Research Article

Development of a Defined Approach for Eye Hazard Identification of Solid Chemicals According to the Three UN GHS Categories

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Abstract

Currently there are two OECD-adopted defined approaches (DA) for eye hazard identification of non-surfactant liquids (OECD TG 467). The current study aimed to develop a DA for eye hazard identification of solid chemicals according to the three UN GHS categories (Cat.1, Cat. 2, No Cat.): the DAS. The DAS combines two test methods described in OECD TG 437 and TG 492. The DAS was developed based on in-depth statistical analysis of a database on solids containing *in vitro* and historically curated *in vivo* Draize eye test data. The performance of the DAS was assessed by comparing the predictions with the classification based on *in vivo* Draize eye test data, on the one hand, and with the performance criteria established by the OECD expert group, on the other hand. In a first tier of the DAS, the SkinEthic™ HCE EIT method (TG 492) is used to distinguish No Cat. from classified substances. For classified substances, the BCOP LLBO method (TG 437) is used to identify Cat. 1, and the remaining solids are predicted Cat. 2. In summary, 77.4% Cat. 1 (N=31), 52.3% Cat. 2 (N=18), and 70.0% of No Cat. (N=60) solids were correctly identified compared to the classification based on the Draize eye test. The percentage of correct predictions met the minimum OECD performance values of 75% Cat. 1, 50% Cat. 2, and 70% No Cat., and the percentage of mispredictions was below the established maximum values. Therefore, inclusion of the DAS in OECD TG 467 has been achieved.

Plain language summary

Defined approaches combine information from different non-animal testing methods in a specific way and interpret the results according to a fixed procedure. Such defined approaches are already available as full replacements of animal testing to assess the eye hazard of liquid chemicals (OECD Test Guideline 467). This study used two OECD-adopted *in vitro* methods, based on human cells and corneas from cattle, to create a defined approach that can be used for solid chemicals. The performance of the procedure was assessed against data from previous animal tests for 109 solid chemicals. The results have already led to this defined approach being adopted by the OECD TGs programme for inclusion in TG 467. With the adoption of the new defined approach, non-animal human relevant strategies are now available for eye hazard assessment of liquids and solids, reducing the need for animal testing.

1 Introduction

In recent decades, many efforts have been made to develop new approach methodologies (NAMs) for eye hazard identification according to the United Nations Globally Harmonized System of Classification (UN, 2023). Since 2009, several Test Guidelines (TGs) have been adopted by the Organisation for Economic Co-operation and Development (OECD) for the identification of test chemicals inducing serious eye damage (UN GHS Cat. 1) or for the identification of test chemicals not requiring classification for eye irritation and serious eye damage hazards (UN GHS No Cat.). In Guidance Document (GD) No. 263 on an integrated approach to testing and assessment (IATA) for serious eye damage and eye irritation, it is proposed to use data generated with these NAMs together, combined with other information sources such as physicochemical properties and *in silico* and read-across predictions from chemical analogues (OECD, 2019).

In this context, the publication of the Draize eye test Reference Database (DRD) by Cosmetics Europe was an important step towards understanding which of the *in vivo* effects are responsible for driving UN GHS classification (Barroso et al., 2017). The advantage of selecting substances from the DRD is that the database contains 681 independent historical *in vivo* studies (634 individual chemicals) conducted according to OECD TG 405 (OECD,

2023a), covering all drivers of classification based on the observed tissue effects, relevant chemical classes, and physical states. In addition, the authors proposed a number of key criteria to be considered when selecting reference chemicals from the DRD for the evaluation of defined approaches for eye hazard identification.

All these efforts are finally starting to pay off, resulting in full replacement of the *in vivo* Draize eye test. In June 2022, the SkinEthic™ Human Corneal Epithelium (HCE) Time-to-Toxicity (TTT) was adopted by the OECD as a full replacement of the *in vivo* Draize eye test for eye hazard identification according to UN GHS for both liquids and solids (TG 492B; OECD, 2022a). Likewise, two defined approaches (DAs) for eye hazard identification of non-surfactant liquids were accepted (DAL-1 and DAL-2) and integrated into a new OECD TG (TG 467 Part I and Part II; OECD, 2022b). The DAL-1 is based on the combination of a Reconstructed human Cornea-like Epithelium test method (OECD TG 492 RhCE, EpiOcular™ Eye Irritation Test or SkinEthic™ HCE EIT for liquids; OECD, 2023b) and the Bovine Corneal Opacity and Permeability (BCOP) test method using the laser light-based opacitometer (TG 437, LLBO; OECD, 2023c) as well as four physicochemical properties of the chemical (water solubility, octanol-water partition coefficient, vapor pressure, and surface tension) (Alépée et al., 2019a). The DAL-2 is based on the combination of the Short Time Exposure (STE) test method (TG 491; OECD, 2023d) and the BCOP LLBO (Alépée et al., 2019b; OECD, 2023c; SCCS, 2023[1]). Next, a separate DA was developed to identify the eye hazard of liquid, semi-solid and solid chemicals having surfactant (SF) properties. The DASF is based on the combination of a RhCE test method (TG 492, EpiOcular™ EIT or SkinEthic™ HCE EIT) and a modification of the STE test method (Alépée et al., 2023). The DASF is currently under OECD consideration and was recently accepted by the Working Party of Hazard Assessment as an IATA Case Study to illustrate the use of the DASF for eye hazard identification of surfactants.

As the DAs listed above are applicable to liquids and/or surfactants only, the purpose of the current study was to develop a DA for eye hazard identification according to the three categories of the UN GHS (UN GHS Cat.1, Cat. 2 and No Cat) for solid chemicals (i.e., non-pipettable test chemical). During the development phase, several OECD adopted test methods were excluded from consideration for the DAS because of their limited applicability with respect to solids, i.e., the Isolated Chicken Eye (ICE) test method has a high false negative rate for solids when used to identify Cat. 1 (TG 438; OECD, 2023e); non-surfactant solids are excluded from the applicability domain of the STE when used to identify No Cat. (OECD, 2023d); the Fluorescence Leakage (FL) test method can only be used for the identification of Cat. 1 water-soluble chemicals[2] (TG 460; OECD, 2023f); in the Vitrigel® EIT method, test chemicals are dissolved or suspended in culture medium and acidic preparations ($pH \leq 5$) and rapid phase separation is outside the applicability domain (TG 494; OECD, 2021)[2]; the Ocular Irritation® test method is only applicable to solids whose 10% solution/dispersion pH is in the range of $4 \leq pH \leq 9$ (TG 496; OECD 2023g)[2]. Knowing that solids are not considered outside the applicability domain of the BCOP test method[2] (TG 437; OECD, 2023c), and no restrictions are known for the RhCE[2] (TG 492; OECD, 2023a) test methods, only these OECD-adopted NAMs were considered as potential components of a testing strategy for solid chemicals.

The DA for solids (DAS) was developed based on a set of 71 solid chemicals. Additional solids were then selected for testing to obtain a more comprehensive set of 109 solids representing the different drivers of UN GHS classification. The performance of the DAS was assessed by comparing the predictions with the classification based on historical *in vivo* Draize eye test data, on the one hand, and with the performance criteria established by the OECD expert group on eye/skin irritation/corrosion and phototoxicity, on the other hand (GD 354; OECD, 2022c).

2 Materials and methods

Reference chemicals

The set of reference chemicals to support the review of the DAS is composed of 109 neat solids with high-quality Draize eye test data and is listed in Table 1. The set covers a wide range of applications and chemical classes, includes small and large, hydrophobic and hydrophilic molecules with a range of 112 different organic functional groups (OFG) defined according to OECD QSAR Toolbox analysis version 3.2.[3] Summary statistics describing the chemical space of the reference chemicals are shown in Table 2.

Data sources

Historical Draize eye test data on solids were selected from the DRD according to the key principles described by Barroso et al. (2017). The criteria used for chemicals that should not be selected according to the key principles are listed in chapter 3.1.5. of the OECD supporting document (GD 354, Chapter 3; OECD, 2022c). For each Draize eye test study, detailed information was available on ocular tissue effects driving classification *in vivo* (Tab. 1).

Abbreviations: BCOP, bovine corneal opacity and permeability; CASRN, Chemical Abstracts Service Registry Number; Cat. 1, UN GHS classification for chemicals causing irreversible effects on the eye/serious damage to the eye; Cat. 2, UN GHS classification for chemicals causing reversible effects on the eye/eye irritation, sub-categorized in 2A (irritant to eyes, eye effects are not fully reversible within 7 days of observation) and 2B (mild irritant to eyes, eye effects fully reversible within 7 days of observation); CO, corneal opacity; DA, defined approach; DAL, defined approach for liquids; DAS, defined approach for solids; DASF, defined approach for surfactants; DRD, Draize eye test Reference Database; EIT, eye irritation test; EITS, eye irritation test solids; GD, Guidance Document; HCE, human corneal epithelium; IATA, integrated approach to testing and assessment; LLBO, laser light-based opacitometer; NAM, new approach methodology; No Cat., chemicals not classified for serious eye damage or eye irritation under GHS/EU CLP; NPCM, no prediction can be made; OECD, Organisation for Economic Co-operation and Development; OFG, organic functional group; RhCE, reconstructed human cornea-like epithelium; STE, short time exposure; TG, Test Guideline; TP, true positive; TTT, time-to-toxicity; UN GHS, United Nations Globally Harmonized System of Classification and Labelling of Chemicals

[1] https://health.ec.europa.eu/system/files/2023-12/sccs_o_273_final.pdf

[2] Eye irritation – PETA Science Consortium International e.V. https://www.thepsci.eu/eye-irritation-2/ (accessed 15.01.2024)

[3] https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm

Tab. 1: List of solid chemicals

No.	Chemical name	CAS RN	UN GHS	Main driver	Set
1	2-Benzyl-4-chlorophenol	120-32-1	Cat 1	CO mean ≥ 3 // CO pers D21	Training
2	2-Hydroxy iso-butyric acid	594-61-6	Cat 1	CO mean ≥ 3	Training
3	4,4'-(4,5,6,7-Tetrabromo-3H-2,1-benzoxathiol-3-ylidene) bis[2,6-dibromophenol] S,S-dioxide	4430-25-5	Cat 1	CO mean ≥ 3	Training
4	4-([2-Sulphatoethy] sulphony)-aniline	2494-89-5	Cat 1	CO mean ≥ 3	Test
5	4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	Cat 1	CO mean ≥ 3	Training
6	alpha-Ketoglutaric acid	328-50-7	Cat 1	CO mean ≥ 3	Training
7	Dibenzoyl-L-tartaric acid	2743-38-6	Cat 1	CO mean ≥ 3	Training
8	Imidazole	288-32-4	Cat 1	CO mean ≥ 3 // CO pers D21	Training
9	Promethazine HCL	58-33-3	Cat 1	CO mean ≥ 3	Test
10	1-Naphthalene acetic acid Na salt	61-31-4	Cat 1	CO pers D21	Training
11	3,4-Dichlorophenyl isocyanate	102-36-3	Cat 1	CO pers D21	Test
12	3,4,5,6-Tetrachloro-2-(1,4,5,8-tetrabromo-6-hydroxy-3-oxoxanthen-9-yl)-benzoic acid	18472-87-2	Cat 1	CO pers D21	Test
13	Captan 90-concentrate	133-06-2	Cat 1	CO pers D21	Training
14	Lauric acid	143-07-7	Cat 1	CO pers D21	Training
15	m-Phenylene diamine	108-45-2	Cat 1	CO pers D21	Training
16	p-tert-Butylphenol	98-54-4	Cat 1	CO pers D21	Training
17	Sodium perborate tetrahydrate	10486-00-7	Cat 1	CO pers D21	Test
18	Sodium salicylate	54-21-7	Cat 1	CO pers D21	Training
19	3,4-Dimethyl-1H-pyrazole	2820-37-3	Cat 1	CO pers D21	Test
20	Benzoic acid	65-85-0	Cat 1	CO pers D21	Training
21	1,2,4-Triazole Na salt	41253-21-8	Cat 1	CO = 4	Test
22	1,2-Benzisothiazol-3(2H)-one	2634-33-5	Cat 1	CO = 4	Training
23	1,3-Diiminobenz (f)-isoindoline	65558-69-2	Cat 1	CO = 4	Test
24	beta-Resorcyclic acid	89-86-1	Cat 1	CO = 4	Test
25	Chlorhexidine	55-56-1	Cat 1	CO = 4	Training
26	Chlorophenacyl	6305-04-0	Cat 1	CO = 4	Test
27	Granuform (Chemical name: Paraformaldehyde)	30525-89-4	Cat 1	CO = 4	Training
28	N-(2-Methylphenyl)-iminodicarbonimidic diamide (1-(o-Tolyl)biguanide)	93-69-6	Cat 1	CO = 4	Training
29	N-Acetyl-DL-methionine	1115-47-5	Cat 1	CO = 4	Training
30	Sodium hydrogen sulphate	7681-38-1	Cat 1	CO = 4	Test
31	Triethanolamine orthovanadate	13476-99-8	Cat 1	CO = 4	Training
32	(2R,3R)-3-((R)-1-(Tert-butyldimethylsiloxy)ethyl)-4-ox-oazetidin-2-yl acetate	76855-69-1	Cat 2	CO mean ≥ 1	Training
33	3,3'-Dithiopropionic acid	1119-62-6	Cat 2	CO mean ≥ 1	Training
34	4-Carboxybenzaldehyde	619-66-9	Cat 2	CO mean ≥ 1	Training
35	Dibenzyl phosphate	1623-08-1	Cat 2	CO mean ≥ 1	Training
36	gamma-(Aminocarbonyl)-N-methyl-N,N-bis(1-methyletheyl)-gamma-phenyl-, iodide	71-81-8	Cat 2	CO mean ≥ 1	Test
37	1,3-bis-(2,4-Diaminophenoxy) propane tetrachloride	74918-21-1	Cat 2	Conj mean ≥ 2	Test
38	1,5-Naphthalenediol	83-56-7	Cat 2	Conj mean ≥ 2	Training
39	2-Amino-3-hydroxy pyridine	16867-03-1	Cat 2	Conj mean ≥ 2	Training
40	4-Amino-3-nitrophenol	610-81-1	Cat 2	CO mean ≥ 1	Test

No.	Chemical name	CAS RN	UN GHS	Main driver	Set
41	Ammonium nitrate	6484-52-2	Cat 2	Conj mean ≥ 2	Training
42	Sodium benzoate	532-32-1	Cat 2	Conj mean ≥ 2	Training
43	2,6-Dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate	96568-04-6	Cat 2	CO mean ≥ 1	Training
44	1,4-Dibutoxybenzene	104-36-9	Cat 2	Conj mean ≥ 2	Test
45	2-Hydroxy-1,4-naphthoquinone	83-72-7	Cat 2	Conj mean ≥ 2	Training
46	Camphene	79-92-5	Cat 2	Conj mean ≥ 2	Test
47	m-Dinitrobenzene	99-65-0	Cat 2	Conj mean ≥ 2	Training
48	p-Nitrobenzoic acid	62-23-7	Cat 2	Conj mean ≥ 2	Training
49	Sodium monochloroacetate	3926-62-3	Cat 2	Conj mean ≥ 2	Test
50	Iodosulphuron-methyl-sodium	144550-36-7	No Cat	CO > 0 **	Test
51	Sodium bisulphite	7631-90-5	No Cat	CO > 0 **	Training
52	1-Phenyl-3-pyrazolidone	92-43-3	No Cat	CO > 0	Training
53	2,4-Dichloro-5-sulphamoyl-benzoic acid	2736-23-4	No Cat	CO > 0	Test
54	Acrylamidopropyltrimonium chloride/acrylamide copolymer	75150-29-7	No Cat	CO > 0	Test
55	Betaine monohydrate	590-47-6	No Cat	CO > 0	Test
56	DL-Glutamic acid	19285-83-7	No Cat	CO > 0	Test
57	Ethylenediaminetetraacetic acid dipotassium salt (EDTA di-K salt)	25102-12-9	No Cat	CO > 0	Training
58	Iminodibenzyl	494-19-9	No Cat	CO > 0	Training
59	Magnesium carbonate	56378-72-4	No Cat	CO > 0	Training
60	Propyl-4-hydroxybenzoate	94-13-3	No Cat	CO > 0	Training
61	N,N-Dimethyl guanidine sulphate	598-65-2	No Cat	CO = 0 **	Test
62	[3-Chloro-4-[(3-fluorobenzyl)oxy]phenyl](6-iodoquinazolin-4-yl)amine	231278-20-9	No Cat	CO = 0	Training
63	1,5-Di(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene	33089-61-1	No Cat	CO = 0	Training
64	1H-Indole-2,3-dione	91-56-5	No Cat	CO = 0	Training
65	1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl) urea	101-20-2	No Cat	CO = 0	Test
66	1-(4-Phenyl-phenoxy)-1-(1,2,4-triazole-1)-3,3-dimethylbutan-2-ol	55179-31-2	No Cat	CO = 0	Training
67	1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol	72956-09-3	No Cat	CO = 0	Training
68	2′,6′,8-Trifluoro-5-methoxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulphonanilide	145701-23-1	No Cat	CO = 0	Training
69	2,2'-[[3-Methyl-4-[(4-nitrophenyl)azo]phenyl]imino]bisethanol	3179-89-3	No Cat	CO = 0	Test
70	2,2'-[6-(4-Methoxyphenyl)-1,3,5-triazine-2,4-diyl]bis[5-[(2-ethylhexyl)oxy]-phenol]	187393-00-6	No Cat	CO = 0	Training
71	2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) (97.9%)	103597-45-1	No Cat	CO = 0	Training
72	2,3-Dimethyl-2,3-dinitrobutane	3964-18-9	No Cat	CO = 0	Training
73	2,5,6-Triamino-4-pyrimidinol sulphate	1603-02-7	No Cat	CO = 0	Training
74	2,6-Dihydroxy-3,4-dimethylpyridine	84540-47-6	No Cat	CO = 0	Training
75	2-(Diphenylacetyl)-1,3-indandione	82-66-6	No Cat	CO = 0	Test
76	2-Aminophenol	95-55-6	No Cat	CO = 0	Test
77	2-Anilino-4,6-dimethylpyrimidine	53112-28-0	No Cat	CO = 0	Test
78	2-Mercaptopyrimidine	1450-85-7	No Cat	CO = 0	Training

No.	Chemical name	CAS RN	UN GHS	Main driver	Set
79	3,4-Dimethoxybenzaldehyde	120-14-9	No Cat	CO = 0	Test
80	3,5-Dihydroxyacetophenone	51863-60-6	No Cat	CO = 0	Training
81	3H-Pyrazole-3-one, 2(4-aminophenyl),4-dihydro-5-(1-pyrrolindinyl)	30707-77-8	No Cat	CO = 0	Training
82	3-((Benzylthio)methyl)-6-chloro-1,1-dioxide	91-33-8	No Cat	CO = 0	Training
83	3-(2-Chloro-thiazol-5-ylmethyl)-5-methyl[1,3,5]oxa-diazinan-4-ylidene-N-nitroamine	153719-23-4	No Cat	CO = 0	Test
84	4'-Aminoazobenzene-4-sulphonic acid	104-23-4	No Cat	CO = 0	Test
85	4,4'-Methylene bis-(2,6-di-tert-butylphenol)	118-82-1	No Cat	CO = 0	Test
86	4,4'-Sulfonylbisbenezenamide	80-08-0	No Cat	CO = 0	Training
87	Aluminium hydroxide	21645-51-2	No Cat	CO = 0	Training
88	Anthracene	120-12-7	No Cat	CO = 0	Training
89	Benzoflex S-312 (Chemical name: Neopentyl glycol dibenzoate)	4196-89-8	No Cat	CO = 0	Training
90	beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	55219-65-3	No Cat	CO = 0	Training
91	Chlorpyrifos	2921-88-2	No Cat	CO = 0	Training
92	EPIKURE 1061	2716-10-1	No Cat	CO = 0	Training
93	Gluconolactone	90-80-2	No Cat	CO = 0	Training
94	Hexamethylenetetraamine	100-97-0	No Cat	CO = 0	Training
95	Hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl) benzoate	302776-68-7	No Cat	CO = 0	Test
96	Methyl p-hydroxybenzoate	99-76-3	No Cat	CO = 0	Training
97	Myristyl myristate	3234-85-3	No Cat	CO = 0	Training
98	Phenothiazine	92-84-2	No Cat	CO = 0	Training
99	Phenylbutazone	50-33-9	No Cat	CO = 0	Training
100	Phenylthiourea	103-85-5	No Cat	CO = 0	Test
101	Potassium tetrafluoroborate	14075-53-7	No Cat	CO = 0	Training
102	Silicic acid	1343-98-2	No Cat	CO = 0	Training
103	Sodium tripolyphosphate (Grade E, anhydrous) (10 mg)	7758-29-4	No Cat	CO = 0	Test
104	Tetrabromobisphenol A	79-94-7	No Cat	CO = 0	Training
105	Theobromine	83-67-0	No Cat	CO = 0	Training
106	Theophylline sodium acetate	8002-89-9	No Cat	CO = 0	Test
107	Tris(2-ethylhexyl)-4,4',4''-(1,3,5-triazine-2,4,6-triyltriimino) tribenzoate	88122-99-0	No Cat	CO = 0	Test
108	Trisodium mono-(5-(1,2-dihydroxyethyl)-4-oxido-2-oxo-2,5-dihydro-furan-3-yl) phosphate	66170-10-3 437-74-1	No Cat	CO = 0	Training
109	Xanthinol nicotinate		No Cat	CO = 0	Test

CO, corneal opacity; CO pers D21, CO persistence on day 21; Conj, conjunctival; mean, mean scores calculated from gradings at 24, 48, and 72 hours after instillation of the test chemical; CO > 0, CO scores > 0 in at least one animal and at least one observed time point; CO = 0, CO scores equal to 0 at all observation times in all animals. Studies marked with ** are studies for which at least one animal had a mean of the scores of days 1-3 above the classification cut-off for at least one endpoint but not in enough animals to generate a classification.

Tab. 2: Summary of the physicochemical property ranges that describe the chemical space of the chemicals tested using the DAS

UN GHS	MW	Melting point (C°)	Water solubility (mg/mL)	LogP	Vapor pressure (mmHg)
	Min – Max	Min – Max	Min – Max	Min – Max	Min – Max
Cat. 1	68.1 – 985.1	43 – 237	0.001 – 578	-1.79 – 8.0	0 – 2.96
Cat. 2	80.0 – 480.4	46 – 233	< 0.001 – 1000	-1.56 – 4.64	0 – 2.53
No Cat.	78.0 – 823.1	42 – 357	< 0.001 – 589	-3.36 – 9.12	0 – 1.91
Overall	68.1 – 985.1	42 – 357	< 0.001 – 1000	-3.36 – 9.12	0 – 2.96

MW, molecular weight; LogP, octanol-water coefficient; Min – Max, minimum and maximum values

Data on the SkinEthic™ HCE EITS and the BCOP LLBO test methods were taken from several peer-reviewed publications (Alépée et al., 2016, 2019a,b; Verstraelen et al., 2017; Van Rompay et al., 2018; Adriaens et al., 2021). Additional solids were tested to fill the remaining data gaps for the SkinEthic™ HCE EITS and BCOP LLBO test method, resulting in an evaluation of 109 solids with the SkinEthic™ HCE EITS and 105 solids with the BCOP LLBO. Four solids were not tested with the BCOP LLBO because they were not commercially available or because they were very expensive (Tab. S1[4]). The solids were tested according to the OECD TG 437 (BCOP) and OECD TG 492 (RhCE). The protocols of the test methods are published in the DB-ALM dataset (SkinEthic™ HCE EITS: DB-ALM Protocol n° 191)[5] and MethodsX (BCOP LLBO: Van Rompay et al., 2020). An overview of the prediction models for the individual test methods that are part of the DAS is shown in Table 3.

Since data were also available for the EpiOcular™ EIT (N = 106) and the BCOP OP-KIT (N = 67) test methods (Tab. S1[4]), two validated reference methods included in OECD TG 492 and 437, respectively, the performance of these methods was also assessed. Data on the EpiOcular™ EIT and the BCOP OP-KIT test methods were taken from several peer-reviewed publications (ICCVAM, 2006; Barroso et al., 2014; Verstraelen et al., 2017; Kandarova et al., 2018; Van Rompay et al., 2018; Adriaens et al., 2021). An overview of the prediction models for the individual test methods is shown in Table 4.

Development of the DAS

The DAS was developed based on the results of 71 neat solids (training set) that were available for the different components of the DAS. In a next step, the performance of the DAS was assessed for the test set (N = 38). No changes were made to the data interpretation procedure (DIP) after assessing the performance of the test set, as no further improvement to the DIP was possible based on the performance of the training and test set results shown separately in Table S2[6]. The identification of the chemicals that were used in the training set and the test set is available in Table 1 and Table S1[4].

The performance of the DAS was assessed by comparing the prediction results with the classification based on historical *in vivo* Draize eye test data. For each chemical, the predicted class was obtained by considering all available results of each *in vitro*

Tab. 3: Prediction models of the DAS components according to UN GHS Category

TG 492		TG 437	
UN GHS	SkinEthic™ HCE EITS	UN GHS	BCOP LLBO
		Cat. 1	Opacity > 145 and/or OD > 2.5
NPCM	Tissue viability ≤ 50%	NPCM	All other combinations
No Cat.	Tissue viability > 50%	No Cat.	LIS ≤ 30

OD, optical density; LIS, LLBO irritancy score = mean opacity (read-out LLBO in lux/7 value) + (15 x mean permeability)

Tab. 4: Prediction models of the EpiOcular™ EIT and the BCOP OP-KIT test method

TG 492		TG 437	
UN GHS	EpiOcular™ EIT	UN GHS	BCOP OPKIT
		Cat. 1	IVIS > 55
NPCM	Tissue viability ≤ 60%	NPCM	3 < IVIS ≤ 55
No Cat.	Tissue viability > 60%	No Cat.	IVIS ≤ 3

IVIS, *in vitro* irritancy score = mean opacity (read-out OP-KIT) + (15 x mean permeability OD490 value)

[4] doi:10.14573/altex.2401191s1

[5] http://jeodpp.jrc.ec.europa.eu/ftp/jrc-opendata/EURL-ECVAM/datasets/DBALM/LATEST/online/DBALM_docs/

[6] doi:10.14573/altex.2401191s2

Tab. 5: Performance metrics for the assessment of the predictivity of a DA of non-surfactant liquid test chemicals for eye hazard identification (OECD GD 354, 2022c)

UN GHS	Defined approach		
	Cat. 1	Cat. 2	No Cat.
Cat. 1	≥ 75%	≤ 25%	≤ 5%
Cat. 2	≤ 30%	≥ 50%	≤ 30%
No Cat.	≤ 5%	≤ 30%	≥ 70%

test method. The performance of the DAS to distinguish between the three UN GHS categories was compared against minimum performance values for each UN GHS category that were accepted by the OECD Expert Group on Eye/Skin Irritation/Corrosion and Phototoxicity (GD 354; OECD, 2022c). The percentage of correct predictions when compared to the UN GHS classification based on the Draize eye test should be at least 75% for Cat. 1, 50% for Cat. 2, and 70% for No Cat. (Tab. 4). The balanced accuracy, which is the average of the proportion of correct predictions of each UN GHS category, was reported as overall measure of accuracy. All analyses were performed with R version 4.3.1.[7]

Minimum performance values

The minimum performance criteria were based on the uncertainty of the *in vivo* Draize eye test. The low acceptance value of at least 50% concordance for Cat. 2 was based on the variability of the Draize eye test, especially for the mild to moderate range. The between-test variability for non-surfactant liquids and solids which resulted in at least a) one Cat. 1 classification among all repeat studies, b) one Cat. 2 classification among all repeat studies, and c) one No Cat. classification among all studies was as follows:

a) 41.7% (5/12) of the chemicals with at least one Cat. 1 study could be equally identified as Cat. 2, therefore the overall concordance of classifications was 58.3% (7/12) for Cat. 1.
b) 50% (5/10) of the chemicals with at least one Cat. 2 study could be equally identified as Cat. 1, and 20% (2/10) could be equally identified as No Cat., therefore the overall concordance of classifications was 30% (3/10) for Cat. 2.
c) 11.1% (2/18) of the chemicals with at least one No Cat. study could be equally identified as Cat. 2 or higher, therefore the overall concordance of classifications was 88.9% (16/18) for No Cat.

Based on these *in vivo* observations, the minimum performance criteria values to be met were discussed and approved by the OECD Working Group of National Co-ordinators of the TGs programme and included in the supporting document (GD 354; OECD, 2022c) (Tab. 5).

3 Results

3.1 Performance of the individual NAMs

Table 6 shows the performance of the individual NAMs. Based on these results, it can be concluded that the SkinEthic™ HCE EITS is the most promising test method to identify No Cat., with 70.0% (N = 60) agreement with the UN GHS classification based in the Draize eye test. The EpiOcular™ EIT has a correct prediction rate of 56.8% for identifying No Cat. Note that results for two solids were not available, but if predicted as No Cat., this would still result in a concordance of 58.2% (N = 60), which is below the minimum value of 70% correct predictions for No Cat. compared to the UN GHS classification based on the Draize eye test. The

Tab. 6: Performance of the individual NAMs

UN GHS	TG 492				TG 437				
		N	NPCM	No Cat.		N	Cat. 1	Cat. 2	No Cat.
Cat. 1	SkinEthic™ HCE EITS	31 (30)	100% (100%)	0% (0%)	LLBO	31 (23)	77.4% (78.3%)	19.4% (17.4%)	3.2% (4.3%)
	EpiOcular™EITS	30	100%	0%	OP-KIT	23	66.9%[a]	28.8%	4.3%
Cat. 2	SkinEthic™ HCE EITS	18	81.8%	18.2%	LLBO	18 (16)	30.6% (28.1%)	38.9% (43.8%)	30.6% (28.1%)
	EpiOcular™EITS	18	94.4%	5.6%	OP-KIT	16	25.4%	44.3%	31.3%
No Cat.	SkinEthic™ HCE EITS	60 (58)	30.0% (31.0%)	70.0% (69.0%)	LLBO	55 (28)	5.5% (7.1%)	50.9% (61.2%)	43.6% (31.6%)
	EpiOcular™EITS	58	43.2%	56.8%[b]	OP-KIT	28	0.3%	52.6%	47.1%

The values in brackets correspond to the performance for the same set of reference solids.
[a] OECD acceptance criteria ≥ 75% not met. [b] OECD acceptance criteria ≥ 70% not met.

[7] https://www.r-project.org/

BCOP LLBO test method is best suited to identify Cat. 1 with a correct prediction rate of 77.4% (N = 31). For the same set of 23 Cat. 1 solids, the agreement between the UN GHS classification based on the Draize eye test and BCOP predictions was 66.9% for the OP-KIT, while it was 78.3% for the LLBO.

A summary of the predictions for individual solids for the suitable candidates for the DAS is given in Table S1[4]. The within and between laboratory reproducibility of both test methods was assessed during their respective multicenter studies with at least two participating laboratories (Alépée et al., 2016; Verstraelen et al., 2017; Adriaens et al., 2021; Van Rompay et al., 2020). In the context of the current study, more than one result was available for 92 out of 109 solids for the SkinEthic™ HCE EITS test method; in 95.7% the prediction was the same (88/92). Multiple BCOP LLBO results were available for 48 solids and in 93.7% (45/48) the prediction was the same (Tab. S1[4]).

3.2 Performance of the DAS

The bottom-up scheme of the DAS is presented in Figure 1. Solids that result in a tissue viability > 50% with the SkinEthic™ HCE EITS test method are classified No Cat. Solids that result in a tissue viability ≤ 50% are evaluated based on the BCOP LLBO test method in a second step. Solids that result in an opacity > 145 and/or OD > 2.5 are predicted Cat. 1, and the remaining solids are assigned Cat. 2. The minimum performance values established by the OECD experts (Tab. 5) were met for the bottom-up approach: 77.4% Cat. 1, 52.3% Cat. 2, and 70.0% No Cat. were correctly identified when compared to the UN GHS classification based on the Draize eye test, resulting in a balanced accuracy of 66.6% (Tab. 7). When using the BCOP LLBO in a first step (top-down approach, Tab. 7), the minimum criteria were not met as two No Cat. solids that were predicted as No Cat. with the SkinEthic™ HCE EITS test method were predicted Cat. 1 with the BCOP LLBO (No. 53: CAS RN 2736-23-4 and No. 87: CAS RN 21645-51-2, Tab. S1[4]). Note that the BCOP LLBO can also be used to identify No Cat. (LIS ≤ 30, Tab. 3). This is however not recommended since one Cat. 1 solid (No. 23: CAS RN 140-66-9, Tab. S1[4]) and two Cat. 2 solids (No. 36: CAS RN 71-81-8 and No. 46: CAS RN 79-92-5, Tab. S1[4]) that resulted in No Prediction Can be Made (NPCM) with SkinEthic™ HCE EIT were predicted No Cat. with the BCOP LLBO. For this combination the minimum performance values were not met (Tab. 7).

The reproducibility of the DAS was evaluated on 46 solids for which multiple results were available for both test methods, resulting in concordant predictions in 89.1% (41/46) solids. Two Cat. 1 solids (No. 15: CAS RN 108-45-2 and No: 18: CAS RN 54-21-7, Tab. S1[4]) resulted in discordant predictions based on the BCOP LLBO. Two Cat. 2 solids resulted in discordant predictions. One solid (No. 38: CAS RN 83-56-7, Tab. S1[4]) resulted in discordant predictions for the BCOP LLBO (once a Cat. 1 and once NPCM) and the SkinEthic™ HCE EITS test method (7 times NPCM and 4 times No Cat.). The second Cat. 2 solid (No. 47: CAS RN 99-65-0, Tab. S1[4]) was 10 times predicted No Cat. and once NPCM with the SkinEthic™ HCE EITS. One No Cat. solid (No. 73: CAS RN 1603-02-7, Tab. S1[4]) resulted 4 times in NPCM and 5 times in No Cat. with the SkinEthic™ HCE EITS test method.

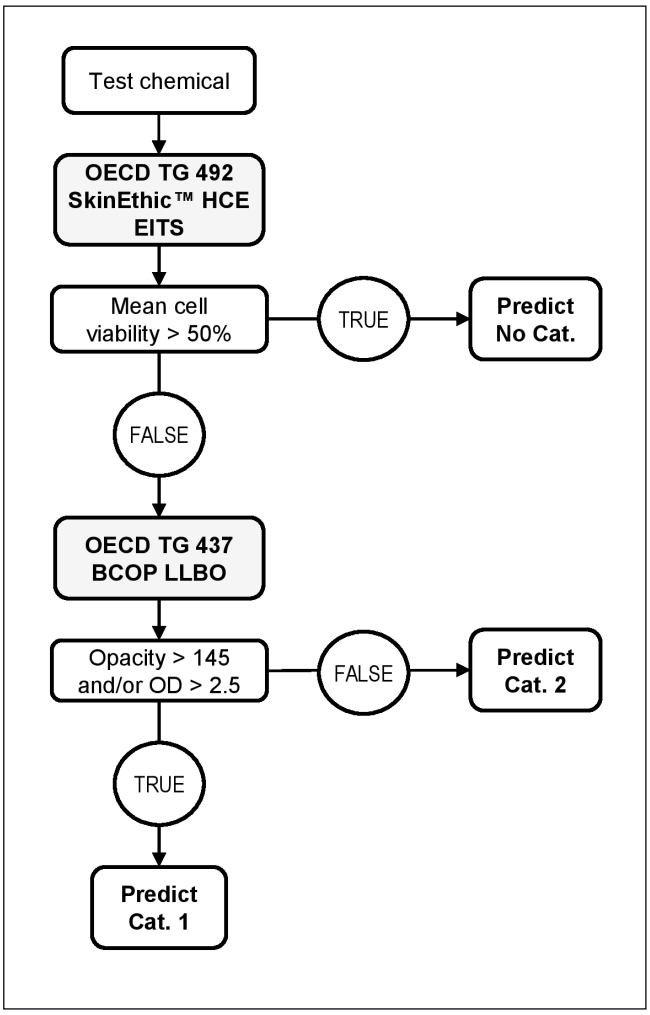

Fig. 1: Scheme of the DAS: Step 1, SkinEthic™ HCE EITS test method used to identify No Cat., and step 2, BCOP LLBO used to identify Cat. 1

4 Discussion

The goal of this study was to develop a DA for eye hazard identification of solids based on a combination of OECD-adopted test methods. For this purpose, the test methods included in OECD TG 492 (RhCE) and TG 437 (BCOP) were considered to identify UN GHS No Cat. and Cat. 1, respectively. The EpiOcular™ HCE EIT did not meet the OECD acceptance criteria for eye hazard identification according to the three UN GHS categories of at least 70% correct No Cat. identification compared to the classification based on the Draize eye test. Although the LabCyte CORNEA-MODEL24 EIT and the MCTT HCE™ EIT test method are two

Tab. 7: Performance of the DAS (N = 109): bottom-up (BU) approach, start with SkinEthic™ HCE EITS followed by BCOP LLBO and top-down (TD) approach: start with BCOP LLBO followed by SkinEthic™ HCE EITS

UN GHS	Prediction DAS		
	Cat. 1	Cat. 2	No Cat.
Cat. 1 (N = 31)	BU and TD: 77.4%	BU and TD: 22.6%	BU and TD: 0.0%
Cat. 2 (N = 18)	BU: 29.5% TD: 30.6%	BU: 52.3% TD: 52.3%	BU: 18.2% TD: 17.2%
No Cat. (N = 60)	BU: 1.7% TD: 5.3% [a]	BU: 28.3% TD: 30.4% [a]	BU: 70.0% TD: 64.3% [a]

Note: The proportion given is based on a weighted calculation which takes into account (where they exist) multiple results from an individual information source for a given chemical and applies a correction factor so that all chemicals have a weight of 1.
[a] Results based on 56 No Cat. solids. For 2 solids, no supplier was available, and 2 solids were very expensive and were therefore not tested.

RhCE test methods included in OECD TG 492, they were not considered as possible components of the DAS due to the limited amount of publicly available data on solids. Furthermore, the specificity of the LabCyte CORNEA-MODEL24 EIT test method (LABCYTE BRD, 2017[8]) based on the same set of 19 solids was 57.9% compared to 68.4% for SkinEthic™ HCE EIT. The MCTT HCE™ EIT test method looks promising, with a specificity of 82.4% based on 17 solids (Yang et al., 2017) of which 88.2% were predicted as No Cat. with the SkinEthic™ HCE EIT method. The sensitivity based on the same set of 20 classified solids was 95% and 88.2%, respectively. Therefore, the MCTT HCE™ EIT test method can probably be considered a potential me-too NAM if (preferably all) data gaps are filled to gain sufficient confidence. The BCOP OP-KIT predicted 66.9% of UN GHS Cat. 1 as Cat. 1, which is below the minimum of at least 75% concordance with the classification based on the Draize eye test. Overall, only the SkinEthic™ HCE EITS (OECD TG 492) and the BCOP LLBO (OECD TG 437) met the acceptance criteria and were therefore included as test methods in the DAS.

Furthermore, it is recommended to use the bottom-up approach described in IATA GD 263 (start with SkinEthic™ HCE EITS) to avoid false positives with the top-down approach when compared with the UN GHS classification based on the Draize eye test (No Cat. predicted Cat. 1 with BCOP LLBO; CAS RN 2736-23-4 and 21645-51-2). Also, the BCOP LLBO should not be used to identify No Cat. since some *in vivo* eye irritants that resulted in NPCM with the SkinEthic™ HCE EITS were predicted No Cat. with the BCOP LLBO (LIS < 30; CAS RN 140-66-6, 71-81-8, and 79-92-5).

Overall, the percentage of correct predictions met the minimum performance values of 75% Cat. 1, 50% Cat. 2, and 70% No Cat. established by the OECD experts. The SkinEthic™ HCE TTT is a stand-alone method applicable to liquids and solids, adopted as TG 492B (OECD, 2022a). The method met the OECD acceptance criteria for the predictivity of eye hazard identification according to UN GHS. The SkinEthic™ HCE TTT yielded similar results (correct prediction: 74.4% of 29 Cat. 1, 55.3% of 19 Cat. 2, and 71.7% of 33 No Cat.) compared to the DAS (77.4% of 31 Cat. 1, 52.3% of 18 Cat. 2, and 70.0% of 60 No Cat.). In total, data on 72 solids were available for both the DAS and the SkinEthic™ HCE TTT, and the performance compared to the reference classification was similar for both NAMs (Tab. S3[6]). The agreement in prediction between the NAMs was higher for UN GHS Cat. 1 (83.3%, 20/24 solids) and UN GHS No Cat. (81.3%, 26/32 solids). For UN GHS Cat. 2, 62.5% (10/16) of the solids resulted in the same prediction for both NAMs. More overpredictions were observed with the DAS while more false negatives were observed in the SkinEthic™ HCE TTT compared to the classification based on the Draize eye test (Tab. S3[6]). This discordance in prediction between the NAMs is not related to the driver of classification; it is related to the difference in the methods. The DAS is a combination of the two NAMs SkinEthic™ HCE EITS (identify No Cat.) and BCOP LLBO (identify Cat. 1). The SkinEthic™ HCE TTT method uses the same tissue construct as the SkinEthic™ HCE EITS but the protocols are different. In the SkinEthic™ HCE TTT method, the tissues are exposed for 30 min and 120 min to 80 mg of the neat solid with no post-incubation period, while in the SkinEthic™ HCE EITS, the tissues are exposed for 4 h to 30 mg of the neat solid followed with an 18-h post-exposure incubation period. As a result, five solids (No. 36, 39, 64, 103, 109) were predicted No Cat. with the SkinEthic™ HCE TTT (in all runs or in the majority of the runs) while they resulted in NPCM with the SkinEthic™ HCE EITS. In the DAS, UN GHS Cat. 1 and Cat. 2 solids were then distinguished from each other based on the organotypic BCOP LLBO, explaining a further discrepancy in the predictions based on the SkinEthic™ HCE TTT. Nevertheless, two options are available that meet the acceptance criteria to distinguish between the 3 UN GHS categories for the identification of eye hazard of solids.

For the Draize eye test Cat. 1 solids, 77.4% (N = 31) were predicted Cat. 1 with the DAS. The under-prediction rate for the *in vivo* Cat. 1 driver of classification CO mean ≥ 3 was low (11.1%) whereas this was 27.3% for the drivers CO = 4 and CO persistence D21 (Tab. 8). For the Draize eye test Cat. 2 solids, 52.3% (N = 18) were predicted Cat. 2 with the DAS. The over-

8 https://www.jacvam.jp/files/list/04/04_07_D2.pdf

Tab. 8: Performance of the DAS grouped by driver of classification (UN GHS Cat. 1 and Cat. 2) and by subgroup (UN GHS No Cat.)

UN GHS	Driver	N	Prediction DAS		
			Cat. 1	Cat. 2	No Cat.
Cat. 1	CO mean ≥ 3	9	88.9%	11.1%	0.0%
	CO = 4	11	72.7%	27.3%	0.0%
	CO pers D21	11	72.7%	27.3%	0.0%
Cat. 2	CO mean ≥ 1	7	42.9%	42.9%	14.3%
	Conj mean ≥ 2	11	21.1%	58.3%	20.7%
No Cat.	CO > 0 ** and CO = 0 **	3	0.0%	100%	0.0%
	CO > 0	9	0.0%	44.4%	55.6%
	CO = 0	48	2.1%	22.9%	75.0%

CO, corneal opacity; CO pers D21, CO persistence on day 21; Conj, conjunctival; mean, mean scores calculated from gradings at 24, 48, and 72 hours after instillation of the test chemical; CO > 0, CO scores > 0 in at least one animal and at least one observed time point; CO = 0, CO scores equal to 0 in all observation times in all animals. Studies marked with ** are studies for which at least one animal had a mean of the scores of days 1-3 above the classification cut-off for at least one endpoint but not in enough animals to generate a classification.

Tab. 9: Predictive performance of the DAS on the basis of the organic functional groups for which at least 5 solids were evaluated for a specific UN GHS category (Cat. 1, Cat. 2, No Cat.)

UN GHS	Predicted class	Aryl	Carboxylic acid	Phenol	Aryl halide	Ether	Carboxylic acid ester
		N = 31	N = 18	N = 17	N = 16	N = 10	N = 9
Cat. 1	Cat. 1	6.0/6	7.5/8	5.5/7	4.0/5	NA	1.0/1
	Cat. 2	0.0/6	0.5/8	1.5/7	1.0/5	NA	0.0/1
	No Cat.	0.0/6	0.0/8	0.0/7	0.0/5	NA	0.0/1
Cat. 2	Cat. 1	1.0/4	1.0/5	1.3/2	0.0/1	1.0/2	0.0/1
	Cat. 2	2.0/4	4.0/5	0.3/2	1.0/1	0.0/2	1.0/1
	No Cat.	1.0/4	0.0/5	0.4/2	0.0/1	1.0/2	0.0/1
No Cat.	Cat. 1/Cat. 2	4.0/21	3.0/5	3.0/8	0.0/10	1.0/8	2.0/7
	No Cat.	17.0/21	2.0/5	5.0/8	10.0/10	7.0/8	5.0/7

UN GHS	Predicted class	Alcohol	Benzyl	Fused carbocyclic aromatic	Ketone	tert-Butyl	Aromatic amine
		N = 7	N = 7	N = 7	N = 5	N = 5	N = 5
Cat. 1	Cat. 1	1.0/2	2.0/2	3.0/3	1.0/2	1.0/1	NA
	Cat. 2	1.0/2	0.0/2	0.0/3	1.0/2	0.0/1	NA
	No Cat.	0.0/2	0.0/2	0.0/3	0.0/2	0.0/1	NA
Cat. 2	Cat. 1	NA	1.0/1	1.3/2	0.0/1	0.0/1	NA
	Cat. 2	NA	0.0/1	0.3/2	1.0/1	0.0/1	NA
	No Cat.	NA	0.0/1	0.4/2	0.0/1	1.0/1	NA
No Cat.	Cat. 1/Cat. 2	1.0/5	0.0/4	0.0/3	1.0/2	0.0/3	0.0/5
	No Cat.	4.0/5	4.0/4	3.0/3	1.0/2	3.0/3	5.0/5

prediction rate was higher for the solids that were classified Cat. 2 based CO mean ≥ 1 (42.9%) compared to Conjunctiva mean ≥ 2 (21.1%, Tab. 8). The false negative rates for the *in vivo* Cat. 2 drivers were 14.3% and 20.7%, respectively. The concordance between the prediction of the DAS and the UN GHS No Cat. classification based on the Draize eye test was 70.0% (N = 60). The No Cat. identification rate for solids that induced no opacity in the Draize eye test (CO = 0) was higher (75.0%) compared to the subgroup CO > 0 (55.6%, Tab. 8). No Cat. solids from the subgroup CO > 0 induced CO scores > 0 in at least one animal and at least one observed time point. Furthermore, studies marked with ** are studies for which at least one animal had a mean of the scores of days 1-3 above the classification cut-off for at least one endpoint but not in enough animals to generate a classification. The reference set contained three solids with these characteristics, all were predicted Cat. 2 with the DAS (Tab. 8). In general, chemicals that induce some opacity in the Draize eye test (CO > 0) are more often over-predicted with *in vitro* methods (Verstraelen et al., 2017; Kandarova et al., 2018; Van Rompay et al., 2018; Alépée et al., 2019a).

Discrepancies between the UN GHS classification based on the Draize eye test and the DAS prediction were further investigated in terms of water solubility. Five *in vivo* irritants had a water solubility < 0.01 mg/mL, 2 out of 3 Cat. 1 solids and 2 Cat. 2 solids were correctly identified, indicating that poor solubility is not an issue. Note that under-predictions were also observed for solids with a water solubility > 10 mg/mL, 4 out of 10 *in vivo* Cat. 1 solids were sometimes or constantly under-predicted with the DAS.

The relationship between organic functional groups (OFGs) and misprediction with the DAS was also investigated. Only OFGs for which at least 5 solids were evaluated for a specific UN GHS category are discussed (Tab. 9). Solids with an aryl, carboxylic acid, phenol or aryl halide were the only *in vivo* Cat. 1 chemicals with at least 5 solids. The concordance for Cat. 1 solids with an aryl function (N = 6) was 100% and for the other OFGs (carboxylic acid, phenol or aryl halide) the majority (> 75%) of the solids was predicted Cat. 1. Solids with a carboxylic acid are the only *in vivo* Cat. 2 chemicals with at least 5 solids being tested with 80% (N = 5) predicted Cat. 2 with the DAS. For No Cat., the false positive rate was 60% for carboxylic acids (N = 5) and 37.5% for phenols (N = 8) when compared to the UN GHS classification based on the Draize eye test. For all other solids with at least 5 UN GHS No Cat. chemicals the false positive rate was ≤ 20% (aryl, aryl halide, ether, carboxylic acid ester, alcohol, aromatic amine). About 81% of the *in vivo* No Cat. solids with an aryl group (N = 21) were predicted as No Cat. with the DAS. Overall, the number of substances per OFG with results for DAS is limited, and therefore it is not possible to exclude any OFGs from the applicability domain of the DAS.

In conclusion, it was demonstrated in the context of the IATA concept that the DAS is a reliable defined approach for eye hazard assessment of solids according to UN GHS and can be considered as a full replacement NAM of the *in vivo* Draize test. The DAS was adopted by the OECD Working Group of National Co-ordinators of the TGs programme in April 2024, and a revision of TG 467 will be released later this summer.

References

Adriaens, E., Verstraelen, S., Desprez, B. et al. (2021). Overall performance of bovine corneal opacity and permeability (BCOP) laser light-based opacitometer (LLBO) test method with regard to solid and liquid chemicals testing. *Toxicol In Vitro 70*, 105044. doi:10.1016/j.tiv.2020.105044

Alépée, N., Leblanc, V., Adriaens, E. et al. (2016). Multi- laboratory validation of SkinEthic HCE test method for testing serious eye damage/eye irritation using liquid chemicals. *Toxicol In Vitro 31*, 43-53. doi:10.1016/j.tiv.2016.02.014

Alépée, N., Adriaens, E., Abo, T. et al. (2019a). Development of a defined approach for eye irritation or serious eye damage for neat liquids based on cosmetics Europe analysis of in vitro RhCE and BCOP test methods. *Toxicol In Vitro 59*, 100-114. doi:10.1016/j.tiv.2019.04.011

Alépée, N., Adriaens, E., Abo, T. et. al. (2019b). Development of a defined approach for eye irritation or serious eye damage for liquids, neat and in dilution, based on cosmetics Europe analysis of in vitro STE and BCOP test methods. *Toxicol In Vitro 57*, 154-163. doi:10.1016/j.tiv.2019.02.019

Alépée, N., Adriaens, E., Abo, T. et al. (2023). Development of a defined approach for eye hazard identification of chemicals having surfactant properties according to the three UN GHS categories. *Toxicol In Vitro 89*, 105576. doi:10.1016/j.tiv.2023.105576

Barroso, J., Alépée, N., Cole at al. (2014). The EURL ECVAM – Cosmetics Europe Prospective Validation Study of Reconstructed Human Cornea-Like Epithelium (RhCE)-Based Test Methods for Identifying Chemicals Not Requiring Classification and Labelling for Serious Eye Damage/Eye Irritation – Validation. doi:10.2787/41680

Barroso, J., Pfannenbecker, U., Adriaens, E. et al. (2017). Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: The Draize eye test Reference Database (DRD). *Arch Toxicol 91*, 521-547. doi:10.1007/s00204-016-1679-x

ICCVAM (2006). Background Review Document: Current Status of in Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability Test Method. (NIH Publ. No. 06-4512).

Kandarova, H., Letasiova, S., Adriaens, E. et al. (2018). CON4EI: EpiOcular™ eye irritation test (EpiOcular™ EIT) for hazard identification and labelling of eye irritating chemicals. *Toxicol In Vitro 49*, 21-33. doi:10.1016/j.tiv.2017.07.002

OECD (2019). Guidance Document on Integrated Approaches to testing and assessment (IATA) for serious eye damage and eye irritation. *OECD Series on Testing and Assessment, No. 263*. OECD Publishing, Paris. https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

OECD (2021). Test No. 494: Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage. *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/9f20068a-en

OECD (2022a). Test No. 492B: Reconstructed Human Cornea-like

Epithelium (RHCE) Test Method for Eye Hazard Identification. *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/0d603916-en

OECD (2022b). Test No. 467: Defined Approaches for Serious Eye Damage and Eye Irritation. *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/28fe2841-en

OECD (2022c). Supporting Document for Evaluation and Review of Test Guideline 467 on Defined Approaches (DAs) for Serious Eye Damage / Eye Irritation, OECD Environment, Health and Safety Publications. *Series on Testing and Assessment, No 354*. OECD Publishing, Paris. https://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmenttestingforhumanhealth.htm

OECD (2023a). Test No. 405: Acute Eye Irritation/Corrosion. *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/9789264185333-en

OECD (2023b). Test No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage. *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/9789264242548-en

OECD (2023c). Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/9789264203846-en

OECD (2023d). Test No. 491: 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 Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/9789264242432-en

OECD (2023e). Test No. 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 Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/9789264203860-en

OECD (2023f). Test No. 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants. *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/9789264185401-en

OECD (2023g). Test No. 496: In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. doi:10.1787/970e5cd9-en

UN (2023). United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Tenth revised edition. ST/SG/AC.10/30/Rev.10. New York and Geneva: United Nations. https://unece.org/sites/default/files/2023-07/GHS%20Rev10e.pdf

Van Rompay, A. R., Alépée, N., Nardelli, L. et al. (2018). CON4EI: SkinEthic™ human corneal epithelium eye irritation test (SkinEthic™ HCE EIT) for hazard identification and labelling of eye irritating chemicals. *Toxicol In Vitro 49*, 11-20. doi:10.1016/j.tiv.2017.06.012

Van Rompay, A. R., Adriaens, E. and Verstraelen S. (2020). Laser light-based opacitometer 'Peira LLBO 180': A new and validated opacitometer for use in the bovine corneal opacity and permeability (BCOP) eye irritation test method. *MethodsX 7*, 101085. doi:10.1016/j.mex.2020.101085

Verstraelen, S., Maglennon, G., Hollanders, K. et al. (2017). CON4EI: Bovine corneal opacity and permeability (BCOP) test for hazard identification and labelling of eye irritating chemicals. *Toxicol In Vitro 44*, 122-133. doi:10.1016/j.tiv.2017.06.028

Yang, H., Kim, D. E., Jang, W. H. et al. (2017). Prevalidation trial for a novel in vitro eye irritation test using the reconstructed human cornea-like epithelial model, MCTT HCE™. *Toxicol In Vitro 39*, 58-67. doi:10.1016/j.tiv.2016.11.010

Conflict of interest

The authors declare that there are no conflicts of interest.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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