Position paper on Reconsideration of the ICE Decision Criteria for identification of UN GHS No Category test chemicals for eye hazard

Chantra Eskes, SeCAM

Contents

I BACKGROUND

1. During the revisions of TG 438 on the ICE test method conducted in 2012-2013 to identify chemicals not requiring classification for eye irritation and/or serious eye damage (UN GHS No Category), it was proposed that the criteria “I;II;II” was used for the identification of UN GHS No Category chemicals. However, as a concession to avoid false negatives as much as possible, the criteria “I;II;II” was considered as an indicator of the category “No prediction can be made”.

2. Since the revisions from 2013, the criteria for acceptance of test methods to identify UN GHS No Category chemicals have changed. This is based on the work performed by Adriaens and colleagues (2014) who showed that with the Draize *in vivo* eye irritation test itself, at least 11% of chemicals classified *in vivo* as UN GHS / EU CLP Category 1 could

equally be identified as Category 2, and that about 12% of the Category 2 chemicals could equally be identified as non-classified chemicals. Such values take into account nevertheless only the *in vivo* within-test variability, and not variability between tests and between laboratories, suggesting that the actual values might be higher when considering also potential variability between tests and between laboratories for the same chemical.

3. During the OECD Working Group of the National Coordinators for the Test Guidelines (WNT) meeting held in April 2015, two New Test Guidelines were adopted on i) the Reconstructed Human Cornea-like Epithelium (RhCE) test method (OECD TG 492, 2015) and on ii) the Short Term Exposure (STE) test method (OECD TG 491, 2015). These two TG show higher false negative rates as compared to TG 438 revised in 2013 (see table 1).

Table 1. Comparison of the predictive capacity of ICE within TG 438 (2013) with the newly adopted TG 491 (2015) and TG 492 (2015) for the identification of chemicals that do not require classification according to the UN GHS classification system.

	ICE	RhCE	STE TG 491	
	TG 438 (n=152)	TG 492 (n=112)	(n=130)*	(n=101)*,**
False negatives	**1% (1/73)**	**4% (n=57)**	**12% (9/73)**	**2% (1/54)**
False positives	33% (26/79)	37% (n=55)	19% (11/57)	19% (9/48)
Accuracy	82% (125/152)	80% (n=112)	85% (110/130)	90% (92/102)

* Water-soluble chemicals or chemicals forming a uniform suspension

** Excluding highly volatile substances and solid substances other than surfactants

4. During the same OECD WNT meeting, it was agreed to revise the Decision Criteria of the ICE test method for the identification of UN GHS No Category chemicals based on the current acceptance standards. Such work is to be conducted in conjunction with the agreed revisions of TG 438 and GD 160 to include histopathology as an additional endpoint for the identification of UN GHS Cat. 1 detergent and cleaning products and of surfactants, a proposal led by The Netherlands.

5. Table 2 provides an overview of the ICE Decision Criteria for classification highlighting the proposed revisions.

Table 2: Overview of the criteria proposed for *in vitro* GHS classifications for the ICE test method. Highlighted in red are the main revisions proposed.

<table>
<tr><th>GHS classification</th><th>OECD GD 160 & ICCVAM BRD from 2009</th><th>OECD TG 438 from 2013</th><th>Revised proposed criteria for TG 438</th><th>Revised proposed criteria for GD 160</th></tr>
<tr><td>NC</td><td>3xI
2xI, 1xII</td><td>3xI
2xI, 1xII</td><td>3xI
2xI, 1xII
1xI, 2xII</td><td>3xI
2xI, 1xII
1xI, 2xII</td></tr>
<tr><td>Cat. 2B</td><td>3xII
2xII, IxI
2xII, 1xIII
2xI, 1xIV
1xI, 1xII, 1xIII</td><td rowspan="2">No prediction can be made</td><td rowspan="2">No prediction can be made</td><td>3xII
2xII, 1xIII
2xI, 1xIII*
1xI, 1xII, 1xIII</td></tr>
<tr><td>Cat. 2A</td><td>3xIII
2xIII, 1xII
2xIII, 1xIV
2xIII, 1xI
2xII, 1xIV
1xII, 1xIII, 1xIV</td><td>3xIII
2xIII, 1xII
2xIII, 1xIV
2xIII, 1xI
2xII, 1xIV
1xII, 1xIII, 1xIV
2xI, 1xIV*
1xI, 1xII, 1xIV*
1xI, 1xIII, 1xIV*</td></tr>
<tr><td>Cat. 1</td><td>3xIV
2xIV, 1XIII
2xIV, 1XII
2xIV, 1XI</td><td>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)</td><td>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)</td><td>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)</td></tr>
</table>

* Criteria not comprised in previous documents, perhaps due to the fact that these are combinations less likely to occur. However, as in the evaluation from 2013 such combinations did occur, and as such, it was proposed to allocate them to specific GHS classification categories.

II PREDICTIVE CAPACITY OF THE ICE TEST METHOD WITH THE REVISED DECISION CRITERIA

6. An evaluation was conducted to assess the impact of using the ICE criteria "I;II;II" to identify UN GHS No Category chemicals versus 'no prediction can be made'. For this purpose, the existing dataset used in the revisions of TG 438 in 2013 (OECD GD 188) was re-evaluated based on the proposed criteria shown in Table 2. Table 3 shows those chemicals for which a different classification would be obtained when applying the criteria "I;II;II" to identify UN GHS No Category chemicals as opposed to 'no prediction can be made'. These consisted of 10 chemicals out of the 152 in the existing dataset. Eight were UN GHS No Category chemicals that became correctly predicted chemicals with the use of the revised Decision Criteria, and two were UN GHS classified chemicals that became mispredicted UN GHS No Category chemicals.

7. Furthermore, newly available parallel *in vivo* and ICE *in vitro* datasets on detergent and cleaning products (Cazelle et al., 2014, 2015), on surfactants (A.I.S.E., unpublished data) and on agrochemicals (Buda et al., 2013) were also taken into consideration. The dataset on detergent and cleaning products consisted of 48 detergent and cleaning products, out of which 11 have Draize *in vivo* data (see table 4), and 38 have *in vivo* data generated with the Low Volume Eye Test (LVET)[1] (see annex I). The dataset on surfactants consisted of 11 surfactants out of which 8 have Draize *in vivo* data (see table 5) and 3 had LVET data (see annex I). Finally, the dataset on agrochemicals consisted of 5 chemicals, but this dataset could not be used due to the lack of reported *in vivo* data and classification.

8. Table 6 shows the predictive capacity values obtained for the ICE test method when using the criteria "I;II;II" to predict the UN GHS No Category chemicals for i) the existing dataset used in the revisions of TG 438 in 2013 (OECD GD 188), and ii) for the existing dataset combined with the new datasets having Draize *in vivo* data (Annex II shows the obtained predictive capacity if the existing dataset is combined with the new datasets based on both LVET and Draize *in vivo* data).

9. These findings show that the false positives and overall accuracy are improved with the ICE revised decision criteria, whereas the false negative results remain comparable to the rates obtained with the newly accepted TG 492.

[1] The LVET is a refinement of the Draize test that has been accepted by EURL-ECVAM to be used as a reference method for the validation of *in vitro* test methods for raw materials (surfactants) and finished products of the specific use domain of household detergent and cleaning products (ESAC, 2009)

Table 3: Chemicals from the ICE existing dataset (OECD GD 188, 2013) for which a different classification is derived when using the criteria "I;II;II" to identify a UN GHS No Category classification (highlighted in shadowed boxes).

N. OECD GD 188	Test substance	CASRN	Chemical Class	Product Category	Physical state	Purity (%)	Concen-tration tested	*In Vivo* Draize GHS Cat	Overall *In Vitro* ICE prediction (GHS)	*In Vitro* ICE predictions (GHS)*	ICE Categories**	Original reference
62	Quinacrine	69-05-6	Amine/ Amidine, Hetero-cyclic, Polycyclic compound	Anti-infective (anti-helmentic)	solid	n.p.	neat	Cat. 1	NC ~~2B~~	NC ~~2B~~ NC 2A NC ~~2B~~	II;II;I I;I;I III;III;II II;I;II	Balls et al. (1995)
36	Ethyl-2-methylacetoacetate	609-14-3	Ketone, Ester	Not classified	liquid	97	undiluted	Cat. 2B	NC ~~2B~~	NC NC ~~2B~~ 2B NC	I;I;I II;I;II II;II;II II;I;I	Balls et al. (1995)
37	Ethyl trimethyl acetate	3938-95-2	Ester	Solvent	liquid	99	undiluted	NC	NC ~~2B~~	NC ~~2B~~ 2A NC NC ~~2B~~	II;I;II III;III;III/IV I;I;I II;I;II	Balls et al. (1995)
105	TNO-32 (Ink-4)	n.p.	Not classified	Dyes	liquid	n.p.	undiluted	NC	NC ~~2B~~	NC ~~2B~~	II;I;II	Prinsen (1996)
137	TNO-64 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	emulsion	n.p.	undiluted	NC	NC ~~2B~~	NC ~~2B~~	II;II;I	Prinsen (2005)
144	TNO-71 (Fluoroallyl acrylate copolymer)	n.p.	Not classified	Copolymer	emulsion	n.p.	undiluted	NC	NC ~~2B~~	NC ~~2B~~	II;I;II	Prinsen (2005)
150	TNO-77 (Raw material liquid)	n.p.	Not classified	Raw material	liquid	n.p.	undiluted	NC	NC ~~2B~~	NC ~~2B~~	II;I;II	Prinsen (2005)
157	TNO-84 (Surfactant liquid)	n.p.	Not classified	Soaps; Surfactants	n.p.	n.p.	undiluted	NC	NC ~~2B~~	NC ~~2B~~	II;II;I	Prinsen (2005)
160	TNO-87 (Enzyme liquid)	n.p.	Not classified	Enzyme solution	liquid	n.p.	undiluted	NC	NC ~~2B~~	NC ~~2B~~	II;II;I	Prinsen (2005)
174	Triethanolamine	102-71-6	Amine/ Amidine, Alcohol	Cleaner; Cosmetic ingredient; Intermediate for waxes, cutting oils	liquid	99	undiluted	NC	NC ~~2B~~	NC ~~2B~~	II;II;I	Prinsen and Koëter (1993)

Cat.: Category; NC: No Category; n.p.: not provided

* Based on the Decision Criteria as described in OECD GD 160 (2011) for all predictions other than "I;II;II"; **Expressed as fluorescein retention, corneal opacity and corneal swelling categories respectively.

Table 4: A.I.S.E. detergent and cleaning products having parallel Draize *in vivo* and ICE *in vitro* data.

A.I.S.E. Formulation	Physical state	*In vivo* EU CLP	ICE Categories*	ICE UN GHS prediction**
Acidic extreme pH # 1	L	Draize – Cat. 1	III;IV;II	Cat. 2A (borderline Cat 1)
Alkaline extreme pH # 1	L	Draize – Cat. 1	IV;III;IV	Cat. 1
Alkaline extreme pH # 2	L	Draize – Cat. 1	IV;IV;III	Cat. 1
Alkaline extreme pH # 3	Gel	Draize – Cat. 1	IV;IV;III	Cat. 1
Alkaline extreme pH # 4	L	Draize – Cat. 1	IV;III;IV	Cat. 1
Alkaline extreme pH # 6	L	Draize – Cat. 2A	IV;IV;IV	Cat. 1
Alkaline extreme pH # 7	L	Draize – Cat. 2A	IV;IV;IV	Cat. 1
HDWL 13	L	Draize – Cat. 2A	II;II;II	Cat. 2B
HDWL 14	L	Draize – Cat. 2A	II;I;II	No Cat.
HDWL 17	L	Draize - NC	I;II;II	No Cat.
Acidic extreme pH # 2	L	Draize - NC	III;III;II	Cat. 2A

Cat.: Category; HDWL: Hand dishwash liquid; histo: histopathology; L: liquid; NC: No Category; S: solid; * Expressed as fluorescein retention, corneal opacity and corneal swelling categories respectively.;
** Based on the Decision Criteria as described in OECD GD 160 (2011) for all predictions other than "I;II;II".

Table 5: Additional surfactants not included in the ICE existing dataset (OECD GD 188, 2013) tested within the A.I.S.E. *In Vitro* program with parallel Draize *in vivo* and ICE *in vitro* data

Chemical name	CAS	Physical state	Type of surfactant	*In vivo* GHS Cat.	ICE Categories*	ICE UN GHS prediction**
Distearyldimethylammonium chloride (100%)	107-64-2	S	Cationic	Draize – Cat. 1	IV;IV;II	Cat. 1
Coco alkyl dimethyl betaine (~30%)	68424-94-2	L	Amphoteric	Draize – Cat. 1	III;III;II	Cat. 2A
Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides (~28%)	308062-28-4 (1643-20-5)	L	Non-ionic	Draize – Cat. 1	III;III;II	Cat. 2A
Benzalkonium chloride (1%)	63449-41-2	L	Cationic	Draize – Cat. 1	III;II;II	Cat. 2B
Benzethonium chloride (10%)	121-54-0	L	Cationic	Draize – Cat. 1	IV;IV;III	Cat. 1
Cetyl pyridinium bromide (1%)	140-72-7	L	Cationic	Draize – Cat. 2A	III;III;II	Cat. 2A
N-Lauroyl sarcosine Na salt (10%)	137-16-6*	L	Anionic	Draize – Cat. 2A	III;III;II	Cat. 2A
Polysorbate 20 (Tween 20) (100%)***	9005-64-5	L	Non-ionic	Draize and LVET - NC	II;II;I	No Cat.

Cat.: Category; histo: histopathology; L: liquid; LVET; Low Volume Eye Test NC: No Category; S: solid
* Expressed as fluorescein retention, corneal opacity and corneal swelling categories respectively.
** Based on the Decision Criteria as described in OECD GD 160 (2011) for all predictions other than "I;II;II".
*** Tween 20 was also tested in the existing ICE dataset, and found to be borderline *in vitro* chemical using the revised Decision Criteria (2x NC and 2x 2B). With the additional dataset from the A.I.S.E. study, the chemical becomes correctly predicted as UN GHS No Category.

Table 6. Performances of the ICE with the revised Decision criteria in which "I;II;II" is used to predict UN GHS No Category chemicals as compared to the *in vivo* classification obtained with the Draize rabbit eye test

	ICE TG 438 (n=152)	ICE Revised Decision Criteria - Validation dataset (n=152)	ICE Revised Decision Criteria - Validation dataset + AISE Draize dataset* (n=170)	RhCE - TG 492 (n=112)	STE - Draft TG (n=101)*,**
False negatives	1.37% (1/73)	4.11% (3/73)	**4.49% (4/89)**	**4% (n=57)**	2% (1/54)
False positives	32.91% (26/79)	22.78% (18/79)	**22.22% (18/81)**	**37% (n=55)**	19% (9/48)
Accuracy	82.24% (125/152)	86.18% (131/152)	**87.05% (148/170)**	**80% (n=112)**	90% (92/102)

* Includes surfactants, detergent and cleaning products.

III DISCUSSION ON THE FALSE NEGATIVES

10. Three chemicals were identified as false negatives when using the revised 'I;II;II' criteria for the identification of UN GHS No Category out of the 111 classified chemicals and mixtures available. These were in addition to the chemical TNO-94 already mispredicted as false-negatives using the criteria 'I;I;II' as discussed during the revisions of OECD TG 438 in 2013. The additional three under-predicted chemicals were:

- Ethyl-2-methylacetoacetate (CAS 609-14-3) from the ICE existing dataset (OECD GD 188, 2013);
- The Hand Dishwash Liquid 14 from the A.I.S.E. dataset (Cazelle et al., 2014); and
- Quinacrine (CAS 69-05-6) from the ICE existing dataset, originally coming from the EC/HO study (Balls et al., 1995; OECD GD 188, 2013).

Their detailed *in vivo* data are shown in Annex III.

11. Ethyl-2-methylacetoacetate (CAS 609-14-3) is a borderline *in vivo* chemical with a UN GHS Cat. 2B classification (based on the EURL-ECVAM template and its original dataset from ECETOC, 1998; see Annex I). As the Draize rabbit eye test is based on subjective observation and grading of effects, these may vary according to the observer. Few single difference in corneal score can have consequences in the final classification of the test material, which is the case of Ethyl-2-methylacetoacetate (see corneal opacity scores at day 3 in Annex I).

12. The Hand Dish wash Liquid 14 is an *in vivo* Draize UN GHS Cat. 2A from the A.I.S.E. dataset (Cazelle et al., 2014; see Annex I for details).

13. Quinacrine (CAS 69-05-6), is a solid drug therapeutic agent, classified *in vivo* as UN GHS Cat. 1 (based on the EURL-ECVAM template and its original dataset from ECETOC, 1998; see Annex I). It is to be noted, that during the EC/HO study two of the four laboratories conducting the ICE test used a different type of slit-lamp to the one 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 vary depending on the participating laboratory to the EC/HO study (lab 22: -2.8 to 46.6; lab 27: 7 to 224; lab 24: 1.4 to 68.9; and lab 25: 0 to 151.7) [2]. Since the different ranges of corneal swelling at the time of the EC/HO study could have impacted the *in vitro* predictions, and due to the clear differences in ranges observed for laboratories 25 and 27, it was agreed during the revision of the ICE test method in 2012-2013 to perform two analyses, one including all four laboratories, and one which excluded laboratories 27 and 25. If such dataset is excluded from the evaluation of the *in vitro* dataset of Quinacrine, the chemical would become a UN GHS Cat. 2 *in vitro* predicted chemical (see Table 7).

Table 7. Detailed *in vitro* dataset of Quinacrine

N. OECD GD 188	Test substance	CAS	*In Vivo* Draize GHS Cat	Overall *In Vitro* ICE prediction (GHS)	*In Vitro* ICE predictions (GHS)*	ICE Cat.**	Fluorescein Retention Score	Corneal Opacity Score	Corneal Swelling Score	Laboratories with questionable swelling values
62	Quinacrine	69-05-6	Cat. 1	Cat. 2	NC	II;II;I	1.2	0.6	4.1	
					~~NC~~	~~I;I;I~~	~~0.2~~	~~0.2~~	~~12~~	Excluded lab
					2A	III;III;II	2	2	11.49	
					~~NC~~	~~II;I;II~~	~~1~~	~~0.5~~	~~6.8~~	Excluded lab

* Based on the Decision Criteria as described in OECD GD 160 (2011) for all predictions other than "I;II;II".
**Expressed as fluorescein retention, corneal opacity and corneal swelling categories respectively.

14. Furthermore, Quinacrine was also predicted as UN GHS No Category with the BCOP test method in the EC/HO study (mean *In Vitro* Score of 1.55 which is below the 3.0 threshold for the identification of UN GHS No Category). However, in another study (Gautheron et al., 1994) the same chemical was correctly predicted by the BCOP (Unpublished annexes to OECD GD 189, 2013). It is therefore questionable whether the quality of the Quinacrine tested during the EC/HO might not have been altered and/or damaged.

15. Finally, solids have been reported to lead to variable and extreme exposure conditions in the *in vivo* Draize eye irritation test, resulting in responses which may not reflect the true irritation potential of chemicals in human accidental exposure (Prinsen, 2006). Before the adoption of the revised TG 405 in 2002, eyes exposed to solids were not rinsed for up to 24 hours (which was the case of the reference dataset used for Quinacrine from ECETOC, 1998). Since rabbits blink and tear, it was unclear how much of the compound would be present and for how long it stayed in contact with the eye (Prinsen, 2006). In addition, blinking can induce mechanical damage *in vivo* especially for solids, contributing to a higher

[2] TG 438 (2013) addresses the use of different pachymeters. The use of proficiency chemicals together with the appropriate slit width is considered sufficient to guarantee the assessment of swelling in the expected range. Labs 27 and 25 from the EC/HO study are no longer active in the area of toxicity testing. Labs who want to introduce the ICE at their facility usually contact TNO for advice on the slit-lamp microscope to be purchased.

degree of irritation (Prinsen, 2006). Since Quinacrine has a solubility of 10-50 mg /ml, it could be that not all the amount applied was solubilised in the tears (which have a volume of 7-8 µl in rabbits), causing possible abrasion that facilitate the penetration of the chemical into the lower layers of the eyes, affecting e.g., the iris.

16. Existing data on Quinacrine tested *in vivo* in the Draize test in which washing took place both at 24h and 1h after exposure (see table 8), show that washing one hour after exposure resulted in less strong effects, although still considered a UN GHS Cat. 1 *in vivo* due to the moderate corneal opacity and neovascularisation observed at day 14 in all three rabbits. Furthermore, existing data on Quinacrine using the LVET test (on the same batch of the chemical used to perform the 1h washing Draize test), which is a refinement of the standard Draize eye testbased on the application of the test sample directly to the cornea of the eye of the animal instead of the conjunctival sac (0.01 mL versus 0.1 mL), showed the chemical to induce considerable less severe effects not warranting classification as UN GHS Cat. 1 (see table 8). Such findings suggest that if the Quinacrine solid material was applied using more realistic conditions to the human accidental exposure (e.g. application directly to the cornea instead of the conjuctival sac, washing of the eyes), its effect were less strong.

Table 8: *In vivo* responses following exposure to the solid material Quinacrine (Prinsen, unpublished data)

Quinacrine		No. of animals*	Opacity Score	Iris	Conjunctivae			MMAS	Classification	
					Redness	Swelling	Discharge		*In vivo* UN-GHS	*In vitro* (ICE) EC/UN-GHS
1 hr	Draize 24 h[a]	3/3	4	2†	1	3	2	62		
	Draize 1 h[b,d]	3/3	1	1	1.3	2	2	36		
	LVET[c,d]	3/3	0.3	0	1	0	0.7	10		
24 h	Draize 24 h	3/3	3	1	2	2	1.7	46		
	Draize 1 h	3/3	2	1	2.7	3	2	60		
	LVET	3/3	0.7	0	1	0	0	15		
48 h	Draize 24 h	3/3	3	1.7	3	2	2	52		
	Draize 1 h	3/3	2	1	2.7	3	2.3	62		
	LVET	0/3	0	0	0	0	0	0		
72 h	Draize 24 h	3/3	2	2	3	3	2	66	Cat. 1	No Cat. 1
	Draize 1 h	3/3	2	1	2.7	2.3	2.3	60	Cat. 1	
	LVET	0/3	0	0	0	0	0	0	No Cat. 1	
7 days	Draize 24 h	3/3	3	2†	2	2	1	70		
	Draize 1 h	3/3	2	0	1	2	0.3	47		
	LVET									
14 days	Draize 24 h	3/3	3	2†	2	2	2	82		
	Draize 1 h	3/3	2‡	0	1	0	0	42		
	LVET									
21 days	Draize 24 h									
	Draize 1 h									
	LVET									

[a] ECETOC (1998); [b] TNO study 3415/01 (February 2002); [c] TNO report V6215/01 (July 2005); [d] Data from the Draize 1h and the LVET study were obtained using the same batch of chemical; * = number of animals showing eye effects/total number of animals in test; † = dubious score; estimated because of opacity present; ‡ = neovascularization

IV CONCLUSIONS

17. The overall ICE accuracy and false positive rates were improved when using the ICE revised Decision Criteria, i.e., using the criteria "I;II;II" to identify UN GHS No Category chemicals (instead of 'no prediction can be made' as in the current TG 438 (2013)). Furthermore, although its false negative rate increase, it was still found to be comparable to the rates obtained with the newly adopted TG 492.

18. In particular, the use of the revised Decision Criteria allowed to decrease the false positive rates from 33% (26/79) to 22% (18/81) when considering all dataset available on the ICE test method having existing Draize rabbit eye data.

19. The revised Decision Criteria led to three additional false negative predictions as compared to the criteria adopted in 2013, among which one was a UN GHS Cat. 1 chemical, i.e., Quinacrine. However, its *in vitro* results seem uncertain. Furthermore, the *in vivo* classification seems to derive from effects that would not occur in human accidental exposure. As a consequence, it is questioned whether it is relevant to take into account such a compound when making a decision about the usefulness of the revised ICE Decision Criteria.

V REFERENCES

Adriaens E., Barroso J., Eskes C., Hoffmann S., McNamee P., Alépée N., Bessou-Touya S., De Smedt A, de Wever B., Pfannenbecker U., Tailhardat M., Zuang V. (2014). Draize test for serious eye damage / eye irritation: importance of the endpoints evaluated with regard to UN GHS / EU CLP classification. Archives of Toxicology 88, 701-723

Balls M., Botham P.A., Bruner L. H., Spielmann H. (1995). The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicology *In Vitro* 9: 871-929.

Buda I., Budai P., Szabó R., Lehel J. (2013). *In vitro* eye corrosion study of agrochemicals on isolated chicken eye. Comm. Appl. Biol. Sci, Ghent University 78, 177-181

Cazelle E., Eskes C., Hermann M., Jones P., McNamee P., Prinsen M., Taylor H., Wijnands M.V.W. (2014). Suitability of histopathology as an additional endpoint to the isolated chicken eye test for classification of non-extreme pH detergent and cleaning products. Toxicology In Vitro 28, 657-666.

Cazelle E., Eskes C., Hermann M., Jones P., McNamee P., Prinsen M., Taylor H., Wijnands M.V.W. (2015). Suitability of the Isolated Chicken Eye Test for Classification of Extreme pH Detergents and Cleaning Products. Toxicology In Vitro 29, 609-616.

ECETOC (1998). Eye Irritation Reference Chemicals Data Bank. Technical Report n. 48 (2), European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

ESAC (2009). Statement on the use of existing Low Volume Eye Test (LVET) data for Weight-of-Evidence decisions on classification and labelling of cleaning products and their main ingredients. Available at: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/validation-regulatory-acceptance/topical-toxicity/eye-irritation#1-ecvam-validated-test . Accessed on 19.06.2013.

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.

Gautheron P., Giroux J., Cottin M., Audegond L., Morilla A., Mayordomo-Blanco L., Tortajada A., Haynes G., Vericat J.A., Pirovano R., Tos E.G., 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: 381-392.

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 hazard to the human eye. Toxicology and Applied Pharmacology 55, 501-513.

OECD GD 160 (2011) Guidance Document on "The Bovine Corneal Opacity and Permeability (BCOP) and Isolated Chicken Eye (ICE) Test Methods: Collection of Tissues for Histological Evaluation and Collection of Data on Non-Severe Irritants. Series on Testing and Assessment no. 160. ENV/JM/MONO(2011)45, 63 pp.

OECD GD 188 (2013). Streamlined Summary Document Supporting OECD Test Guideline 438 on the Isolated Chicken Eye for Eye Irritation/Corrosion. Series on Testing and Assessment No. 188. OECD Guideline for the Testing of Chemicals, Paris, France.

OECD GD 189 (2013). Annexes to Streamlined Summary Document Supporting OECD Test Guideline 437 on the Bovine Corneal Opacity and Permeability for Eye Irritation/Corrosion. Series on Testing and Assessment No. 189. OECD Guideline for the Testing of Chemicals, Paris, France.

OECD TG 438 (2013). Test Guideline 438: Isolated Chicken Eye test method for identifying i) chemicals inducing serious eye damage and ii) chemicals not requiring classification for eye irritation or serious eye damage. OECD Guideline for the Testing of Chemicals, Paris, France.

OECD TG 491 (2015). Short Time Exposure *In Vitro* Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. OECD Guideline for the Testing of Chemicals, Paris, France.

OECD TG 492 (2015). Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage. OECD Guideline for the Testing of Chemicals, Paris, France.

Prinsen M.K. (1996). The chicken enucleated eye test (CEET): A practical (pre)screen for the assessment of eye irritation/corrosion potential of test materials. Food Chem Toxicol 34:291-296.

Prinsen M.K. (2005). *In vitro* and in vivo data for 94 substances tested in the isolated chicken eye test. Unpublished data provided directly to NICEATM by MK Prinsen, TNO Nutrition and Food Research Institute.

Prinsen M.K. (2006). The Draize Eye Test and *in vitro* alternatives; a left-handed marriage? Toxicology *in Vitro* 20,78-81.

Prinsen M.K., Koëter B.W.M. (1993). Justification of the enucleated eye test with eyes of slaughterhouse animals as an alternative to the Draize eye irritation test with rabbits. Food Chem Toxicol 31:69-76.

ANNEX I

Additional surfactants (n=3) and A.I.S.E. detergent and cleaning products (n=37) having parallel LVET *in vivo* and ICE *in vitro* data.

Formulations and surfactants	Physical state	*In vivo* EU CLP[(1)]	ICE Categories[(2)]	ICE UN GHS prediction[(3)]	ICE + histo[(4)]
Alkaline extreme pH # 5	S	LVET – Cat. 1	II(IV);II(IV);II	Cat. 1	Cat. 1
HDWL 1	L	LVET – Cat. 1	II;II;II	Cat. 2B	Cat. 1
HDWL 2	L	LVET – Cat. 1	II;III;III	Cat. 2A	Cat. 1
HDWL 3	L	LVET – Cat. 1	II;II;II	Cat. 2B	Cat. 1
HDWL 4	L	LVET – Cat. 1	II;II;II	Cat. 2B	Cat. 1
HDWL 5	L	LVET – Cat. 1	II;II;II	Cat. 2B	Cat. 1
Laundry powder 1	S	LVET – Cat. 1	II;II;II	Cat. 2B	No Cat. 1
Laundry liquid 1	L	LVET – Cat. 1	III;III;III	Cat 2A	Cat. 1
Laundry liquid 2	L	LVET – Cat. 1	II;II;II	Cat. 2B	No Cat. 1
50% active N,N-Dimethyl-N-coco-N-(3-sulfopropyl) ammonium betaine (CAS 68201-55-8)	L	LVET – Cat. 1	III;II;III	Cat. 2A	No Cat.1*****
APC 1	L	LVET – Cat. 2A	III;IV;III	Cat 2A	No Cat. 1
APC 2	L	LVET – Cat. 2A	IV;III;III	Cat 2A (borderline Cat 1)	Cat. 1
HDWL 6	L	LVET – Cat. 2A	II;III;II	Cat. 2B	Cat. 1
HDWL 7	L	LVET – Cat. 2A	II;III;II	Cat. 2B	Cat. 1
HDWL 8	L	LVET – Cat. 2A	II;II;II	Cat. 2B	Cat. 1
HDWL 9	L	LVET – Cat. 2A	II;II;II	Cat. 2B	No Cat. 1
HDWL 10	L	LVET – Cat. 2A	II;III;II	Cat. 2B	No Cat. 1
HDWL 12	L	LVET – Cat. 2A	II;III;II	Cat. 2B	No Cat. 1
Laundry liquid 3	L	LVET – Cat. 2A	II;II;II	Cat. 2B	No Cat. 1
HDWL 11	L	LVET – Cat. 2B	II;II;II	Cat. 2B	No Cat. 1
Laundry powder 2	S	LVET – Cat. 2A	II;II;II	Cat. 2B	No Cat. 1
Laundry powder 3	S	LVET – Cat. 2B	II;II;II	Cat. 2B	No Cat. 1
APC 3	L	LVET - NC	II;III;II	Cat 2A	No Cat. 1
APC 4	L	LVET - NC	III;III;III	Cat 2A	No Cat. 1
Acidic extreme pH # 3	L	LVET - NC	III;III;II	Cat. 2A	Cat. 1
Acidic extreme pH # 4	L	LVET - NC	III;III;II	Cat. 2A	Cat. 1
Acidic extreme pH # 5	L	LVET - NC	III;IV;III	Cat. 2 A (borderline Cat 1)	Cat. 1
Acidic extreme pH # 6	L	LVET - NC	III;III;III	Cat. 2A	Cat. 1
Acidic extreme pH # 7	L	LVET - NC	III;III;III	Cat. 2A	Cat. 1
Acidic extreme pH # 8	L	LVET - NC	III;III;II	Cat. 2A	No Cat. 1
Acidic extreme pH # 9	L	LVET - NC	III;III;II	Cat. 2A	No Cat. 1
Alkaline extreme pH # 8	L	LVET - NC	IV;III;III	Cat. 2A (borderline Cat 1)	Cat. 1
Alkaline extreme pH # 9	L	LVET - NC	III;IV;III	Cat. 2A (borderline Cat 1)	No Cat. 1
HDWL 15	L	LVET - NC	II;I;II	No Cat.	No Cat. 1
HDWL 16	L	LVET - NC	III;III;IV	Cat 2A (borderline Cat 1)	Cat. 1
Laundry liquid 4	L	LVET - NC	II;III;II	Cat 2B	No Cat. 1
Laundry powder 4	S	LVET - NC	II;II;II	Cat. 2B	No Cat. 1
Laundry powder 5	S	LVET - NC	II;II;II	Cat. 2B	No Cat. 1
Sodium Lauryl Sulfate (5 %) (CAS 151-21-3)	L	LVET - NC	III;II;II	Cat. 2B	No Cat.1
50% active N,N-Dimethyl-N-coco-N-(3-sulfopropyl) ammonium betaine (30 %) (CAS 68201-55-8)	L	LVET - NC	III;II;II	Cat. 2B	No Cat.1

APC: All purposes cleaner; Cat.: Category; HDWL: Hand dishwash liquid; histo: histopathology; L: liquid; LVET; Low Volume Eye Test; NC: No Category; S: solid; [(1)]LVET classification derived using the Draize criteria for classification; [(2)]Expressed as fluorescein retention, corneal opacity and corneal swelling categories respectively; [(3)]Based on the Decision Criteria as described in OECD GD 160 (2011) for all predictions other than "I;II;II"; [(4)]Based on histopathological criteria developed by A.I.S.E.(Cazelle et al., 2014, 2015)

ANNEX II

Performances of the ICE with the revised Decision criteria in which “I;II;II” is used to predict UN GHS No Category chemicals, and including the validation dataset as well as the AISE new dataset on surfactants and detergents and cleaning products having both Draize and LVET data.

	ICE Revised Decision Criteria - Validation dataset + AISE Draize dataset* (n=170)	AISE dataset* having LVET *in vivo* data (n=40)	ICE Revised Decision Criteria - Validation dataset + AISE full dataset* (Draize + LVET) (n=210)	RhCE - TG 492 (n=112)	STE - Draft TG (n=101)*,**
False negatives	4.49% (4/89)	0.0% (0/22)	**3.60% (4/111)**	**4% (n=57)**	2% (1/54)
False positives	22.22% (18/81)	94.4% (17/18)	**35.35% (35/99)**	**37% (n=55)**	19% (9/48)
Accuracy	87.05% (148/170)	57.5% (23/40)	**81.43% (171/210)**	**80% (n=112)**	90% (92/102)

* Includes surfactants, detergent and cleaning products.

ANNEX III
In vivo data of false negative predictions

Chemical	Ethyl-2-methyl acetoacetate				
CAS-Nr	609-14-3	no. of animals	3	Data entry	CES
Data source	ECETOC	Reference		Date	13.07.06
Testing lab		study duration	7		
Species/strain		physial state		Quality check	CCG
Concentration	100%	amount	100 µl	Date	31.07.06
pH		purity	97.00%	Extra QC	CES
Chemical source	Fluka	MMAS	18.0	Date	21.08.2015

Classifications			
EU DSD	not classified		
EU CLP (UN GHS)	category 2	UN GHS subcategory (optional)	B
US EPA	category III		

SUMMARY	EU DSD			UN GHS / EU CLP			
Persistence (YES/NO/?, days)	NO			NO	7		
	mean/median	R36	R41	Majority	Cat. 2	Cat. 1	cornea of 4?
Cornea Opacity	1.00	0	0	1.00	1	0	0
Iris	0.00	0	0	0.00	0	0	
Conjunctiva Redness	1.33	0		1.33	0		
Chemosis	1.00	0		1.00	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	Notes:	mean	cornea of 4?	max. score
Cornea Opacity	0		1	1	0	0			0																0.67	0	1
Area involved	0		1	1	0	0			0																		
Iris	0		0	0	0	0			0																0.00		0
Conjunctiva Redness	2		1	2	1	1			0																1.33		2
Chemosis	1		1	1	1	1			0																1.00		1
Discharge	3		3	3	1	0			0																		
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																		

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
Cornea Opacity	0		1	1	1	0			0																1.00	0	1
Area involved	0		2	2	1	0			0																		
Iris	0		0	0	0	0			0																0.00		0
Conjunctiva Redness	2		1	1	1	1			0																1.00		2
Chemosis	2		1	1	0	0			0																0.67		2
Discharge	3		1	1	0	0			0																		
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																		

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	1		1	1	1	0			0																1.00	0	1
Area involved	1		3	2	1	0			0																		
Iris	0		0	0	0	0			0																0.00		0
Conjunctiva Redness	2		1	2	1	0			0																1.33		2
Chemosis	2		1	1	1	0			0																1.00		2
Discharge	3		2	1	1	0			0																		
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																		

Chemical	HDWL 14				
CAS-Nr	na	no. of animals	3	Data entry	
Data source		Reference		Date	30.nov.10
Testing lab		study duration	21 days		
Species/strain	Rabbit/ New Zealan	physial state	L viscous	Quality check	CES
Concentration	Applied neat	amount	0,1 ml	Date	21.08.2015
pH	7	purity	na	Extra QC	
Chemical source		MMAS		Date	

Classifications			
EU DSD	R36		
EU CLP (UN GHS)	category 2	UN GHS subcategory (optional)	A
US EPA	category II		

SUMMARY	EU DSD			UN GHS / EU CLP			
Persistence (YES/NO/?, days)	NO			NO	21		
	mean/median	R36	R41	Majority	Cat. 2	Cat. 1	cornea of 4?
Cornea Opacity	2.00	1	0	2.00	1	0	0
Iris	0.00	0	0	0.00	0	0	
Conjunctiva Redness	2.00	0		2.00	1		
Chemosis	3.00	1		3.00	1		

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
Cornea Opacity	0		0	0	2				2							0							0		0.67	0	2
Area involved	0		0	0	1				1							0							0				
Iris	0		0	0	0				0							0							0		0.00		0
Conjunctiva Redness	2		2	2	2				2							1							0		2.00		2
Chemosis	3		3	3	3				3							1							0		3.00		3
Discharge	3		2	2	2				2							1							0				
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																			21				

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
Cornea Opacity	0		2	2	2				2							2							0		2.00	0	2
Area involved	0		2	2	2				2							1							0				
Iris	0		0	0	0				0							0							0		0.00		0
Conjunctiva Redness	1		2	2	2				2							1							0		2.00		2
Chemosis	3		3	3	3				3							2							0		3.00		3
Discharge	3		2	2	2				2							1							0				
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																			21				

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	2	2				2							0							0		2.00	0	2
Area involved	0		1	1	1				1							0							0				
Iris	0		0	0	0				0							0							0		0.00		0
Conjunctiva Redness	2		2	2	3				2							2							0		2.33		3
Chemosis	3		3	3	3				3							2							0		3.00		3
Discharge	3		2	2	2				2							2							0				
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																			21				

Chemical	Quinacrine				
CAS-Nr	69-05-6	no. of animals	3	Data entry	CCG
Data source	ECETOC	Reference		Date	12.07.06
Testing lab		study duration	14		
Species/strain		physial state		Quality check	CES
Concentration	100%	amount	100 mg	Date	13.07.06
pH		purity	not provide	Extra QC	SH
Chemical source	Sigma	MMAS	82.0	Date	14.07.06

Classifications			
EU DSD	SCNM		
EU CLP (UN GHS)	category 1	UN GHS subcategory (optional)	
US EPA	SCNM		

SUMMARY	EU DSD			UN GHS / EU CLP			
Persistence (YES/NO/?, days)	?			?	?		
	mean/median	R36	R41	Majority	Cat. 2	Cat. 1	c
Cornea Opacity	2.67	1	0	2.67	1	0	
Iris	1.67	1	0	1.67	1	1	
Conjunctiva Redness	2.67	1		2.67	1		
Chemosis	2.33	1		2.33	1		

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?
Cornea Opacity	4		3	3	2				2			3				3									2.67	1
Area involved	2		2	2	4				4			4				4										
Iris	2		1	1	2				1			2				2									1.33	
Conjunctiva Redness	1		2	3	3				3			2				2									2.67	
Chemosis	3		2	2	3				3			2				2									2.33	
Discharge	2		1	2	2				2			1				2										
Irreversible effects at d21 (No = 0; Yes = 1 ; unknown = ?)	EU DSD, EU CLP and UN GHS	?																								
	US EPA	?	EU DSD, EU CLP and UN GHS full reversibility after ... days	?																						

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?
Cornea Opacity	4		3	3	2				2			3				3									2.67	1
Area involved	1		2	1	4				4			3				4										
Iris	2		1	2	2				1			2				2									1.67	
Conjunctiva Redness	1		2	3	3				3			2				2									2.67	
Chemosis	3		2	2	3				2			2				2									2.33	
Discharge	2		2	2	2				1			1				2										
Irreversible effects at d21 (No = 0; Yes = 1 ; unknown = ?)	EU DSD, EU CLP and UN GHS	?																								
	US EPA	?	EU DSD, EU CLP and UN GHS full reversibility after ... days	?																						

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?
Cornea Opacity	4		3	3	2				2			3				3									2.67	1
Area involved	3		2	1	4				4			3				4										
Iris	2		1	2	2				1			2				2									1.67	
Conjunctiva Redness	1		2	3	3				3			2				2									2.67	
Chemosis	3		2	2	3				3			2				2									2.33	
Discharge	2		2	2	2				2			1				2										
Irreversible effects at d21 (No = 0; Yes = 1 ; unknown = ?)	EU DSD, EU CLP and UN GHS	?																								
	US EPA	?	EU DSD, EU CLP and UN GHS full reversibility after ... days	?																						