.	liquid	n.p.	50%	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	1	II	0	I	0	I	Prinsen (2005)	-
143	TNO-70 (Fluoroallyl acrylate copolymer)	n.p.	emulsion	n.p.	undiluted	2A/n.p.	2A	2A	n.a.	2A	2	2A	2A	2A	2A	2	III	1	II	20	III	Prinsen (2005)	-
144	TNO-71 (Fluoroallyl acrylate copolymer)	n.p.	emulsion	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	2/NC	2B	2B	2B	2B	1	II	0.5	I	13	II/III	Prinsen (2005)	-
145	TNO-72 (Fluoroallyl acrylate copolymer)	n.p.	emulsion	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	1.5	II	0.5	I	5	I	Prinsen (2005)	-
146	TNO-73 (Fluoroallyl acrylate copolymer)	n.p.	emulsion	n.p.	undiluted	2A/n.p.	2A/1 (2A)	2A	1	1	2	1	1	2A/1 (2A)	1	2.7	IV	2	III	18	II/III	Prinsen (2005)	The ICE test showed 'loosening of corneal epithelium' which justifies an *in vitro* Cat. 1 classification
147	TNO-74 (Raw material powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.5	I	0	I	0	I	Prinsen (2005)	-
148	TNO-75 (Fluoroallyl acrylate copolymer)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	2	I	Prinsen (2005)	-
149	TNO-76 (Ferro powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	2	I	Prinsen (2005)	-
150	TNO-77 (Raw material liquid)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	NC	2B	2B	NI	2B	1	II	0.5	I	7	II	Prinsen (2005)	-
151	TNO-78 (Raw material liquid)	n.p.	liquid	n.p.	undiluted	2B/n.p.	2B	2B	n.a.	2B	2	2B	2B	2B	2B	1.3	II	1	II	15	II/III	Prinsen (2005)	-
152	TNO-79 (Silicon resin powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	2	2B	2B	2B	2B	1	II	1	II	10	II	Prinsen (2005)	-
153	TNO-80 (Raw material powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.3	I	0	I	-1	I	Prinsen (2005)	-
154	TNO-81 (Surfactant liquid)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	1	I	Prinsen (2005)	-
155	TNO-82 (Surfactant liquid)	n.p.	n.p.	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	-2	I	Prinsen (2005)	-
156	TNO-83 (Surfactant liquid)	n.p.	n.p.	n.p.	undiluted	2B/n.p.	2B	2B	n.a.	2B	2	2B	2B	2B	2B	0.8	II	0.7	II	10	II	Prinsen (2005)	-
157	TNO-84 (Surfactant liquid)	n.p.	n.p.	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	NC	2B	2B	NI	2B	0.7	II	0.7	II	2	I	Prinsen (2005)	-
158	TNO-85 (Surfactant liquid)	n.p.	n.p.	n.p.	undiluted	1/n.p.	2B (1)	1	n.a.	2B	2	2A/2B	2B	2B	2A/2B	2	III	1.3	II	14	II/III	Prinsen (2005)	-
159	TNO-86 (Surfactant liquid)	n.p.	n.p.	n.p.	undiluted	NI/n.p.	NI	SCNM	NC	2B	2	2B	2B	2B	2B	1	II	1	II	7	II	Prinsen (2005)	-
160	TNO-87 (Enzyme liquid)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	2B	NC	2B	2B	2B	2B	0.7	II	1	II	1	I	Prinsen (2005)	-
161	TNO-88 (Miscellaneous liquid)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.3	I	0.7	II	3	I	Prinsen (2005)	-
162	TNO-89 (Ferro powder)	n.p.	solid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.2	I	0.7	II	1	I	Prinsen (2005)	-
163	TNO-90 (Enzyme solution)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0	I	2	I	Prinsen (2005)	-
164	TNO-91 (Enzyme solution)	n.p.	liquid	n.p.	undiluted	NI/n.p.	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0	I	0.2	I	1	I	Prinsen (2005)	-
165	TNO-92 (raw material solid)	n.p.	solid	n.p.	undiluted	1/n.p.	1	SCNM	1	2B	2	2A/2B	2B	2B	2A/2B	0.8	II	1.7	III	16	II/III	Prinsen (2005)	-
166	TNO-93 (Antifouling paint)	n.p.	emulsion	n.p.	undiluted	1/n.p.	1	SCNM	1	2A	2	2A	2A	2A	2A	3	IV	2	III	17	II/III	Prinsen (2005)	-
167	TNO-94 (anti-fouling paint)	n.p.	liquid	n.p.	undiluted	1/n.p.	1	1	n.a.	NI	NC	NC	NI	NI	NC	1	II	0.5	I	2	I	Prinsen (2005)	-
168	Toluene	108-88-3	liquid	99	undiluted	NI	NI	NC	n.a.	2B	2	2B	2A	2B (2A)	2A	1.4	II	1	II	5.2	II	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
168	Toluene	108-88-3	liquid	99	undiluted					2A	2	2A				2	III	1.3	II	29	II/IV	Balls et al. (1995)	
168	Toluene	108-88-3	liquid	99	undiluted					2A/2B	2	2A/2B				1.33	II	2	III	13.87	II/III	Balls et al. (1995)	
168	Toluene	108-88-3	liquid	99	undiluted					2A	2	2A				1	II	2	III	58.2	IV	Balls et al. (1995)	

ANNEX 3 (cont' 11) – Compiled dataset of GHS *in vivo* and *in vitro* classifications and *in vitro* individual data on the ICE test method

Highlighted in orange are the *in vitro* classifications which do not seem to be in agreement with the respective DIP.

NO	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concen-tration tested	*In Vivo* Draize GHS (reported by ICCVAM)	*In Vivo* Draize GHS (reported in SSD)	*In Vivo* Draize GHS (sug-gested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (ICCVAM BRD - criteria OECD GD160)	*In Vitro* ICE (revised criteria on TG 438)	*In Vitro* ICE (sug-gested)	Overall *In Vitro* ICE (reported in ICCVAM BRD)	Overall *In Vitro* ICE (reported in SSD)	Overall *In Vitro* ICE (sug-gested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score	Corneal Swelling Category	Reference	Notes
169	Toluene	108-88-3	liquid	99.9	undiluted	n.p.	NI	NC	n.a.	2B	NC	2B	2B	NI	2B	1.1	II	1.4	II	4	I	Prinsen and Koëter (1993)	-
170	Triacetin	102-76-1	liquid	99	undiluted	NI	NI	NC	n.a.	NI	NC	NC	NI	NI	NC	0.5	I	0.4	I	4	I	Prinsen and Koëter (1993)	-
171	Tributyltin chloride	1461-22-9	liquid	96	undiluted	n.p.	n.p. (1)	no raw data	n.a.	1	1	1	1	1	1	3	IV	2.5	III	48	IV	Prinsen and Koëter (1993)	-
172	Trichloroacetic acid (3%)	76-03-9	liquid	RG	3%	NI	NI	NC	n.a.	2A/2B	2B	2B	2A	2B (2A)	2A	2.4	III	1.2	II	13.2	II	Balls et al. (1995)	*The in vitro* classification proposed in SSD seems based on results of one lab only
172	Trichloroacetic acid (3%)	76-03-9	liquid	RG	3%					2A	2	2A				2.3	III	2	III	38	IV	Balls et al. (1995)	
172	Trichloroacetic acid (3%)	76-03-9	liquid	RG	3%					2A	2	2A				1.5	II	2.5	III	27.88	III/IV	Balls et al. (1995)	
172	Trichloroacetic acid (3%)	76-03-9	liquid	RG	3%					2A	2	2A				1.7	III	2	III	26.4	III/IV	Balls et al. (1995)	
173	Trichloroacetic acid (30%)	76-03-9	liquid	RG	30%	1	1	1	n.a.	1	1	1	1	1	1	3	IV	4	IV	32	III/IV	Balls et al. (1995)	-
173	Trichloroacetic acid (30%)	76-03-9	liquid	RG	30%					1	1	1				3	IV	4	IV	153	IV	Balls et al. (1995)	-
173	Trichloroacetic acid (30%)	76-03-9	liquid	RG	30%					1	1	1				3	IV	4	IV	(b)	(b)	Balls et al. (1995)	-
174	Triethanolamine	102-71-6	liquid	99	undiluted	NI/n.p.	NI	NC	n.a.	2B	NC	2B	2B	2B	2B	0.9	II	0.7	II	4	I	Prinsen and Koëter (1993)	The *in vitro* classification proposed in SSD does not correspond to criteria proposed in revised TG 438
175	Triton X-100 (5%)	9002-93-1	liquid	98	5%	2A/2B	2A	2A	n.a.	2B	2	2B	2A	2B (2A)	2A	1	II	0.6	II	9.8	II	Balls et al. (1995)	-
175	Triton X-100 (5%)	9002-93-1	liquid	98	5%					2A [(a)]	2	2A				1.3	II	0	I	38	IV	Balls et al. (1995)	-
175	Triton X-100 (5%)	9002-93-1	liquid	98	5%					2B [(a)]	2	2B				2	III	0	I	3.97	I	Balls et al. (1995)	-
175	Triton X-100 (5%)	9002-93-1	liquid	98	5%					2A	2	2A				1	II	2	III	39.6	IV	Balls et al. (1995)	-
176	Triton X-100 (10%)	9002-93-1	liquid	98	10%	1/2A	(2A) 1	1	n.a.	2B	NC	2B	2A/2B	2B (2A/2B)	2B	1.4	II	0.1	I	9.9	II	Balls et al. (1995)	-
176	Triton X-100 (10%)	9002-93-1	liquid	98	10%					2A/2B	2	2A/2B				1	II	0.8	II	29	III/IV	Balls et al. (1995)	-
176	Triton X-100 (10%)	9002-93-1	liquid	98	10%					2A	2	2A				2.67	IV	1.17	II	20.2	III	Balls et al. (1995)	-
176	Triton X-100 (10%)	9002-93-1	liquid	98	10%					2B	2	2B				1.7	III	1	II	11.2	II	Balls et al. (1995)	-
177	Triton X-500 (5%)	n.p.	liquid	n.p.	undiluted	n.p./NI	n.p. (2A)	2A	n.a.	2B	2	2B	2B	2B	2B	1	II	0.7	II	14	II/III	Prinsen (2000)	-
177	Triton X-500 (5%)	n.p.	liquid	n.p.	undiluted					2B	2	2B				1	II	0.7	II	14	II/III	Prinsen (2000)	-
177	Triton X-500 (5%)	n.p.	liquid	n.p.	undiluted					2B	2	2B				1	II	0.7	II	13	II/III	Prinsen (2000)	-
177	Triton X-500 (5%)	n.p.	liquid	n.p.	undiluted					2B	2	2B				1	II	0.8	II	8	II	Prinsen (2000)	-
177	Triton X-500 (5%)	n.p.	liquid	n.p.	undiluted					2B	2	2B				1	II	0.7	II	11	II	Prinsen (2000)	-
178	Tween 20	9005-64-5	liquid	98	undiluted	NI	NI	NC	n.a.	2B	NC	2B	2B	2B	2B	1	II	1	II	3.6	I	Balls et al. (1995)	-
178	Tween 20	9005-64-5	liquid	98	undiluted					2B (a)	2	2B				0.2	I	0	I	31	III/IV	Balls et al. (1995)	-
178	Tween 20	9005-64-5	liquid	98	undiluted					2B	2	2B				2.5	III	1	II	5.63	II	Balls et al. (1995)	-
178	Tween 20	9005-64-5	liquid	98	undiluted					2B	NC	2B				1	II	0.5	I	6.7	II	Balls et al. (1995)	-

n.a.= not applicable; NC = not classified; NI = non-irritating; n.p.= not provided; RG = Reagent Grade; SC = classification assigned on the basis of skin corrosion assay; SCNM = Study criteria not met.

[(a)] combination of effects not defined by ICCVAM and OECD GD 160 proposed criteria for classification; [(b)] solubility uncertain

ANNEX 4

Literature review on the limitations of the *in vivo* Draize rabbit eye test

Extract from a Guidance Document prepared by Eskes C. on behalf of the Swiss Federal Office of Public Health, 2010[5]

While the current *in vivo* Draize eye irritation test method has been widely used, the test has often been reason for criticism. Besides the fact that the Draize test can be very painful to the rabbits, some of the drawbacks referred in literature and detailed below are:

- The type of exposure which does not reflect a potential human accidental exposure;
- The ill-defined duration of exposure to the test material;
- The limited reproducibility within and between the laboratories that may be due to the ill-defined duration of exposure, but also to the subjectivity in the allocation of the respective scores and to the rabbits individual variability;
- The differences in physiology and sensitivity to tested substances between rabbit and human eyes;
- Ethical issues.

1. *In vivo* exposure to test substance

The Draize rabbit eye test was initially developed to evaluate products that come into contact with the eye and ocular adnexa such as ophtamological preparations and cosmetics. Only later it has been incorporated into testing guidelines for industrial chemicals, household products or pesticides to estimate accidental exposure to the human eye (Wilhelmus, 2001[9]). However, such exposure conditions, i.e., in the conjunctival sac and manual eyelid closure that prolong the duration and extend of exposure, are not consistent with accidental exposures of the human eye which generally occur by direct contact with the corneal surface (Griffith *et al.*, 1980[10]). It is believed that the response experienced by humans would be more readily duplicated in animals by corneal application than by conjunctival instillation (ILSI, 1996[11]).

Another criticism is that in the Draize rabbit eye test the precise exposure times and/or delivery of dosage remain actually unknown. It might depend on the time the animals take to flush the test material from the eyes, and on the test material properties. For example, for aqueous and non-viscous formulations the standard instillation results in a rapid removal of the material within seconds/minutes through blinking with the nictitating membrane (third eye-lid) and grooming by the rabbit. This contrasts with the situation for sticky pastes for example, which cannot be removed that easily. In these cases, the contact time may vary from a couple of minutes to 24 h, because rinsing the eye is not allowed before the 24 hours reading.

The most dramatic variation in contact time and dosage occurs with solids. Even if applied as a 0.1 ml equivalent (the content of the cul-de-sac), the actual amount of a powder/solid that stays in contact with the eye is unpredictable. The contact time may also vary from a couple

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[9] Wilhelmus, K.R. (2001). The Draize eye test. *Survey of Ophthalmology* **45**, 493-515.

[10] Griffith JF, Nixon GA, Bruce RD, Reer PJ, Bannan EA (1980). Dose-response studies with chemical irritants in the albino rabbit eye as basis for selecting optimum testing conditions for predicting haard to the human eye. *Toxicology and Applied Pharmacology* **55**, 501-513.

[11] ILSI Health and Environmental Sciences Institute (1996). Replacing the Draize eye irritation test : scientific background and research needs. *J. Toxicol. Cut. & Ocular Toxicol.* **15**, 211-234.

of minutes to 24 h depending on the guidelines and their application. Indeed, if generally rinsing the eye is not allowed before the 24 hours reading, in the updated OECD TG 405 from 2002 an exception is made for solids, which may be washed 1 hour after exposure. Still enclosure of test materials in the conjunctival cul-de-sac in combination with mechanical damage, could have devastating effects. For example in the case of poorly water-soluble solids with distinct cytotoxic properties, swelling of the conjunctivae may occur making it even more difficult for the animal to remove the test material. Such forced continuous exposure for up to 24 hours may result in a complete closure of the eye lids by the abundant production of colloidal discharge which often forms a sealing crust. Upon opening these sealed eye-lids, purulent discharge, and other inflammatory debris are released. The degree of swelling of the conjunctivae can be sufficiently severe such that removal of any remains of the test substance is hardly possible anymore. Also in the case of less severe effects, the eye can become vulnerable to microbiological infection, or secondary inflammation process, causing initial mild to moderate effects during the first days after exposure developing into more severe and prolonged effects during the 21 day observation period.

Such uncertainty in the true amount, duration and delivery of the test material, may lead to variations on the way animals lacrimate and flush out the test material resulting in possible variation in the reactions from the animals even before the scoring of effects takes place (Prinsen, 2006[12]).

2. Variability of the Draize eye irritation test

Several studies report on a limited within- and between-laboratory reproducibility of the Draize rabbit eye test, found especially in the middle range of mild to moderate irritating compounds (Wilhelmus, 2001[8]; ILSI, 1996[10]; Balls *et al.*, 1999[13]). In addition to the variations linked to the exposure conditions as described above (e.g., rate of release of the test product from the delivery vehicle, the amount of reflex tearing, the exposure duration and timing of post-exposure irrigation) other described sources of variation include the subjective scoring systems and the differences in the individual animal responses of the same species and strain (Wilhelmus, 2001[8]; Eskes *et al.*, 2005[14]; Balls *et al.*, 1999[12]).

The largest study on variability is perhaps the one from Weil and Scala (1971[15]) who studied 9 substances tested in up to 24 laboratories. The authors show that the Draize eye test can produce quite variable results among laboratories as well as within certain laboratories. Certain materials were rated as the most irritating tested by some laboratories and, contrariwise, as the least irritating by others. Figure 1 represents an extract of Weil and Scala's findings, where the maximum and minimum eye irritation scores found for the different tested substances and laboratories are given (the minimum and maximum attributable rates being 0 and 110 respectively). As it can be seen, the test material with the smallest variability had scores varying from 0 to 63. The authors suggest that the primary reason for the observed extreme variation between laboratories is in the reading of reactions. Unconscious bias or definite tendencies to over- or under-read reactions or misinterpret the meaning of descriptive terms may have accounted for that. In addition, variation in

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[12] Prinsen M.K. (2006). The Draize eye test and in vitro alternatives, a left-handed marriage? *Toxicology In Vitro* **20**,78-81.

[13] Balls, M., Berg, N., Bruner, L.H., Curren, R., deSilva, O., Earl, L.K., Esdaile, D.J., Fentem, J.H., Liebsch, M., Ohno, Y., Prinsen, M.K., Spielmann, H. & Worth, A.P. (1999). Eye irritation testing: the way forward. The report and recommendations of ECVAM workshop 34. *ATLA* **27**, 53-77.

[14] Eskes C., Bessou S, Bruner L, Curren R, Harbell J, Jones P., Kreiling R, Liebsch M, McNamee P, Pape W, Prinsen M, Seidle T, Vanparys P, Worth A, Zuang V (2005). Subchapter 3.3. Eye Irritation. In *Alternative (non-animal) Methods for Cosmetics Testing: Current Status and Future Prospects* (Eskes C., Zuang V. eds). *ATLA* **33**, Suppl. 1, 47-81

[15] Weil C.S., Scala A. (1971). Study of intra- and inter- laboratory variability in the results of rabbit eye and skin irritation tests. *Toxicology and Applied Pharmacoogy* **19**, 276-360.

interpretation of and performance of the procedures was also reported as a component for the observed interlaboratory variability (Weil and Scala, 1971[14]).

TABLE 12

MINIMUM-MAXIMUM SCORE FOR EYES OF INDIVIDUAL RABBITS—24 HR REFERENCE PROCEDURE

Laboratory No.*	Material A	F	G	J	K	L	M	N	P	Any material
14	38–58	58–60	26–60	56–60	18–62	40–68	52–60	21–60	26–52	18–68
29	25–65	58–70	37–90	28–71	14–43	32–53	30–37	23–38	6–36	6–90
4	30–62	46–94	0–25	9–57	59–99	24–65	37–83	37–63	26–63	0–99
12	17–44	30–59	11–90	39–59	14–63	39–63	34–46	37–46	39–46	11–90
1	23–59	35–57	55–86	23–48	10–37	31–59	23–45	6–18	18–64	6–86
10	9–33	40–57	39–59	26–55	8–42	40–57	6–59	2–53	9–35	2–59
31	25–55	26–35	13–53	19 33	8–52	24–53	9–72	9–57	9–37	8–72
22	13–37	30–41	4–79	6–37	10–35	29–39	0–39	0–35	10–35	0–79
2	4–100	25–54	0–23	4–57	4–49	44–63	4–92	8–61	0–65	0–100
9	20–37	26–34	12–61	0–43	10–37	26–37	30–37	18–34	11–32	0–61
11	11–28	11–37	37–71	2–37	6–39	14–39	32–37	23–34	2–34	2–71
23	15–35	24–35	0–61	35–41	8–41	30–39	26–39	9–34	18–37	0–61
25	35–39	23–35	68–92	10–36	6–39	11–37	33–37	4–35	10–37	4–92
21	9–43	74–80	22–56	49–83	10–12	32–76	0–6	6–15	0–8	0–83
5	8–27	6–31	17–29	20–31	18–26	20–24	17–25	20–27	17–31	6–31
19	0–12	15–19	33–70	9–43	17–55	22–53	0–45	20–49	6–13	0–70
16	15–25	8–18	8–110	17–23	18–30	19–25	8–22	8–23	4–16	4–110
24	13–21	8–37	35–63	8–37	6–23	19–34	6–23	15–21	8–17	6–63
7	2–36	42–50	40–72	11–26	11–30	0–11	0–4	0–2	0–2	0–72
13	0–37	2–39	0–57	0 30	0–14	6–39	6–16	6–16	12–32	0–57
18	0–28	16–21	17–62	2–13	6–45	7–16	16–26	2–12	10–18	0–62
8	0–29	8–18	55–86	2–47	2–11	2–14	6–15	4–25	6–35	0–86
27	2–23	14–24	2–66	0–26	all 4	7–26	2–29	2–31	4–21	0–66
30	2–6	12–19	2–30	8–16	13–22	13–25	2–42	13–15	0–4	0–42
Any laboratory	0–100	2–80	0–110	0–83	0–99	0–76	0–83	0–63	0–65	—

* Laboratories ordered by sum of ranks of 24-hr medians as in Table 26.

Figure 1: Extract from Weil and Scala (1971[14]). Minimum and maximum scores for eye irritation of individual rabbits.

Marzulli and Ruggles (1973[16]) have studied 7 test materials in 10 laboratories and have confirmed the findings from Weil and Scala. Although the authors suggest that laboratories were able in most cases to distinguish eye irritants from non irritants, statistically significant differences were found between collaborators with regard to the tissue readings. A quantification of the Draize rabbit eye test variation has been done by Cormier *et al*. (1996[17]). The authors have estimated the coefficient of variation (CV) of the Draize Maximum Average Scores (MAS) obtained for 1 material tested in several laboratories in 13 studies, and found it to be in the order of 38%. The authors also calculated the CVs from the MAS scores obtained at the 24h observation time in the work of Weil and Scala (1971[14]), and have found it to range from 42 to 59% for the 9 test materials tested in 24 laboratories.

Gettings and co-workers (1996[18]) have studied 25 surfactant-based formulations using 6 replicate animals tested in different randomized blocks to reflect within-laboratory between-test variability. They have found a higher standard error for the middle range of mild to

[16] Marzulli FN, Ruggles DI (1973). Rabbit eye irritation test: collaborative study. *J. Ass. Off. Analyt. Chem.* **56**, 905-914.

[17] Cormier EM, Parker RD, Henson C, Cruze LW, Merritt AK, Bruce RD, Osborne R (1996). Determination of the intra- and inter-laboratory reproducibility of the Low Volume Eye Test and its statistical relationship to the Draize tes. *Reg. Tox. Pharmac.* **23**, 156-161.

[18] Gettings, S.D., Lordo, R.A., Hintze, K.L., Bagley, D.M., Casterton, P.L., Chudkowski, M., Curren, R.D., Demetrulias, J.L., DiPasquale, L.C., Earl, L.K., Feder, P.I., Galli, C.L., Glaza, S.M., Gordon, V.C., Janus, J., Kurtz, P.J., Marenus, K.D., Moral, J., Pape, W.J.W., Renskers, K.J., Rheins, L.A., Roddy, M.T., Rozen, M.G., Tedeschi, J.P. & Zyracki, J. (1996). The CTFA evaluation of alternatives program: an evaluation of *in vitro* alternatives to the Draize primary eye irritation test. (phase III) Surfactant-based formulations. *Food and Chemical Toxicology* **34**, 79-117.

moderate irritants. Earl and co-workers (1997[19]) made a review of existing studies on the Draize rabbit eye test variability and also show that variability, as measured by the standard deviation (SD) of the MAS, was consistently found to be greatest in the middle ranges of irritancy , and the lowest at the extremes of the scales. Ohno and co-workers (1999[20]) also confirmed these findings and showed that variation in Draize scores was larger for mild and moderate irritants (MAS scores from 15 to 50) with standard deviations going up to 50 over the MAS scores. The authors also showed that variability could be due to differences in the grading techniques depending on the institutes and scientist, but also due to the sensitivity of the rabbit eyes from the individual animals and/or strains.

3. Inter-species differences and predictive capacity of the Draize eye irritation test

Rabbits are often preferred over other animals for their large eyes with well-described anatomy and physiology, ease of handling, and availability. However, the eyes of rabbits appear to present several anatomical and physiological differences with regard to the human eye, they are not able to measure ocular pain and sting, and are in general more sensitive to irritating substances than the eyes of humans (Roggeband *et al*., 2000[21]; Gershbein and McDonald, 1977[22]; Wilhelmus, 2001[8]; ILSI, 1996[10]).

Physiologically, the rabbit eyes show lower tear production, less developed blink reflex, a thinner cornea (0.4 for rabbits versus 0.53 to 0.54 for humans) and a larger corneal surface area (Beckeley, 1965[23]; Wilhelmus, 2001[8]; ILSI, 1996[10]). Furthermore, the Bowman's layer is not present in the rabbit eyes, and rabbits have larger conjunctival sac which allows for larger test volumes to be instilled than what could be accounted for on human accidental exposure (Wilhelmus, 2001[8]; Curren and Harbell, 1998[24]; ILSI, 1996[10]). On the other hand, rabbits have a nictating membrane that functions as a third eyelid and may help removing irritating substances from the corneal surface although in a different way than in humans. Rabbit eyes were also reported to have different constituents of the tear film, and different ocular pigmentation (Wilhelmus, 2001[8]; ILSI, 1996[10]). These elements could all contribute for differences in the responses of the rabbit eyes to irritants with respect to the human eyes.

Indeed, several studies have shown that rabbits seem to be amongst the most sensitive species that react to ocular irritation insults. Gershbein and McDonald (1977[21]) have studied the corneal irritancy developed by various species by testing four shampoos and two cationic detergents. They found that corneal sensitivity was highest in the rabbit, hamster and mouse; intermediate in the rat and guinea pig, and possibly lowest in the dog, cat, rhesus monkey and chicken. Beckeley and co-workers (1969[25]) compared the ocular irritation effects of a soap formulation in humans, monkeys and rabbits. The authors showed that rabbits over-predicted the human responses, whereas the monkey was more accurately predicting the

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[19] Earl LK, Dickens AD, Rowson MJ (1997). A critical analysis of the rabbit eye irritation test variability and its impact on the validation of alternative methods. *Toxicology in vitro* **11**, 295-304.

[20] Ohno, Y., Kaneko, T., Inoue, T., Morikawa, Y., Yoshida, T., Fuji, A., Masuda, M., Ohno, T., Hayashi, M., Momma, J., Uchiyama, T., Chiba, K., Ikeda, N., Imanashi, Y. & Itakagaki, H. (1999). Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (1) Overview of the validation study and Draize scores for the evaluation of the tests. *Toxicology in Vitro* **13**, 73-98.

[21] Roggeband R, York M, Pericoi M, Braun W (2000). Eye irritation responses in rabbit and man after single applications of equal volumes of undiluted model liquid detergent products. *Food and Chemical Toxicology* **38**, 727-734.

[22] Gershbein LL, McDonald JE (1977).Evaluation of the corneal irritancy of test shampoos and detergents in various animal species. *Food and Cosmetics Toxicology* **15**, 131-134.

[23] Beckeley JH (1965). Comparative eye testing: man vs. animal. *Toxicology and Applied Pharmacology* **7**, 93-101.

[24] Curren R., Harbell J. (1998). In vitro alternatives for ocular irritation. *Environmental Health Perspectives* **106**, 485-492.

[25] Beckeley JH, Russel TJ, Rubin LF (1969). Use of the Rhesus monkey for predicting human response to eye irritants. *Toxicology and Applied Pharmacology* **15**, 1-9.

nature of the human hazard. Bito (1984[26]) also described the rabbit eye as the most sensitive of the species studied, whereas the primate eyes were the least sensitive, and proposed an evolutionary reasoning for such differences.

Griffith and co-workers (1980[9]) have compared the effects of 21 test materials in rabbits and man. The authors showed that the rabbit eye develops more intense responses to many chemicals than does the human eye, and that the period of recovery extends beyond that seen in typical chemical exposures in man. Freeberg and co-workers (1984[27]) showed that rabbits produced more severe eye responses than those reported from human eye accidents with ten household consumer products. The same group of authors have further compared the reactions of rabbits and humans to four household products (Freeberg *et al*., 1986[28]), and showed again that the Draize eye test was poorly predictive of the human recovery time, i.e., the rabbit and human 'mean time to clear' presented a correlation of 0.35 to 0.40. Roggeband and co-workers (2000[20]) confirmed such findings, by testing two undiluted liquid detergents in 29 human volunteers and 12 rabbits. The authors found that effects in the rabbit were greater than the effects observed in man.

4. Ethical issues

Perhaps because of the fact that the Draize rabbit eye test can be very painful and result in readily visible suffering, trauma and reactions in the rabbit eyes, animal activists have often used this assay as a symbol for cruelty. In the 1980s, the animal right activist Henry Spira specifically targeted the Draize eye test by publishing a full-page advertisement in the New York Times asking, "How many rabbits does Revlon blind for beauty's sake?" (figure 1). In addition, the existence of extensive literature on the limitations of the assay and on its poor scientific quality, as well as the major developments which took place since the '80s to advance alternatives to reduce, replace and refine the Draize rabbit eye (Balls *et al*., 1999[12]; Eskes *et al*., 2005[13]), make some authors consider that the ethical balance weights against the Draize eye test (Wilhelmus, 2001[8]; Rowan, 1980[29]).

[26] Bito LZ (1984). Species differences in the responses of the eye to irritation and trauma: a hypothesis of divergence in ocular defense mechanisms, and the choice of experimental animals for eye research. *Exp. Eye Res.* **39**, 807-829.

[27] Freeberg FE, Griffith JF, Bruce RD, Bay PHS (1984). Correlation of animal test methods with human experience for household products. J. *Toxicol. Cut. & Ocular Toxicol.* **1**, 53-64.

[28] Freeberg, F.E., Nixon, G.A., Reer, P.J., Weaver, J.E., Bruce, R.D., Griffith, J.F. & Sanders, L.W. (1986). Human and rabbit eye responses to chemical insult. *Fundamental and Applied Toxicology* **7**, 626-634.

[29] Rowan A. (1980). The Draize test: a critique and proposals for alternatives. Institute for the Study of Animal problems, Washington DC.

Figure 1: Full-page advertisement seeking to influence public opinion about the Draize eye test in the New York Times, appeared on 15 April 1980 and 7 October 1980 (extract from Wilhelmus, 2001[8]).

ANNEX 5

In vivo data for Dimethyl sulfoxide (CAS 67-68-5) from the study of Gautheron et al. (1994)

Substance	Dimethylsulfoxide (DMSO)				
CAS-Nr		no. of animals	3	Data entry	JBA
Data source	Gautheron 1994	Reference	Gautheron	Date	######
Testing lab	Agence du Médic	study duration	14 days		
Species/strain		physical state	Liquid	Quality check	
Concentration		amount	0.1mL	Date	
pH		purity		Extra QC	
Substance source	Aldrich 27043-1	MMAS	9.7	Date	

Classifications	
EU DSD	not classified
EU CLP (UN GHS)	no category (optiona
US EPA	category III

SUMMARY	EU DSD			UN GHS / EU CLP				US EPA				
Persistence (YES/NO/?, days)	NO			NO	7			NO				
	mean/median	R36	R41	percentile	Cat. 2	Cat. 1	cornea of 4?		max.	day 7-20	day 21	clas
Cornea Opacity	0.00	0	0	0.00	0	0	0	max. cornea	0	0	0	
Iris	0.00	0	0	0.00	0	0		max. iris	0	0	0	
Conjunctiva Redness	1.00	0		1.00	0			max. redness	2	0	0	
Chemosis	0.33	0		0.33	0			max. chemosis	2	0	0	

Animal 1	hour 1	hour 4	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13	day 14	day 15	day 16	day 17	day 18	day 19	day 20	day 21
Cornea Opacity	1		0	0	0	0			0							0							
Area involved																							
Iris	0		0	0	0	0			0							0							
Conjunctiva Redness	1		2	1	0	0			0							0							
Chemosis	2		1	0	0	0			0							0							
Discharge																							
Irreversible effects at d21 (No = 0; Yes = 1; unknown = ?	EU DSD, EU CLP and UN GHS		0																				
	US EPA		0	EU DSD, EU CLP and UN GHS full reversibility after ... days											3								

Notes: animal 1
Material washed after 1 hour exposure

mean	cornea of 4?	max. score
0.00	0	1
0.00		0
1.00		2
0.33		2

reversible (EU DSD, EU CLP, UN GHS)	irreversible (US EPA)
3	0
7	0
3	0
0	

Animal 2	hour 1	hour 4	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13	day 14	day 15	day 16	day 17	day 18	day 19	day 20	day 21
Cornea Opacity	0		0	0	0	0			0							0							
Area involved																							
Iris	0		0	0	0	0			0							0							
Conjunctiva Redness	1		2	2	1	0			0							0							
Chemosis	2		2	0	0	0			0							0							
Discharge																							
Irreversible effects at d21 (No = 0; Yes = 1; unknown = ?	EU DSD, EU CLP and UN GHS		0																				
	US EPA		0	EU DSD, EU CLP and UN GHS full reversibility after ... days											7								

Notes: animal 2
Material washed after 1 hour exposure

mean	cornea of 4?	max. score
0.00	0	0
0.00		0
1.67		2
0.67		2

reversible (EU DSD, EU CLP, UN GHS)	irreversible (US EPA)
-	
-	
-	
#DIV/0!	
#DIV/0!	
#DIV/0!	
FALSO	?

Animal 3	hour 1	hour 4	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13	day 14	day 15	day 16	day 17	day 18	day 19	day 20	day 21
Cornea Opacity	0		0	0	0	0			0							0							
Area involved																							
Iris	0		0	0	0	0			0							0							
Conjunctiva Redness	1		1	1	0	0			0							0							
Chemosis	2		0	0	0	0			0							0							
Discharge																							
Irreversible effects at d21 (No = 0; Yes = 1; unknown = ?	EU DSD, EU CLP and UN GHS		0																				
	US EPA		0	EU DSD, EU CLP and UN GHS full reversibility after ... days											3								

Notes: animal 3
Material washed after 1 hour exposure

mean	cornea of 4?	max. score
0.00	0	0
0.00		0
0.67		1
0.00		2

APPENDIX 4

ADDENDUM TO ISSUE PAPER

Use of the Isolated Chicken Eye (ICE) Test Method (OECD TG 438) to Identify Substances Not Requiring Classification for Serious Eye Damage / Eye Irritation According to UN GHS

Prepared by:

Chantra Eskes
Services & Consultation on Alternative Methods (SeCAM)

For the:

Organisation for Economic Co-operation and Development (OECD)

4 December 2012 – reviewed on 28 March 2013

A. Background

1. Following discussions between the OECD Secretariat, TNO and ICCVAM it was agreed to perform an additional evaluation of the predictive capacity of the ICE test which takes into account the results of individual laboratories. The reason for this is to consider in a different way those cases in where two laboratories may have one *in vitro* classification (e.g., non-classified), whereas two other laboratories may have a different *in vitro* classification (e.g., Eye Cat 2B).

2. In addition, TNO noted that during the EC/HO study two laboratories used a different type of slit-lamp as it is currently recommended in TG 438, which led to different ranges of values for corneal swelling. This range, which is usually of 0 - 60%, was found to be following in the various laboratories that participated to the EC/HO study:

- lab 22: -2.8 to 46.6
- lab 27: 7 to 224
- lab 24: -1.4 to 68.9
- lab 25: 0 to 151.7

Since the different ranges of % corneal swelling can impact the *in vitro* predictions, and due to the clear differences in ranges observed for laboratories 27 and to a lesser extent, also laboratory 25, it was agreed between the OECD Secretariat, TNO and ICCVAM to perform two analyses, one including all four laboratories, and one which excludes laboratories 27 and 25.

B. Predictive Capacities of the ICE test method for identifying chemicals not requiring GHS classification – <u>based on individual laboratory *in vitro* classifications</u>

3. Based on the compiled dataset as shown in the table presented in annex 3 of the Issue Paper (version from 16 Nov 2012) and taking into account the above considerations, the predictive capacities of the ICE test method to identify chemicals not requiring a classification for serious eye damage and eye irritation (i.e., to be used as a initial step of a bottom-up approach) were calculated based on individual laboratory *in vitro* classifications and reported in table 1. The following data interpretations were considered:

- Suggested-2: based on the *in vitro* and *in vivo* data suggested in the present evaluation following review of individual *in vitro* data and raw *in vivo* data and also the 9 additional chemicals for which raw data was not available but could now be reviewed and/or clarified (i.e., inclusion of chemicals n. 2, 9, 25, 66, 117, 121, 122, 123, 124). This evaluation does not comprehend materials for which Study Criteria were Not Met (SCNM) to assign an *in vivo* classification.
- Suggested + Expert Judgment (EJ): as Suggested-2 with in addition all materials that had SCNM for *in vivo* GHS classification, but for which it was possible to estimate an *in vivo* GHS classification with certain confidence (i.e., chemicals n. 5, 30, 38, 46, 57, 59, 85, 107, 109, 135, 159, 165, 166).

4. In addition, two different cases were considered:

- *In vitro* classifications from all laboratories from the EC/HO were considered
- *In vitro* classifications from Laboratories 27 and 25 from the EC/HO were excluded from the analyses due to the different ranges of corenal swelling observed within these two laboratories.

Table 1: Predictive capacities of the ICE test methods *to identify chemicals not requiring a GHS classification for eye irritation* (i.e., to be used as a initial step of a bottom-up approach) - based on individual laboratory *in vitro* classifications

	Concordance	Sensitivity	Specificity	False negatives	False positives
All laboratories					
Suggested-2	78.1% (250/320)	93.9% (184/196)	53.2% (66/124)	6.1% (12/196)	46.8% (58/124)
Suggested + EJ	78.3% (275/351)	93.7% (207/221)	52.3% (68/130)	6.3% (14/221)	47.7% (62/130)
Excl. Labs 27 & 25					
Suggested-2	79.9% (171/214)	92.0% (107/116)	65.3% (64/98)	7.8% (9/116)	34.7% (34/98)
Suggested + EJ	79.4% (185/233)	91.6% (120/131)	63.7% (65/102)	8.4% (11/131)	36.3% (37/102)

C. Misclassifications observed based on individual laboratory *in vitro* classifications

5. The under-predicted classifications (false negatives) obtained based on the results from individual laboratories are shown in table 2. Due to the fact that these results do not necessarily represent the overall *in vitro* classification of a test substance, the additional predictions obtained in other laboratories for the same substance were also reported within table 2.

6. Based on the individual laboratory predictions, a higher percentage of under-predictions as compared to the predictions calculated for the substances (as a mean of laboratory results) was found. This is due to the fact that although one laboratory may generate an under-prediction, it does not necessarily mean that the substance is under-predicted. The under-predicted substances will depend on the results of all participating laboratories. For example, substances 15, 16[30], 62 and 72 (Captan 90, Quinacrine, and Sodium perborate respectively) had only 1 laboratory out of 4 showing under-predictions, so that overall these substances are not to be considered as under-predicted.

7. A number of borderline substances were found, where 2 laboratories estimated the substance as being non-classified, and 2 other laboratories estimated it as a Cat 2B. These were the substances: 36 (ethyl-2-methylacetoacetate, GHS Cat 2B) and 71 (sodium oxalate, GHS Cat 1). In addition, one *in vivo* Eye Cat 1 material was clearly underpredicted as not being classified, which is material 167, or TNO-94, the anti-fouling paint.

8. For the other materials (46 and 50)[30], the mean laboratory classifications would lead to a classified prediction due to the fact that at least in one laboratory an Eye Cat 2A prediction was found, and in another laboratory an Eye Cat 2B was found, bringing the balance towards a prediction of classification.

[30] Correction from 28 March 2013 as stated in table 2.

Table 2. *In vivo* and *in vitro* data of substances showing one or more under-classifications in the various laboratories having tested the substance. Highlighted in gray are the *in vitro* corneal swelling data from laboratories 27 and 25 from the EC/HO study. Note on chemical 16: the *in vitro* classification of lab 1 was corrected on 28 March 2013 (i.e., fluorescein retention cat. II and not I as erroneously stated previously)

No	Substance/Product Name	CASRN	Physical state	Typical purity (%)	Concen-tration tested	*In Vivo* Draize GHS (suggested)	*In Vivo* Draize GHS (suggested based on expert judgement)	*In Vitro* ICE (suggested)	Fluorescein Retention Score	Fluorescein Retention Category	Corneal Opacity Score	Corneal Opacity Category	Corneal Swelling Score
15	Captan 90 concentrate	133-06-2	solid	90	neat	1	n.a.	NC	0	I	0.4	I	1.7
								2A/2B	0.2	I	1	II	27
								2B	0	I	1.33	II	19.17
								2B	1	II	1	II	20
16	4-Carboxybenzaldehyde	619-66-9	solid	95	neat	2A	n.a.	2B	1	II	0.5	I	5.4
								1	1.3	II	3	IV	89
								NC	0.67	II	0.5	I	-1.4
								2A/2B	2	III	1	II	12.7
36	Ethyl-2-methylacetoacetate	609-14-3	liquid	97	undiluted	2B	n.a.	NC	0.4	I	0.3	I	-2.8
								2B	1	II	0	I	7
								2B	0.67	II	1	II	11.52
								NC	1	II	0.5	I	4.5
46	Maneb	12427-38-2	solid	90	neat	SCNM	2A	NC	0	I	0.5	I	2.8
								2A	1	II	2	III	33
								NC	0	I	0.5	I	8.03
								2B	1	II	1	II	6.7
50	Methyl cyanoacetate	105-34-0	liquid	99	undiluted	2A	n.a.	NC	0.4	I	0.3	I	4.5
								2A	0.5	I	0.7	II	44
								NC	0.17	I	0.5	I	4.93
								2B	1	II	1	II	10.7
62	Quinacrine	69-05-6	solid	n.p.	neat	1	n.a.	2B	1.2	II	0.6	II	4.1
								NC	0.2	I	0.2	I	12
								2A	2	III	2	III	11.49
								2B	1	II	0.5	I	6.8
71	Sodium oxalate	62-76-0	solid	>99	neat	1	n.a.	2B	0.7	II	0.7	II	6.3
								2B	0.2	I	0	I	24
								NC	0.5	I	0.5	I	2.62
								NC	1	II	0	I	2.4
72	Sodium perborate, $4H_2O$	10486-00-7	solid	98.6	neat	1	n.a.	NC	0.6	II	0.5	I	3.1
								2B	0.2	I	0.7	II	23
								2B	1.33	II	1	II	7.54
								2B	1	II	0.5	I	14.6
167	TNO-94 (anti-fouling paint)	n.p.	liquid	n.p.	undiluted	1	n.a.	NC	1	II	0.5	I	2

n.a.= not applicable; NC = not classified; n.p.= not provided; SCNM = Study criteria not met.

9. If the results of corneal swelling of the two laboratories 27 and 25 from the EC/HO were excluded, in addition to substance 167 (TNO-94), an additional substance would be under-classified, which is substance 50, methyl cyanoacetate, *in vivo* UN GHS Cat 2A. In contrast, chemical 62, Quinacrine (*in vivo* UN GHS Cat 1) would become correctly predicted as a classified material.

10. Furthermore the following five substances would become borderline regarding *in vitro* predictions (balanced *in vitro* predictions as Cat 2 and NC): 36 (ethyl-2-methylacetoacetate, *in vivo* UN GHS Cat 2B), 46 (Maneb, *in vivo* UN GHS Cat 2A based on expert judgement), 71 (sodium oxalate, *in vivo* UN GHS Cat 1) and 72 (sodium perborate, *in vivo* UN GHS Cat 1). However, these results obtained with the exclusion of two laboratories from the EC/HO study are to be taken with care, since the actual *in vitro* classification is less certain without the parameter of corneal swelling.

D. Conclusions

11. Based on the present analyses, the overall under-prediction rates were found to be between 6.1% to 8.5%. However, although the calculation of the predictive capacity based on individual laboratory *in vitro* classifications allows accounting for the variability in predictions by the different laboratories, it also results on a 'biased' dataset where some substances count four times more as compared to other substances.

12. Here again one substance was found to be clearly under-predicted, which is the *in vivo* UN GHS Cat. 1 material TNO-94, an anti-fouling paint. The SSD reports that "*In the Draize eye irritation test it produced irritating but reversible eye effects in two out of three rabbits. In the third rabbit an unusual adherence of the paint to the cornea occurred which was the reason to humanely sacrifice the animal on day 1.*" In addition, two substances were found to have borderline *in vitro* classifications (ethyl-2-methylacetoacetate, a GHS Cat 2B; and sodium oxalate, a GHS Cat 1).

13. Furthermore, if the corneal swelling results of laboratories 27 and 25 from the EC/HO were excluded, also the GHS Cat 2A substance 50 (methyl cyanoacetate) would be under-classified. However, Quinacrine (*in vivo* UN GHS Cat 1) would become correctly predicted as a classified material. In addition, four substances would have borderline *in vitro* classifications (1 *in vivo* Cat 2B, 2 *in vivo* Cat 2A and 2 *in vivo* Cat 1). However, these results should be taken with care, since the actual *in vitro* classification is less certain without the parameter of corneal swelling.

E. Additional analyses carried out during the Expert Meeting on Eye Irritation (Dec 2012)

14. During the Expert meeting from Dec 2012 it was suggested to perform a 'weighted analyses' per laboratory (e.g., if a material is tested in 4 laboratories, each result is multiplied by the factor 0.25), which was carried out during the meeting and presented below. The false negatives were ≤ 6% and the specificity was ≥ 68%. Regarding false negative chemicals, the only chemical misclassified in all laboratories (i.e., having a factor of 1) was again TNO-94 anti-fouling paint (*in vivo* UN GHS Cat 1). Three[30] substances (including two[30] liquids UN GHS Cat 2 and 1 solid UN GHS Cat 1) had ½ chances of misprediction (i.e., misclassified as non-classified in two laboratories, whereas correct predictions were found in the two other laboratories), and four[30] other substances (three solids in vivo UN GHS Cat 1 and one solid UN GHS Cat 2) had ¼ chances of misclassification (i.e., mispredicted by only one laboratory out of 4 laboratories conducting the test).

Weighted approach	Accuracy[30]	False negatives[30]	False positives
Suggested w/o SC-based class.	80.7% (114.65/142)	5.6% (3.50/63)	30.2% (23.85/79)
Suggested with SC-based class.	81.5% (120.65/148)	5.1% (3.50/69)	30.2% (23.85/79)
Suggested 2a + EJ	80.4% (124.65/155)	5.5% (4.00/73)	32.1% (26.35/82)

class.: classification; SC: eye irritation classification derived from skin corrosive effects

15. Following review of the various possibilities for analyses (i.e., based on individual chemical outcome as shown in the Issue Paper, or on individual laboratory outcome as shown in this Addendum), the OECD Expert Group on Eye Irritation recommended to make use of the analyses based on the outcome of individual chemicals (and not on individual laboratory outcome whether weighted or not). The reason for this recommendation was in order to be in alignment with previous ICCVAM evaluations and to be aligned also with the analyses carried out in the context of the revisions of the BCOP Test Guideline.

APPENDIX 5

Raw *In Vivo* Data from the Anti-fouling organic solvent containing paint TNO-94

Substance	TNO-94 (anti-fouling paint)				
CAS-Nr		no. of animals	3	Data entry	CES
Data source	TNO Prinsen	Reference		Date	03.12.2012
Testing lab	TNO	study duration	6		
Species/strain	rabbit	physial state		Quality check	
Concentration		amount	0.1 ml or	Date	
pH		purity		Extra QC	
Substance source		MMAS	32.7	Date	

SUMMARY	EU			GHS				EPA				
Persistence (YES/NO/?, days)	?			?	?			?				
	mean/median	R36	R41	percentile	Cat. 2	Cat. 1	cornea of 4?		max.	day 7-20	day 21	class
Cornea Opacity	####	###	####	#DIV/0!	#DIV/0!	#DIV/0!	0	max. cornea	0	0	0	4
Iris	####	###	####	#DIV/0!	#DIV/0!	#DIV/0!		max. iris	0	0	0	4
Conjunctiva Redness	####	###		#DIV/0!	#DIV/0!			max. redness	0	0	0	4
Chemosis	####	###		#DIV/0!	#DIV/0!			max. chemosis	0	0	0	4

Cassifications		
EU	#DIV/0!	
GHS	#DIV/0!	#DIV/0!
EPA	SCNM	

Animal 1	hour 1	hour 4	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13	day 14	day 15	day 16	day 17	day 18	day 19	day 20	day 21	Notes:	mean	cornea of 4?	max. score	reversible (EU/GHS)	irreversible (EPA)
Cornea Opacity	0		0	0	0				0							0									#DIV/0!	0	0	1	0
Area involved	0		0	0	0				0							0												1	0
Iris	0		0	0	0				0							0									#DIV/0!		0	?	?
Conjunctiva Redness	1		2	2	3				1							0									#DIV/0!		0		
Chemosis	3		2	2	2				1							0									#DIV/0!		0		
Discharge	0		3	2	2				0							0													
Irreversible effects at d21 (No = 0; Yes = 1 ; unknown = ?):		EU and GHS	0																									1	
		EPA	0	EU & GHS full reversibility after … days						1																				

Animal 2	hour 1	hour 4	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13	day 14	day 15	day 16	day 17	day 18	day 19	day 20	day 21	Notes:	mean	cornea of 4?	max. score	reversible (EU/GHS)	irreversible (EPA)
Cornea Opacity	0		1	2	2				0							0									#DIV/0!	0	0	#DIV/0!	#DIV/0!
Area involved	0		4	2	2				0							0												#DIV/0!	#DIV/0!
Iris	0		1	1	1				0							0									#DIV/0!		0	#DIV/0!	#DIV/0!
Conjunctiva Redness	1		3	3	3				1							0									#DIV/0!		0	#DIV/0!	#DIV/0!
Chemosis	3		2	3	2				1							0									#DIV/0!		0	#DIV/0!	#DIV/0!
Discharge	0		3	3	2				1							0												FALSO	
Irreversible effects at d21 (No = 0; Yes = 1 ; unknown = ?):		EU and GHS	0																										
		EPA	0	EU & GHS full reversibility after … days						1																				

Animal 3	hour 1	hour 4	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13	day 14	day 15	day 16	day 17	day 18	day 19	day 20	day 21	Notes:	mean	cornea of 4?	max. score
Cornea Opacity	0			2																				Observations on Day 1 for			
Area involved	0			2																				Cornea Opacity, Area	#DIV/0!	0	0
Iris	0			1																				Involved and Iris were "not			
Conjunctiva Redness	1		3	3																				observable beause of the very	#DIV/0!		0
Chemosis	3		4	3																				severe swelling of the	#DIV/0!		0
Discharge	0		3	3																				conjunctivae." Test	#DIV/0!		0
Irreversible effects at d21 (No = 0; Yes = 1 ; unknown = ?):		EU and GHS	?																					substance adhered to cornea			
		EPA	?	EU & GHS full reversibility after … days						?														Day 2,3 Ischemic necrosis			
																								on Days 2,3			