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Foreword

This document is supporting the inclusion of defined approaches for surfactants in OECD Test Guideline 467 (TG 467) on Defined Approaches (DAs) for Serious Eye Damage and Eye Irritation. The draft supporting document was approved at the Working Party of the National Coordinators of the Test Guidelines Programme (WNT) in April 2025, after review by the dedicated OECD Expert Group on eye irritation.

Following declassification in July 2025, the Supporting Document is published under the responsibility of the Chemicals and Biotechnology Committee.

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List of acronyms

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

CC: conjunctival chemosis

CO: corneal opacity

Conj: conjunctival effects

CR: conjunctival redness

DA: Defined approach

DAL: Defined approach liquids

DAS: Defined approach solids

DASF: Defined approach surfactants

DIP: data interpretation procedure

DRD: Draize eye test Reference Database

ECHA: European Chemicals Agency

EITL: Eye Irritation Test Liquids

EITS: Eye Irritation Test Solids

EURL ECVAM: European Union Reference Laboratory on Alternatives to Animal Testing

FN: false negative

FP: false positive

GL: guideline

HCE: Human Corneal Epithelium

IATA: Integrated Approaches to Testing and Assessment

IR: iritis

MoA: Modes of Action

MSDS: material safety data sheet

No Cat.: Not requiring UN GHS Classification

OECD: Organisation for Economic Co-operation and Development

RhCE: Reconstructed human Cornea-like Epithelium

SPSF: Standard Project Submission Form

UN GHS: United Nations Globally Harmonized System

WNT: Working Group of National Co-ordinators of the Test Guidelines programme

1 Introduction

1. In June 2022 two defined approaches (DAs) for non-surfactant liquids were accepted (DAL-1 and DAL-2) and were integrated in a new OECD test guideline (TG) for serious eye damage/eye irritation i.e., discrimination between the three United Nations Globally Harmonized System of Classification (UN GHS) categories (Part I and Part II, OECD TG 467 2024). In June 2024, a third DA was accepted to specifically address the eye hazard potential of solids across the 3 UN GHS categories (Part III, OECD TG 467 2024). A fourth DA has been developed that addresses the eye hazard potential of surfactants across the 3 UN GHS categories (Alépée et al., 2023; Alépée et al., 2024; OECD Case study, 2024). The DASF was developed as part of the Long-Range Science Strategy (LRSS) of Cosmetics Europe and is now supported by International Collaboration on Cosmetics Safety (ICCS). The DASF has been integrated in TG 467 as part IV.

2. The earlier developed DAs for non-surfactant liquids and solids did not allow for addressing surfactants. Consequently, the DAs were modified to address neat surfactants and diluted ones. Surfactants are a specific group of chemicals with similar properties, characterized by their amphiphilic nature, having both polar (hydrophilic) and lipophilic (hydrophobic) components. This unique structure drives their primary mode of action, which includes reducing surface tension, forming micelles, and stabilizing emulsions. These actions can also lead to cell membrane disruption, as surfactants can insert themselves into the lipid bilayer of cell membranes, causing increased permeability or even cell lysis. Surfactants can be further classified as anionic, cationic, nonionic, and amphoteric, each with distinct functional groups that determine their specific applications and effects.

3. According to the UN GHS classification system, Category 1 (serious eye damage) refers to the production of tissue damage in the eye, or serious physical decay of vision, which is not fully reversible, occurring after exposure of the eye to a substance or mixture. Category 2 (eye irritation) refers to the production of changes in the eye, which are fully reversible, occurring after the exposure of the eye to a substance or mixture. Based on this definition, the hazard potential of a test chemical has been determined in the Draize eye test (OECD TG 405, 2023) based on its effect on corneal opacity (CO), iritis (IR), conjunctival redness (CR), and conjunctival chemosis (CC). Based on the severity of effects and/or the timing of their reversibility, classifications are derived by the UN GHS (UN 2023). Effects not fully reversed at the end of the 21 day observation period of the Draize test are considered irreversible (Category 1) or not (Category 2). Cat. 2 may be

divided into the optional Categories 2A (effects fully reversible within 21 days) and 2B (effects fully reversible within 7 days). When none of the Cat. 1 or Cat. 2 classification criteria are met, the chemical does not require classification which corresponds with No Category (No Cat.). Note that every time reference is made to in vivo Cat. 1, Cat. 2, and No Cat. in this background review document, those classifications have been derived from testing in albino rabbits according to the Draize eye test method (OECD TG 405). The main data source of the historical data was the Draize eye test Reference Database (DRD) published by Cosmetics Europe (Barroso et al., 2017).

4. A comprehensive analysis to address the main *in vivo* ocular tissue effects that drive UN GHS classification was conducted and the outcomes were used to evaluate the performance of the DASF described in the present document. The analyses identified nine different criteria from the four *in vivo* tissue effects (CO, IR, CR, and CC) that can each independently drive the classification of a chemical (Barroso et al., 2017). Of note, CR and CC were not reported separately but were reported together as conjunctival effects (Conj) because previous analyses revealed that CC rarely drives the classification of chemicals in the absence of CR effects (Adriaens et al., 2014; Barroso and Norman, 2014). Chemicals classified as Cat. 1 were grouped based on (i) severity (mean scores of days 1–3); (ii) persistence of any ocular effect on day 21 in the absence of severity; or (iii) CO = 4 (at any observation time during the study) in the absence of both severity and persistence (or if unknown). Chemicals classified as Cat. 2 were allocated to one of the three following groups based on the main endpoint leading to Cat. 2 classification, i.e., “CO”, “Conj”, and “IR”. Studies with chemicals not requiring classification for serious eye damage/eye irritation (No Cat.) were distributed in four different groups depending on whether they showed CO scores equal to 0 in all animals and all observed time points (CO = 0 and CO = 0**) or not (CO > 0 and CO > 0**). No Cat. 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 (borderline cases) were marked with ** (CO = 0**, CO > 0**). A detailed description of the drivers of classification and use of the terms CO, IR and Conj to describe key effects is provided in the paper of Barroso and co-workers (2017).

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) Retrospective analysis of the Draize test for serious eye damage/eye irritation: importance of understanding the in vivo endpoints under UN GHS/EU CLP for the development and evaluation of in vitro test methods. Arch Toxicol 88:701–723.

Alépée N, Adriaens E, Abo T, Magby J, Mewes KR, Giusti A (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. https://doi.org/10.1016/j.tiv.2023.105576

Alépée N, Mewes KR, Abo T, Cavarzan A, Odriscoll C, Adriaens E (2024). Performance of the DASF compared to other combinations of OECD NAMs for eye hazard identification of surfactants. ALTEX DOI: https://doi.org/10.14573/altex.2406031

Barroso J, Norman K (2014). REACHing for alternatives to animal testing. A webinar series on modern testing strategies for REACH. Webinar 3 of 6 on “Serious Eye Damage and Eye Irritation”, December 4, 2014. http://www.piscltd.org.uk/reaching-alternatives-animal-testing/.

Barroso J, Pfannenbecker U, Adriaens E, Alépée N, Cluzel M, De Smedt A, Hibatallah J, Klaric M, Mewes KR, Millet M, Templier M, McNamee P (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 (2017) 91:521–547.

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

OECD (2024e), No. 391 Case Study on the use of Integrated Approaches for Testing and Assessment for “Eye hazard identification” of “surfactants”, Series on Testing and Assessment, OECD Publishing, Paris https://one.oecd.org/document/ENV/CBC/MONO(2024)6/en/pdf

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

2 Presentation of the analysed DA

Introduction

5. This document supports the integration of a DA for surfactants into OECD TG 467, Defined Approaches for Serious Eye Damage and Eye Irritation as Part IV. The DA for surfactants (hereinafter referred to as DASF) is based on a combination of Reconstructed human Cornea-like Epithelium (RhCE) test methods (OECD TG 492, 2025b, EpiOcular™ EIT or SkinEthic™ HCE EIT), and the modified Short Time Exposure (STE) test method (Alépée et al., 2023), which has now been renamed as $STE^{0.5}$ for surfactants.

6. The DAs currently included in OECD TG 467 (2025c) are limited to non-surfactant liquids (DAL-1 and DAL-2) and solids (DAS). Therefore, a new DA (the DASF) was developed that specifically addresses the assessment of eye hazard of liquid, semi-solid and solid chemicals having surfactant properties[1].

7. The DASF has proven to enable reliable predictions across UN GHS Category 1 (Cat. 1) on "serious eye damage"; and No Category (No Cat.) for chemicals "not requiring classification and labelling" (UN GHS, 2023), but due to the small dataset (10) for Category 2 (Cat.2) "eye irritation", if a surfactant is predicted as Cat.2, care should be taken when drawing conclusions on the prediction. Whilst the components of the DASF can be used to identify chemicals requiring classification of Cat. 1 ($STE^{0.5}$ for surfactants, Alépée et al., 2023) and chemicals that do not require classification for eye irritation or serious eye damage (No Cat.; OECD TG 492, 2025), the DASF also allows to make a Cat. 2 prediction. However, the DASF is not designed to distinguish between Categories 2A and 2B.

8. This supporting document provides information on the evaluation of the proposed DASF for identification of UN GHS Cat. 1, Cat. 2, and No Cat., that is proposed to include as Part IV in OECD TG 467 on DAs for serious eye damage/eye irritation. Much of the information provided in this supporting document has been published in peer reviewed journals (Alépée et al., 2023, 2024).

[1] Surfactant means any substance and/or mixture, which has surface-active properties and which consists of one or more hydrophilic and one or more hydrophobic groups of such a nature and size that it is capable of reducing the surface tension of water, and of forming spreading or adsorption monolayers at the water-air interface, and of forming emulsions and/or microemulsions and/ or micelles, and of adsorption at water-solid interfaces.

DASF

9. The DASF presented in this document describes the combination of the EpiOcular™ EIT or SkinEthic™ HCE EIT according to OECD TG 492 (2025b) and the $STE^{0.5}$ for the identification of the eye hazard potential for surfactants primarily for the purposes of classification and labelling without the use of animal testing. The OECD adopted STE test method (TG 491, 2025a) was optimized to discriminate between UN GHS Cat. 1 and not Cat. 1 surfactants (hereinafter referred to as $STE^{0.5}$ for surfactants). The $STE^{0.5}$ for surfactants differs from the procedure described in OECD TG 491 only in that cell viability in corneal epithelial cells is measured after a 5 minutes exposure to a 0.5% concentration and using a different cut-off value of 20% cell viability for classification.

10. The data interpretation procedure (DIP) applied uses the readout of the prediction models of each of the individual RhCE test methods as defined by TG 492 and the readout of the prediction model of the $STE^{0.5}$ for surfactants. A scheme of DASF is presented in Figure 2.1. (Bottom-Up approach). In the Bottom-Up approach, surfactants are evaluated based on a RhCE test method (EpiOcular™ EIT or SkinEthic™ HCE EIT) in Step 1. Note that two different treatment protocols are used for the RhCE test methods, one for liquids (Eye Irritation Test for Liquids - EITL) and one for solids (EITS). Liquid surfactants that result in a tissue viability > 60% are classified No Cat. and liquid surfactants that result in a tissue viability ≤ 60% are evaluated based on the $STE^{0.5}$ for surfactants in a second step. For solid surfactants the EITS protocol should be used, the threshold tissue viability used to identify No Cat. is > 60% for EpiOcular™ EIT and > 50% for SkinEthic™ HCE EIT. Solid surfactants that result in a tissue viability below the threshold are evaluated based on the $STE^{0.5}$ for surfactants in Step 2. Surfactants that result in a cell viability ≤ 20% are predicted Cat. 1 and the remaining surfactants are assigned Cat. 2. Note that is also possible to start with the $STE^{0.5}$ for surfactants followed by the RhCE test method (Top-Down approach), this scheme is presented in Figure 2.2. Depending on the available data, the most relevant approach should be selected: if the test substance is most likely classified as UN GHS Cat.1, consider the Top-Down approach; if there is high certainty that no classification is needed (UN GHS No Cat), consider the Bottom-Up approach (OECD, 2024c).

Figure 2.1. Bottom-Up approach of the DASF; step 1 EpiOcular™ EITL (Liquids) / EITS (Solids) or SkinEthic™ HCE EITL / EITS test method used to identify No Cat., and step 2 STE$^{0.5}$ for surfactants used to identify Cat. 1.

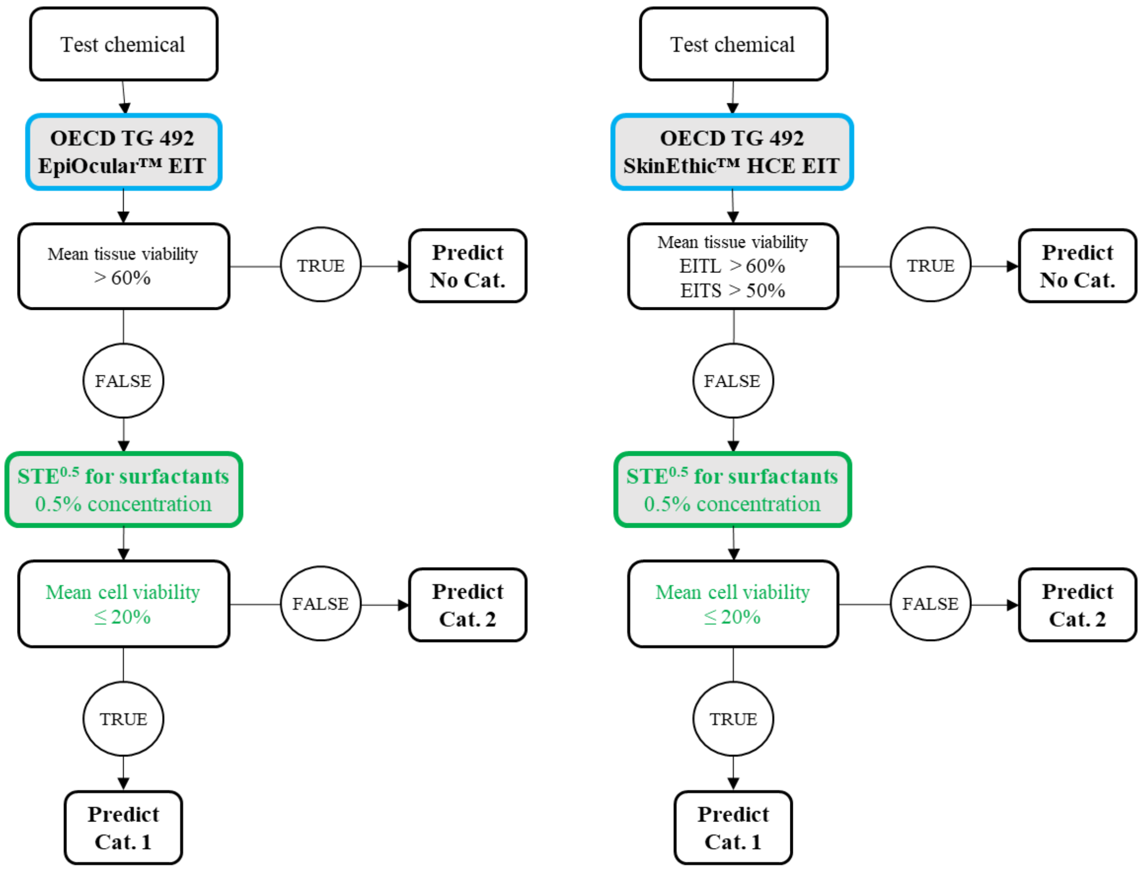
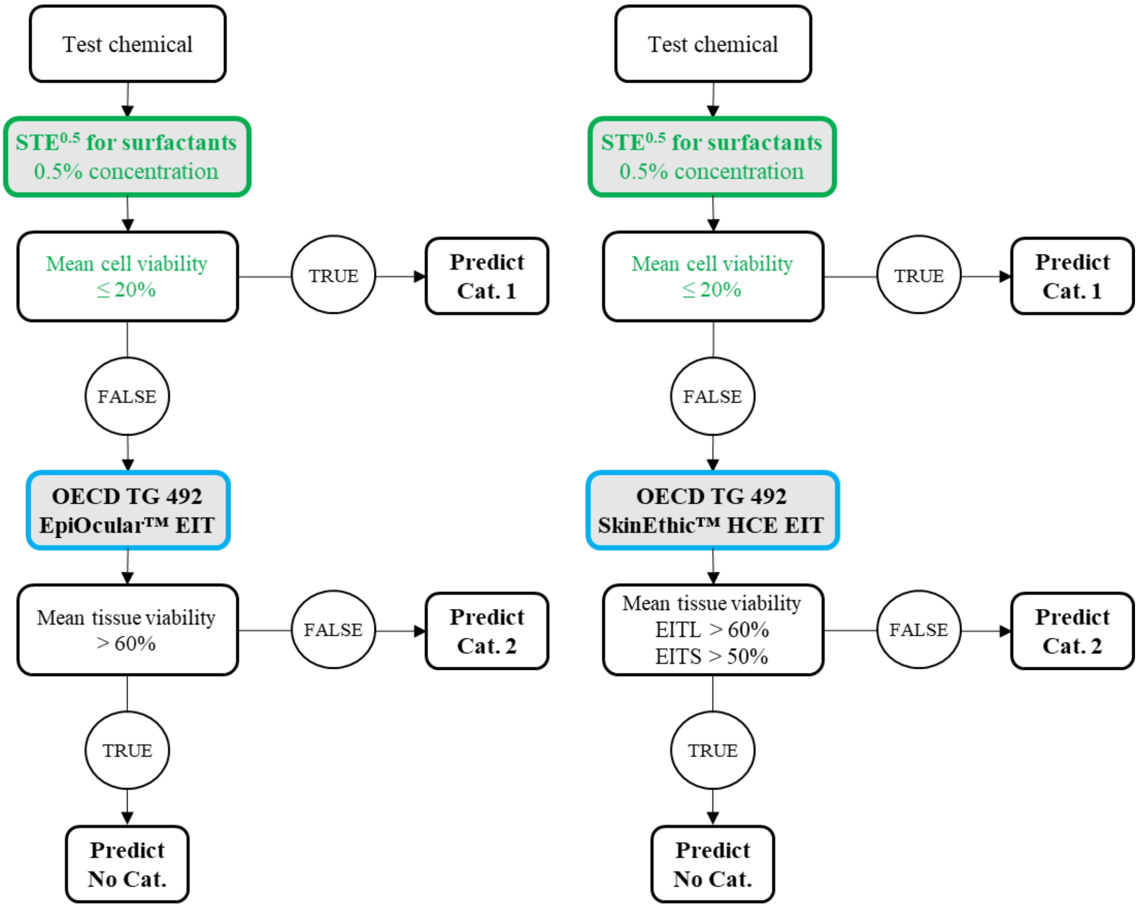


Figure 2.2. Top-Down approach of the DASF; step 1 STE$^{0.5}$ for surfactants used to identify Cat. 1, and step 2 EpiOcular™ EITL / EITS or SkinEthic™ HCE EITL / EITS test method used to identify No Cat.



References

Adriaens E, Verstraelen S, Alépée N, Kandarova H, Drzewiecka A, Gruszka K, Guest R, Willoughby JA, Van Rompay AR (2018). CON4EI: Development of testing strategies for hazard identification and labelling for serious eye damage and eye irritation of chemicals. Toxicol. in Vitro 49, 99–115. https://doi.org/10.1016/j.tiv.2017.09.008.

Alépée N, Adriaens E, Abo T, Magby J, Mewes KR, Giusti A (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. https://doi.org/10.1016/j.tiv.2023.105576

Alépée N, Mewes KR, Abo T, Cavarzan A, Odriscoll C, Adriaens E (2024). Performance of the DASF compared to other combinations of OECD NAMs for eye hazard identification of surfactants. ALTEX DOI: https://doi.org/10.14573/altex.2406031

Barroso J, Pfannenbecker U, Adriaens E, Alépée N, Cluzel M, De Smedt A, Hibatallah J, Klaric M, Mewes KR, Millet M, Templier M, McNamee P (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 (2017) 91:521–547.

OECD (2025a), *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, https://doi.org/10.1787/9789264242432-en.

OECD (2025b), 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, https://doi.org/10.1787/9789264242548-en.

OECD (2025c), No. 467 Defined Approaches for Serious Eye Damage and Eye Irritation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org:10.1787/28fe2841-en

OECD (2024c), *Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation, Third Edition*, OECD Series on Testing and Assessment, OECD Publishing, Paris, https://doi.org/10.1787/cdb440be-en.

UN (2023). United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS). ST/SG/AC.10/30/Rev.10, Revised Edition, New York and Geneva: United Nations. Available at [https://read.un-ilibrary.org/environment-and-climate-change/globally- https://unece.org/sites/default/files/2023-07/GHS%20Rev10e.pdf

3 *In vivo* reference data (Draize eye test)

11. Since the DASF applicability domain is limited to surfactants only, there exists only a limited database with reliable Draize eye test data, particularly for UN GHS Cat. 2 substances. Rigorous curation criteria, as outlined in paragraph 16, were applied to ensure that only surfactants meeting these key criteria were included. To further address this limitation, regulatory agencies, stakeholders, and associations were consulted to expand the dataset. Despite these efforts, only one additional commercially available surfactant, classified as UN GHS Cat. 2 with reliable historical Draize eye test data, was identified. This brings the total number of UN GHS Cat. 2 surfactants to 10. DASF fills an important gap in the DA test guidelines, but due to the small dataset, if a surfactant is predicted as Cat.2, care should be taken when drawing conclusions on the prediction. All surfactants that met the established criteria were incorporated into the dataset. Although the number of surfactants is limited, they share similar properties and mechanism of action. The dataset includes representatives from all four surfactant classes, along with different families within each class, as well as both mono- and multi-constituent substances and UVCBs. This comprehensive approach provides, despite the limited number of surfactants, a representative dataset.

12. The main data source was the Draize eye test Reference Database (DRD) published by Cosmetics Europe (Barroso et al., 2017). Five surfactants were selected from other databases (Blazka et al., 2000 and Scheel et al., 2011). The data from one surfactant came from a Scientific Committee on Consumer Products (SCCP) opinion (SCCP/0917/05, 2006). The data for one surfactant tested neat and in dilution is proprietary.

Criteria applied for the selection of the reference chemicals for the DASF

13. The following criteria were considered when selecting the reference chemicals: (1) the expected applicability of the DASF in terms of UN GHS prediction (No. Cat., Cat. 2, and Cat. 1, (2) important drivers of classification, (3) characterisation of the surfactant, (4) different surfactant classes, and (5) neat surfactants and surfactants diluted in water.

Drivers of classification

14. The chemical selection was performed by taking into account several key criteria that were identified by Barroso and co-workers (2017). One of the key criteria is that the pool of reference chemicals needs to address the main ocular tissue effects that drive classification. In the Draize rabbit eye test, the hazard potential of a test chemical is determined based on its effect on corneal opacity (CO), iritis (IR), conjunctival redness (CR), and conjunctival chemosis (CC). Based on the severity of effects and/or the timing of their reversibility, classifications are derived according to the serious eye damage/eye irritation classification criteria defined by UN GHS (UN 2021).

As described by Barroso and co-workers (2017), there are nine different criteria derived from the four tissue effects (CO, IR, CR, and CC) that can each independently drive the classification of a chemical (Table 3.1).

Table 3.1. Drivers of UN GHS classification

Category 1 Irreversible effects on the eye/serious eye damage						Category 2 Reversible effects on the eye/eye irritation		
Severity (Mean scores of Days 1-3) [a]		Persistence on Day 21			Severe CO	Severity (Mean scores of Days 1-3) [a]		
CO mean ≥ 3	**IR mean > 1.5**	**CO**	**Conj**	**IR**	**CO=4**	**CO mean ≥ 1**	**Conj mean ≥ 2**	**IR mean ≥ 1**
in ≥ 60% of the animals	in ≥ 60% of the animals	in at least one animal	in at least one animal	in at least one animal	in at least one animal	in ≥ 60% of the animals	in ≥ 60% of the animals	in ≥ 60% of the animals

CO: corneal opacity; IR: iritis; Conj: conjunctival redness (CR) and/or conjunctival chemosis (CC)
Drivers with a greyed background correspond with the most important drivers.
[a] Mean scores are calculated from gradings at 24, 48, and 72 hours after instillation of the test chemical

Key criteria considered when selecting reference chemicals

15. Only surfactants for which individual tissue scores for each rabbit from the Draize eye study are available, for which the study criteria were met (Barroso et al., 2017) and which do not have conflicting data in case multiple *in vivo* studies are available were selected. The accessibility of raw data is required to identify if the study criteria (i.e., at least tested in 3 animals) were met and to identify the driver of classification. In addition, the surfactant should be commercially available at a reasonable price or a sample for testing should be available. The surfactants should be well characterized in terms of their purity, number of moles of ethylene oxide (EO), number of moles of propylene oxide (PO), carbon chain length, etc.

Reference chemicals used for the development

16. The set of reference chemicals for the assessment of the predictive capacity of the DASF was composed of 50 surfactants representing 33 unique surfactants, nine were tested neat only, three were tested neat and at least in one dilution, 14 were tested at a single dilution and seven were tested at multiple dilutions (at least two dilutions per surfactant). This information is provided in Annex B.1.

Surfactant class and family

17. The set of surfactants consists of the four different surfactant classes, that is non-ionic (no charge), cationic (+ charge), anionic (- charge), and amphoteric (opposite pH-dependent charge) according to charge of the head group. Each class is represented by mono-constituent and multi-constituent or UVCBs. Several families are represented in the set (e.g. alcohol ethoxylates, alkyl sulfates, sulfosuccinates, …).

Key elements for evaluation of the DASF versus the Draize eye test

18. It was recognized that determination of the most relevant *in vivo* endpoint(s), in particular the effects on cornea, iris or conjunctiva, is extremely important for the development of adequate *in vitro* methods and will allow better understanding of the relationship between the *in vitro* and the *in vivo* data (Scott et al., 2010). A comprehensive in-depth analysis of historical *in vivo* rabbit eye data provided insight into which of the observed *in vivo* effects are important in driving the classification of chemicals for serious eye damage/eye irritation according to the UN GHS, concluding that full replacement of *in vivo* testing for eye hazard will require accounting for the impact of the *in vivo* tissue effects which drive classification (Adriaens et al. 2014). Further, the uncertainty (variability) of the *in vivo* reference data is also recognized as a challenging factor that may hinder the successful development of non-animal approaches and should be considered when evaluating/validating *in vitro* test methods and strategies. It is therefore challenging to align the results from *in vitro* methods to the *in vivo* rabbit test for the middle category (UN GHS Cat 2). A database of Draize data was compiled (Cosmetics Europe Draize eye test Reference Database, DRD) and an evaluation of the various *in vivo* drivers of classification compiled in the database was performed to establish which of these are most important from a regulatory point of view (Barroso et al., 2017). These analyses established the most important drivers for Cat. 1 and Cat. 2 classification and the distribution in different groups for the chemicals that do not require classification.

19. In November (Nov 3, 2020) a teleconference was held with a subgroup of the Expert Group on Skin and Eye irritation to discuss the issue regarding the Modes of Action (MoA). It was concluded that the MoA are unknowable for the majority of the chemicals and most test substances would fall into multiple chemical classes. The MoA for surfactants was also discussed with the Expert Group on Skin and Eye irritation and resulted in the publication

of an Issue Paper on the DASF (Annex C.1). Is was concluded that the MoA for surfactants is better understood. Due to their amphiphilic (polar and lipophilic properties) nature, surfactants form micelles in an aqueous environment. When biological tissues, such as the cornea, are exposed to surfactants, a series of events takes place including cell membrane lysis, protein extraction, and ultimately cell membrane disruption (Helenius and Simons, 1975). In addition to ensuring that the key *in vivo* drivers of classification have been covered by the selected reference chemicals, analysis of the surfactant classes present across the reference test chemicals show that a wide range of functionality has been covered over the UN GHS Cat. 1, Cat. 2 and No Cat. classified chemicals.

20. In conclusion, the assessment of the performance of the individual test methods and the DASF against the Draize eye test has been conducted based on reference surfactants selected such that the important drivers of classification for each UN GHS category and the different surfactant classes are represented. Note that the reference list contains only two amphoteric surfactants, one UN GHS Cat. 1 and one UN GHS Cat. 2. The predictions of the DASF were concordant with the reference classification.

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) Retrospective analysis of the Draize test for serious eye damage/eye irritation: importance of understanding the in vivo endpoints under UN GHS/EU CLP for the development and evaluation of in vitro test methods. Arch Toxicol 88:701–723.

Barroso J, Pfannenbecker U, Adriaens E, Alépée N, Cluzel M, De Smedt A, Hibatallah J, Klaric M, Mewes KR, Millet M, Templier M, McNamee P (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.

Blazka, M.E., Harbell, J.W., Klausner, M., et al.. (2000). Colgate-Palmolive's program to validate the EpiOcular™ human tissue construct model. The Toxicologist 54, 188.

OECD (2005), *Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment*, OECD Series on Testing and Assessment, No. 34, OECD Publishing, Paris, https://doi.org/10.1787/e1f1244b-en.
Scheel, J., Kleber, M., Kreutz, J. et al. (2011). Eye irritation potential: usefulness of the HET-CAM under the Globally Harmonized System of classification and labeling of chemicals (GHS). Regul Toxicol Pharmacol. 59(3), 471–492. https://doi.org/10.1016/j.yrtph.2011.02.003

Scientific Committee on Consumer Products (SCCP) opinion (SCCP/0917/05, 2006). https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_037.pdf

Scott L, Eskes C, Hoffmann S, Adriaens E, Alépée N, Bufo M, Clothier R, Facchini D, Faller C, Guest R, Harbell J, Hartung T, Kamp H, Varlet BL, Meloni M, McNamee P, Osborne R, Pape W, Pfannenbecker U, Prinsen M, Seaman C, Spielmann H, Stokes W, Trouba K, Berghe CV, Goethem FV, Vassallo M, 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. Toxicol In Vitro 24:1–9. doi:10.1016/j.tiv.2009.05.019

4 Evaluation of the Draize eye test uncertainty and reproducibility

21. The Draize eye test is the *in vivo* animal reference test used for benchmarking the predictive performance of serious eye damage/eye irritation DA.

22. This document reports an assessment of the Draize eye test reproducibility that was based on two published comprehensive analyses (Adriaens et al. 2014; Barroso et al. 2017) on the inherent variability of the Draize eye test. The variability of the animal data has to be considered in the evaluation of the uncertainties when comparing DAs' predictions to the benchmark animal predictions.

Within-test variability

23. The impact of the uncertainty of *in vivo* reference data on the evaluation/validation of alternative methods was illustrated by the resampling analysis (within-test variability using individual rabbit data) presented by Adriaens et al. (2014). In total, 2089 studies were used for this analysis.

24. The resampling probabilities were estimated based on the individual rabbit data. Only studies with individual data on at least three rabbits were taken into account. In the resampling approach used in this study, simulated chemicals were created by randomly grouping together three animals that may have been tested with different chemicals.

- First, the different studies were pooled according to UN GHS classification of the tested chemicals. In this way, it was assured that the rabbits used in the various resampling always came from studies with chemicals classified with the same UN GHS category (i.e. No Cat., Cat. 2, or Cat. 1).
- Next, separate resampling analyses were then performed on each of the three individual data pools (the pool of studies within each UN GHS category). Data on 10,000 simulated chemicals were generated, i.e. a random sample of three rabbits was drawn 10,000 times from the data pool without replacement. This means that each animal entered a simulated chemical only once.
- Finally, the UN GHS classification criteria were applied for these simulated chemicals and the predictive capacity (correct classification) was calculated by comparing the theoretical classification (resulting from the resampling approach) with the observed classification.

25. This analysis strongly suggests variability of the Draize eye test results. The resampling analyses based on the simulated chemicals demonstrated an overall probability of

- at least **8%** of solids classified as Cat. 1 by the Draize eye test could be equally identified as Cat. 2 and none of them were identified as No Cat.
- about **13%** of Cat. 2 solids could be equally identified as No Cat.
- the over-classification error for No Cat. and Cat. 2 solids was negligible **(<1 %)**

Between-test variability

26. Cosmetics Europe has compiled a database of Draize data (Draize eye test Reference Database, DRD) from external lists that were created to support past validation activities (Barroso et al. 2017). This database contains 681 independent *in vivo* studies on 634 individual chemicals representing a wide range of chemical classes.

27. For the purpose of this document, an evaluation of the Draize eye test between-test variability was considered. Such analysis was based on surfactants for which more than one independent study was performed by different laboratories. Four surfactants were consistently classified Cat. 1 in the repeat studies. One surfactant was classified once as Cat. 2 and two times as Cat. 1. In addition, one surfactant was consistently classified Cat. 2 in two repeat studies and one surfactant was consistently classified as No Cat. in two repeat studies However, the low number of repeat studies should be taken into account. Therefore reproducibility is also given for liquids, solids, and surfactants 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:

a) 35.3% (6/17) of the chemicals with at least one Cat. 1 study could be equally identified as Cat. 2, therefore the overall concordance of classifications was 64.7% (11/17) for Cat. 1.

b) 50% (6/12) of the chemicals with at least one Cat. 2 study could be equally identified as Cat. 1, and 16.7% (2/12) could be equally identified as No Cat., therefore the overall concordance of classifications was 33.3% (4/12) for Cat. 2.

c) 10.5% (2/19) 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 89.5% (17/19) for No Cat.

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) Retrospective analysis of the Draize test for serious eye damage/eye irritation: importance of understanding the *in vivo* endpoints under UN GHS/EU CLP for the development and evaluation of *in vitro* test methods. Arch Toxicol 88: 701–723.

Barroso J, Pfannenbecker U, Adriaens E, Alépée N, Cluzel M, De Smedt A, Hibatallah J, Klaric M, Mewes KR, Millet M, Templier M, McNamee P (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.

OECD (2022), GD No. 354: 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, OECD Publishing, Paris. Available at [https://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmenttestingforhumanhealth.htm]

5 Analyses of the DASF performance

28. Chapter 5 of this document includes information on the DASF that was supported by the OECD Expert Group to be considered in their programme and was presented at the OECD Expert Group on Eye/Skin Irritation/Corrosion & Phototoxicity (2022). The information in this chapter was organised according to the evaluation framework proposed for the Defined Approaches for liquids (DAL) for eye hazard identification (OECD, 2022). Further, chapter 3 provides details on the criteria applied for the selection of the reference chemicals used to assess the DASF performance, and as agreed during the OECD Expert Group on Eye/Skin Irritation/Corrosion and Phototoxicity meeting of 2019. This information was also shared with the OECD Expert Group in the form of an issue paper on the DASF (Annex C.1).

Performance metrics

29. The performance of the DASF was assessed against the performance metrics that were agreed by the OECD experts for the DA's for non-surfactant liquids (OECD SD 354, 2022 and paragraph 28). The values are reported in Table 5.1.

Table 5.1. Performance metrics for assessment of the predictivity of a DA of non-surfactant liquid test substances for eye hazard identification

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%

DASF

Development of the DIP

30. The DASF was developed based on the results of 32 surfactants (training set) that were available for all (both) components of the DASF.

31. In a next step, the performance of the DASF was assessed for the test set. No changes were made to the DIP after assessing the performance of the test set since no

further improvement to the DIP was possible based on the performance of the training and test set results. The identification of the substances that were used in the training set and the test set is available in Annex B.2 of the current background review document. The distribution of the surfactants by UN GHS category and chemical set is provided in Table 5.2

32. Table 5.2. Distribution of the reference chemicals: number of chemicals tested.

Table 5.2. Distribution of the reference chemicals: number of chemicals tested

UN GHS	Training set	Test set	Total
Cat. 1	15 (4 N, 11 D)	8 (2 N, 6 D)	23 (6 N, 17 D)
Cat. 2	6 (6 D)	4 (4 D)	10 (10 D)
No Cat.	11 (4 N, 7 D)	6 (2 N, 4 D)	17 (6 N, 11 D)
Total	32 (8 N, 23 D)	18 (4 N, 14 D)	**50** (12 N, 38 D)

N: surfactant tested neat; D: surfactant tested in dilution

Note that no neat Cat. 2 surfactant has been identified to date that could be included in the DASF development.

33. The full set of substances evaluated with the DASF (total 50 surfactants) is reported in Annex B.2.

Predictive capacity for the overall set

The predictive performance considering the three UN GHS categories (Cat. 1, Cat. 2, No Cat.) of DASF is reported in

34. Table 5.3. Note that the under-prediction rate is very low, only two UN GHS Cat. 1 surfactants were predicted Cat. 2, and no classified references surfactants were predicted No Cat. with the DASF. In general, the percentage of mispredictions was below the maximum values, except for the *in vivo* No Cat. that were predicted Cat. 1. However, the over-prediction rate of 5.9% (1 out of 17) was only slightly above the maximum threshold of 5%. One UN GHS No Cat. surfactant (Sodium lauryl sulphate, 3%) was predicted Cat. 1. This surfactant caused corneal opacity (CO=2) on day 1 in 3/6 animals, indicating that some irritation was also observed *in vivo*.

Table 5.3. Performance of the DASF based on EpiOcular™ EIT or SkinEthic™ HCE EIT and STE$^{0.5}$ for surfactants (N = 50 surfactants)

UN GHS	DAS		
	Cat 1	Cat 2	No Cat
Cat. 1 (N=23), % [a] (n/N)	*≥ 75%* [b] **91.3% (21.0/23.0)**	*≤ 25%* [b] 8.7% (2.0/23.0)	*≤ 5%* [b] 0.0% (0.0/23.0)
Cat. 2 (N=10), % [a] (n/N)	*≤ 30%* [b] 30.0% (3.0/10.0)	*≥ 50%* [b] **70.0% (7.0/10.0)**	*≤ 30%* [b] 0.0% (0.0/10.0)
No Cat. (N=17), % [a] (n/N)	*≤ 5%* [b] 5.9% [c] (1.0/17.0)	*≤ 30%* [b] 18.1% (3.1/17.0)	*≥ 70%* [b] **76.0% (12.9/17.0)**

79.1 % balanced accuracy

[a] 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 applying a correction factor so that all chemicals have a weight of 1. To improve the readability of the numbers in the table, the numbers n/N have been rounded, so they may deviate slightly from the percentage corresponding to the weighted calculation.

[b] Performance metrics for assessment of the predictivity of a DA for eye hazard identification

[c] One *in vivo* No Cat. surfactant, sodium lauryl sulphate 3%, was predicted Cat. 1. This surfactant belongs to the subgroup CO > 0 **: CO=2 on day 1 in 3/6 animals, CO mean =1.33 for days 1-3 in 1/6 animals (this is above the classification cut-off).

Limitations of individual sources of information

35. The strengths and limitations on individual test methods are described in the corresponding OECD Test Guidelines (OECD TG 491 and TG 492, 2025a and 2025b).

36. It is important to separate these limitations into:

- technical limitations
- limitations in the predictivity for UN GHS categories

Note that the technical limitations for the STE$^{0.5}$ for surfactants are the same as for the STE according to OECD TG 491. Regarding the predictivity limitations of the STE$^{0.5}$ for surfactants, no data was found to assess the performance of DASF for neat Cat. 2 surfactants. If a neat surfactant is predicted as Cat. 2, care should be taken when drawing conclusions on the prediction.

37. The technical limitations may make a chemical not testable in one or more component methods of DASF and may thus limit its applicability domain.

38. The predictivity limitations of some individual test methods for UN GHS categories do not necessarily limit the predictivity of an overarching DA; one of the advantages of DAs is that they are designed to overcome predictivity limitations of single test methods, i.e. the DAs can predict Cat. 2.

References

OECD (2022), GD No. 354: 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, OECD Publishing, Paris. Available at [https://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmenttestingforhumanhealth.htm]

OECD (2025a), *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, https://doi.org/10.1787/9789264242432-en.

OECD (2025b), *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, https://doi.org/10.1787/9789264242548-en.

6 Analyses of the DASF uncertainty and reproducibility

39. The objective of this analysis is to evaluate the uncertainty associated with the performance of the individual test methods (EpiOcular™ EIT, SkinEthic™ HCE EIT and STE$^{0.5}$ for surfactants) and the DASF. The aim was to assess the reproducibility of each information source, and how that propagates to the DASF overall.

40. Transferability, within- and between-laboratory reproducibility (BLR) of EpiOcular™ EIT and SkinEthic™ HCE EIT have been assessed and demonstrated during the respective validation studies (OECD TG 492, 2024). Multiple results are available for 21 surfactants for each RhCE test method (not necessarily the same surfactants, Table 6.1) and are therefore suitable for reproducibility analysis. Also, 43 surfactants were tested with both methods and resulted in identical predictions in 97.7% (42/43). Only one *in vivo* No Cat. surfactant (No. 34 in Table 6.1) was predicted twice No Cat. and once No Prediction Can be Made (NPCM) with EpiOcular™ EIT while this was predicted nine times No Cat. with SkinEthic™ HCE EIT.

41. Transferability, within- and between-laboratory reproducibility (BLR) of OECD TG 491 has been assessed during the respective validation studies (STE review document ICCVAM, 2013). During these validation studies, cell viability was also measured at 0.5% (results not published), so reproducibility at this concentration could be assessed. The BLR for 0.5% concentration, considering the 20% cell viability cut-off, was 97.2% based on 71 compounds, (16 surfactants, 15 solids, and 40 liquids). Specifically for surfactants, 100% concordance in predictions was achieved across laboratories for 20 surfactants (Alépée et al., 2023; Allen et al., 2024) (STE$^{0.5}$ for surfactants columns in Table 6.1).

42. The resulting performance values are shown in Table 6.2 and are based on predictions reported in Table 6.1. The reference benchmark is the UN GHS classification based on the Draize eye test (UN GHS column in Table 6.1).

Table 6.1. Prediction for the individual test methods (proportion of correct predictions, TRUE pred. %). DASF predictions are derived by applying the data interpretation procedure (DIP) to predictions from a single method. [TRUE pred., proportion of predicted results that corresponds with the classification based on the Draize eye test: RhCE test methods = No Cat. versus Cat. 1 + Cat. 2 and STE$^{0.5}$ for surfactants = Cat. 1 versus Cat. 2 + No Cat.].

				SINGLE METHODS								
	Chemicals	**CAS#**	**UN GHS**	**EpiOcular™ EIT**			**SkinEthic™ HCE EIT**			**STE$^{0.5}$ for surfactants**		
				Cat. 1 + Cat. 2	**No Cat.**	**TRUE pred %**	**Cat. 1 + Cat. 2**	**No Cat.**	**TRUE pred %**	**Cat. 1**	**Cat. 2 + No Cat.**	**TRUE pred %**
1	Ethylhexyl acid phosphate ester	12645-31-7	Cat 1	2	0	100	2	0	100	2	0	100
2	Benzalkonium chloride (10%)	63449-41-2	Cat 1	3	0	100						
3	Benzethonium chloride (10%)	121-54-0	Cat 1				9	0	100			
4	Domiphen bromide (10%)	538-71-6	Cat 1	3	0	100	11	0	100			
5	Distearyldimethylammonium chloride	107-64-2	Cat 1	2	0	100	2	0	100	0	2	0
6	Ethyl lauroyl arginate HCl	60372-77-2	Cat 1	3	0	100				2	0	100
7	Cetyl pyridinium bromide (10%)	140-72-7	Cat 1				3	0	100	2	0	100
8	Triton X-100	9002-93-1	Cat 1							4	0	100
9	Benzalkonium chloride (5%)	63449-41-2	Cat 1	2	0	100						
10	Coco amidopropyl betaine (25%)	61789-40-0	Cat 1	9	0	100						
11	Cetyltrimethyl ammonium bromide (10%)	57-09-0	Cat 1	3	0	100	11	0	100			
12	Benzalkonium chloride (1%)	63449-41-2	Cat 1				9	0	100			
13	Di(2-ethylhexyl)sodium sulphosuccinate (10%)	577-11-7	Cat 1				9	0	100	2	0	100
14	Sodium lauryl sulphate (15%)	151-21-3	Cat 1	2	0	100						
15	Stearyltrimethylammonium chloride (10%)	112-03-8	Cat 1				3	0	100			
16	N-Lauroyl sarcosine Na salt (30%)	137-16-6	Cat 1							3	0	100
17	1-Hexadecanaminium, N,N,N-trimethyl-, chloride (25%)	112-02-7	Cat 1	2	0	100	2	0	100			
19	Triton X-100 (10%)	9002-93-1	Cat 1				9	0	100	2	0	100
20	Cetyl pyridinium bromide (6%)	140-72-7	Cat 1	2	0	100	3	0	100			
21	Cetyl pyridinium bromide (1%)	140-72-7	Cat 2				3	0	100	0	3	100
22	Deoxycholic acid Na salt (10%)	302-95-4	Cat 2							2	0	0
23	Triton X-100 (5%)	9002-93-1	Cat 2	4	0	100						
24	Methyl N,N,N-trimethyl-4-[(4,7,7-trimethyl-3-oxobicyclo[2.2.1]hept-2-ylidene)methyl]anilinium sulphate (30%)	52793-97-2	Cat 2	9	0	100						

25	Lauryl sulphobetaine (10%)		Cat 2							0	2	100
26	Benzethonium chloride (1%)		Cat 2							0	2	100
27	1-Hexadecanaminium, N,N,N-trimethyl-, chloride (2%)		Cat 2							0	2	100
28	Sodium lauryl sulphate (3%)	151-21-3	No Cat	2	0	0						
29	N-Lauroyl sarcosine Na salt (3%)	137-16-6	No Cat							0	2	100
30	Cetyl pyridinium bromide (0.1%)	140-72-7	No Cat				0	3	100	0	2	100
31	Polyglyceryl-3-diisooctadecanoate	63705-03-3	No Cat	0	9	100						
32	Steareth-10 allyl ether/acrylates copolymer (30%)	109292-17-3	No Cat	9	0	0						
33	Triton X-100 (1%)	9002-93-1	No Cat				3	0	0	0	2	100
34	Tween 20	9005-64-5	No Cat	0	5	100	0	9	100	0	4	100
35	Tween 80	9005-65-6	No Cat	0	4	100	0	2	100	0	3	100
36	Polyoxyethylene 23 lauryl ether (Brij-35) (10%)	9002-92-0	No Cat				0	3	100	0	2	100
37	Polyethylene glycol (PEG-40) hydrogenated castor oil	61788-85-0	No Cat	0	3	100	0	9	100	0	4	100
38	Cellulose,2-(2-hydroxy-3-(trimethylammonium)propoxy)ethyl ether chloride	68610-92-4	No Cat	1	2	66.7	0	9	100			

Table 6.2. Performance values (surfactants only) – the reproducibility is the overall reproducibility

	Reproducibility	Accuracy	Specificity	Sensitivity
No Cat. / Cat. 1 + Cat. 2				
EpiOcular™ EIT [a]	95.2% (N=21)	94.0% (N=21)	83.3% (N=8)	100% (N=13)
SkinEthic™ HCE EITS [a]	100% (N=21)	95.2% (N=21)	87.5% (N=8)	100% (N=13)

Cat. 1 / Cat. 2 + No Cat.				
STE$^{0.5}$ for surfactants [c]	100% (N=20)	90.0% (N=20)	91.7% (N=12)	87.5% (N=8)

[a] Kaluzhny et al., 2011; Kole et al., 2011; EC EURL ECVAM, 2014; Kandarova et al., 2018;

[b] Alépée et al., 2016 and 2023

[c] Kao Corporation background review document (Takahashi et al., 2009; Sakaguchi et al., 2011; Kojima et al., 2013), Abo et al., 2018, and Allen et al., 2024

References

Abo T, Yuki T, Xu R, Araki D, Takahashi Y, Sakaguchi H, Itagaki H (2018). Expansion of the applicability domain for highly volatile substances on the Short Time Exposure test method and the predictive performance in assessing eye irritation potential. J Toxicol Sci. 43, 407-422. doi: 10.2131/jts.43.407. PMID: 29973473.

Alépée N, Leblanc V, Adriaens E, Grandidier MH, Lelièvre D, Meloni M, Nardelli L, Roper CS, Santirocco E, Toner F, Van Rompay AR, Vinall J, Cotovio J (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. http://dx.doi.org/10.1016/j.tiv.2015.11.012

Alépée N, Adriaens E, Abo T, Magby J, Mewes KR, Giusti A. (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

Allen D, Abo T, Adriaens E, Alépée N, Cavarzan A, Cuciureanu A, Mewes K, O'Driscoll C (2024). Between-laboratory reproducibility of the modified STE to identify UN GHS Category 1 surfactants. Toxicol. Letters 399S2 S72-S364 https://doi.org/10.1016/j.toxlet.2024.07.264

EC EURL ECVAM. (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 Study Report. EUR 28125 EN; doi:10.2787/41680. Available at: [http://publications.jrc.ec.europa.eu/repository/handle/JRC100280].

Kaluzhny Y, Kandárová H, Hayden P, Kubilus J, d'Argembeau-Thornton L, Klausner M. (2011). Development of the EpiOcular(TM) eye irritation test for hazard identification and labelling of eye irritating chemicals in response to the requirements of the EU cosmetics directive and REACH legislation. Altern Lab Anim. 2011, 339-364. doi:10.1177/026119291103900409

Kandarova H, Letasiova S, Adriaens E, Guest R, Willoughby Sr JA, Drzewiecka A, Gruszka K, Alépée N, Verstraelen S, Van Rompay AR (2018). CON4EI: EpiOcularTM eye irritation test (EpiOcularTM EIT) for hazard identification and labelling of eye irritating chemicals. Toxicol. itro 49, 21–33. https://doi.org/10.1016/j.tiv.2017.07.002.

Kojima H, Hayashi K, Sakaguchi H, Omori T, Otoizumi T, Sozu T, Kuwahara H, Hayashi T, Sakaguchi M, Toyoda A, Goto H, Watanabe S, Ahiko K, Nakamura T, Morimoto T (2013). Second-phase validation study of short time exposure test for assessment of eye irritation potency of chemicals. Toxicol In Vitro. 2013 27, 1855-69. doi: 10.1016/j.tiv.2013.05.013.

Kolle SN, Kandárová H, Wareing B, van Ravenzwaay B, Landsiedel R (2011). In-house validation of the EpiOcular(TM) eye irritation test and its combination with the bovine corneal opacity and permeability test for the assessment of ocular irritation. Altern Lab Anim. 39, 365-387. doi:10.1177/026119291103900410

Sakaguchi H, Ota N, Omori T, Kuwahara H, Sozu T, Takagi Y, Takahashi Y, Tanigawa K, Nakanishi M, Nakamura T, Morimoto T, Wakuri S, Okamoto Y, Sakaguchi M, Hayashi T, Hanji T, Watanabe S. (2011). Validation study of the Short Time Exposure (STE) test to assess the eye irritation potential of chemicals. Toxicol In Vitro. 25, 796-809. doi: 10.1016/j.tiv.2011.01.015. Epub 2011 Feb 1. PMID: 21291995.

Takahashi Y, Hayashi T, Watanabe S, Hayashi K, Koike M, Aisawa N, Ebata S, Sakaguchi H, Nakamura T, Kuwahara H, Nishiyama N. (2009). Inter-laboratory study of short time exposure (STE) test for predicting eye irritation potential of chemicals and correspondence to globally harmonized system (GHS) classification. J Toxicol Sci. 2009 34, 611-26. doi: 10.2131/jts.34.611. PMID: 19952497.

7 Detailed performance analysis of individual methods and the DASF against Draize eye test

43. This chapter analyses the performance of the individual methods (EpiOcular™ EIT, SkinEthic™ HCE EIT, and STE$^{0.5}$ for surfactants) and that of DASF, against the curated Draize Eye test reference data. The following methods and DAs were analysed:

- EpiOcular™ EIT
- SkinEthic™ HCE EITS
- STE$^{0.5}$ for surfactants
- DASF

44. The performance of these methods with respect to the whole dataset, by driver of classification, by surfactant class, and for neat and diluted surfactants, is presented in the next chapters, with a specific focus on mispredictions (predictions that are not in accordance with the classification based on the Draize eye test, which is considered as the reference classification). The analyses are meant to provide considerations and support recommendations regarding the use of the DASF based on the performance observed in this dataset. More details regarding the drivers of classification are provided in section 3.1.1. (Drivers of classification) and section 7.3 (Analysis of the performance for surfactant classes with DASF).

All chemicals

45. The full set of substances evaluated with DASF (total 50 different surfactants, representing 33 unique CASRNs) is reported in Annex B (spreadsheet Annex_B.2).

46. The prevalence of *in vivo* classified surfactants (i.e., UN GHS Cat. 1 and Cat. 2) is 66.0% (33/50).

47. The performance of the EpiOcular™ EIT for identifying chemicals not requiring classification for eye irritation or serious eye damage (UN GHS No Cat.) is shown in

48. Table 7.1. **Predictive Capacity of the EpiOcular™ EIT test method for identifying chemicals not requiring classification for eye irritation or serious eye**

damage [UN GHS No Cat. versus Not No Cat. (Cat. 1 + Cat. 2)]. The method has an accuracy of 91.0% (N=48), with 72.9% specificity and 100% sensitivity.

Table 7.1. Predictive Capacity of the EpiOcular™ EIT test method for identifying chemicals not requiring classification for eye irritation or serious eye damage [UN GHS No Cat. versus Not No Cat. (Cat. 1 + Cat. 2)]

	Accuracy (Balanced accuracy)		UN GHS Cat. 1 + Cat. 2			UN GHS No Cat.		
	N	% [a]	N	Sensitivity (%)	FN (%)	N	Specificity (%)	FP (%)
EpiOcular™ HCE EIT	48	91.0 (86.5)	32	100	0	16	72.9	27.1

[a] The proportion in the tables are based on weighted calculation. For each chemical, all results were taken into account and a correction factor was applied so that all chemicals had the same weight (weight of 1).

The performance of the SkinEthic™ HCE EIT for identifying chemicals not requiring classification for eye irritation or serious eye damage (UN GHS No Cat.) is shown in Table 7.2. The method has an accuracy of 91.1% (N=45), with 75.0% specificity and 100% sensitivity.

Table 7.2. Predictive Capacity of the SkinEthic™ HCE EITS test method for identifying chemicals not requiring classification for eye irritation or serious eye damage [UN GHS No Cat. versus Not No Cat. (Cat. 1 + Cat. 2)]

	Accuracy (Balanced accuracy)		UN GHS Cat. 1 + Cat. 2			UN GHS No Cat.		
	N	% [a]	N	Sensitivity (%)	FN (%)	N	Specificity (%)	FP (%)
SkinEthic™ HCE EITS	45	91.1 (87.5)	29	100	0	16	75.0	25.0

[a] The proportion in the tables are based on weighted calculation. For each chemical, all results were taken into account and a correction factor was applied so that all chemicals had the same weight (weight of 1).

49. The performance of the $STE^{0.5}$ for surfactants for identifying chemicals inducing serious eye damage (UN GHS Cat. 1) is shown in Table 7.3. The method has an accuracy of 87.5% (N=48), with 84.0% specificity and 91.3% sensitivity.

Table 7.3. Predictive Capacity of $STE^{0.5}$ for surfactants for identifying chemicals inducing serious eye damage [UN GHS Cat. 1 versus Not Cat. 1 (Cat. 2 + No Cat.)]

	Accuracy (Balanced accuracy)		UN GHS Cat. 1			UN GHS Cat. 2 + No Cat.		
	N	% [a]	N	Sensitivity (%)	FN (%)	N	Specificity (%)	FP (%)
$STE^{0.5}$ for surfactants	48	87.5 (87.7)	23	91.3	8.7	25	84.0	16.0

[a] The proportion in the tables are based on weighted calculation. For each chemical, all results were taken into account and a correction factor was applied so that all chemicals had the same weight (weight of 1).

50. An overview of the surfactants which are mispredicted by the DASF are listed in Table 7.4. *In vivo* Cat. 1 surfactants which are under-predicted compared to the reference classification are the result of an under-prediction by the $STE^{0.5}$ for surfactants. *In vivo* Cat. 2 surfactants which are over-predicted are the result of an over-prediction by the $STE^{0.5}$ for surfactants. False positive *in vivo* No Cat. surfactants are the result of a misprediction by the RhCE test methods (No Prediction Can be Made (NPCM) with EpiOcular™ EIT and SkinEthic™ HCE EIT). One *in vivo* No Cat. surfactant (Sodium lauryl sulphate 3%, CASRN 151-21-3) was predicted Cat. 1 with the DASF. This surfactant resulted in NPCM with the RhCE test methods and was predicted Cat. 1 with the $STE^{0.5}$ for surfactants. Note that none of the *in vivo* Cat.1 reference surfactants was under-predicted as No Cat. with the DASF. There was no FN (False Negative: *in vivo* Cat. 1 or Cat. 2 predicted as No Cat.) identified.

Table 7.4. Mis-predicted surfactants in comparison with UN GHS categories

DRD No.	Chemical	CASRN	Class	UN GHS	Driver	DASF prediction
28	Distearyldimethylammonium chloride (neat)	107-64-2	Cationic	Cat. 1	CO mean ≥ 3	UP (Cat. 2)
66, 67	Benzalkonium chloride (1%)	63449-41-2	Cationic	Cat. 1	CO pers D21	UP (Cat. 2)
186	Deoxycholic acid Na salt (10%)	302-95-4	Anionic	Cat. 2	CO mean ≥ 1	OP (Cat. 1)
209	N-Lauroyl sarcosine Na salt (10%)	137-16-6	Anionic	Cat. 2	CO mean ≥ 1	OP (Cat. 1)
	Isotridecanol, ethoxylated (9 EO) (10%)	69011-36-5	Nonionic	Cat. 2	CO mean ≥ 1	OP (Cat. 1)
265	N-Lauroyl sarcosine Na salt (3%)	137-16-6	Anionic	No Cat.	CO > 0 **	FP (Cat. 2)
266	Sodium lauryl sulphate (3%)	151-21-3	Anionic	No Cat.	CO > 0 **	FP (Cat. 1)
296, 297	Sodium lauryl sulphate (1%)	151-21-3	Anionic	No Cat.	CO > 0	FP (Cat. 2)
475	Triton X-100 (1%)	9002-93-1	Nonionic	No Cat.	CO = 0	FP (Cat. 2)

UP: Under-Predicted surfactants compared to the reference classification; OP: Over-Predicted surfactants compared to the reference classification; FP: False Positives compared to the reference classification; the DASF prediction is provided in parentheses.

CO > 0: in at least one observation time in at least one animal and all animals showing mean scores of days 1–3 below the classification cut-offs for all endpoints, ** Indicates at least one animal with a mean score of days 1–3 above the classification cut-off for at least one endpoint.

N-Lauroyl sarcosine Na salt 3% (subgroup CO > 0 **): CO=1 on day 2 in 3/3 animals, CO mean =1 for days 1-3 in 1/3 animals (this is above the classification cut-off).

Sodium lauryl sulphate 3% (subgroup CO > 0 **): CO=2 on day 1 in 3/6 animals, CO mean =1.33 for days 1-3 in 1/6 animals (this is above the classification cut-off).

Sodium lauryl sulphate 1% (subgroup CO > 0): CO=1 after 4 hours in 1/3 animals in both Draize eye studies.

Analysis of the performance by driver of classification

51. This section focuses on the performance of the individual test methods and DASF by driver of classification. Details on the driver of classification are presented in Chapter 3. The results of the individual test methods are presented in Table 7.5 to The $STE^{0.5}$ for surfactants is used to identify chemicals requiring classification for serious eye damage with DASF. The concordance with the reference classification by driver of classification is shown in Table 7.7. The discordance for the $STE^{0.5}$ for surfactants was low with 14.3% for the driver CO mean ≥ 3 and 8.3% for the driver CO pers D21. For all other Cat. 1 drivers 100% concordance was observed. The discordance with the reference classification for Cat. 2 was 42.9% for CO mean ≥ 1 and 0% for Conj mean ≥ 2. One of the two UN GHS No Cat. surfactants from the subgroup CO > 0 ** was predicted Cat. 1.

52. Table 7.7 summarising the concordance as compared to the Draize eye test benchmark data. The results of the DASF are presented in

53. Table 7.8 summarising TP (true Cat. 1 and true Cat. 2), TN (true No Cat.), over-predictions (OP, *in vivo* Cat. 2 predicted as Cat. 1), under-predictions (UP, *in vivo* Cat. 1 predicted as Cat. 2), FN and accuracy as compared to the Draize eye test benchmark data.

54. The EpiOcular™ HCE EIT test method can be used to identify chemicals that do not require classification for eye irritation or serious eye damage with DASF. The concordance with the reference classification per No Cat. subgroup is shown in Table 7.5. No Cat. solids from the subgroup CO > 0 and CO > 0 ** resulted in 1/2 and 2/2 discordant predictions, respectively. A better concordance with the reference classification was observed for the subgroup CO = 0. Discordant predictions were not observed for the UN GHS Cat. 1 and Cat. 2 surfactants.

Table 7.5. Predictive Capacity of EpiOcular™ HCE EIT for identifying chemicals not requiring classification for eye irritation or serious eye damage [UN GHS No Cat. versus Not No Cat. (Cat. 1 + Cat. 2)]

UN GHS	EpiOcular™ EIT	
Driver of classification	N	Concordant prediction [a]
Cat. 1	**23**	**100**
CO mean ≥ 3	7	100
CO pers D21	12	100
CO = 4	2	100
IR mean > 1.5	1	100
Conj pers D21	1	100
Cat. 2	**9**	**100**
CO mean ≥ 1	7	100
Conj mean ≥ 2	2	100
No Cat.	**16**	**72.9**
CO > 0 **	2	0.0
CO > 0	2	50.0
CO = 0	12	88.9
Accuracy	**48**	90.6

[a] The proportion in the tables are based on weighted calculation. For each chemical, all results were taken into account and a correction factor was applied so that all chemicals had the same weight (weight of 1).

** Indicates at least one animal with a mean score of days 1–3 above the classification cut-off for at least one endpoint.

55. The SkinEthic™ HCE EIT test method can be used to identify chemicals that do not require classification for eye irritation or serious eye damage with DASF. The concordance with the reference classification per No Cat. subgroup is shown in Table 7.6. No Cat. solids from the subgroup CO > 0 and CO > 0 ** resulted in 1/2 and 2/2 discordant predictions. A lower FP rate (8.3%) was observed for the subgroup CO = 0. Discordant predictions were not observed for the UN GHS Cat. 1 and Cat. 2 surfactants.

Table 7.6. Predictive Capacity of SkinEthic™ HCE EIT for identifying chemicals not requiring classification for eye irritation or serious eye damage [UN GHS No Cat. versus Not No Cat. (Cat. 1 + Cat. 2)]

UN GHS	SkinEthic™ HCE EIT	
Driver of classification	N	Concordant prediction [a]
Cat. 1	**20**	**100**
CO mean ≥ 3	6	100
CO pers D21	10	100
CO = 4	2	100
IR mean > 1.5	1	100
Conj pers D21	1	100
Cat. 2	**9**	**100**
CO mean ≥ 1	6	100
Conj mean ≥ 2	3	100
No Cat.	**16**	**75.0**
CO > 0 **	2	0.0
CO > 0	2	50.0
CO = 0	12	91.7
Accuracy	**45**	91.1

[a] The proportion in the tables are based on weighted calculation. For each chemical, all results were taken into account and a correction factor was applied so that all chemicals had the same weight (weight of 1).

** Indicates at least one animal with a mean score of days 1–3 above the classification cut-off for at least one endpoint.

56. The STE$^{0.5}$ for surfactants is used to identify chemicals requiring classification for serious eye damage with DASF. The concordance with the reference classification by driver of classification is shown in Table 7.7. The discordance for the STE$^{0.5}$ for surfactants was low with 14.3% for the driver CO mean ≥ 3 and 8.3% for the driver CO pers D21. For all other Cat. 1 drivers 100% concordance was observed. The discordance with the reference classification for Cat. 2 was 42.9% for CO mean ≥ 1 and 0% for Conj mean ≥ 2. One of the two UN GHS No Cat. surfactants from the subgroup CO > 0 ** was predicted Cat. 1.

Table 7.7. Predictive Capacity of STE$^{0.5}$ for surfactants for identifying chemicals inducing serious eye damage [UN GHS Cat. 1 versus Not Cat. 1 (Cat. 2 + No Cat)]

UN GHS	STE$^{0.5}$ for surfactants

Driver of classification	N	Concordant prediction [a]
Cat. 1	**23**	91.3
CO mean ≥ 3	7	85.7
CO pers D21	12	91.7
CO = 4	2	100
IR mean > 1.5	1	100
Conj pers D21	1	100
Cat. 2	**10**	70.0
CO mean ≥ 1	7	57.1
Conj mean ≥ 2	3	100
No Cat.	**15**	93.3
CO > 0 **	2	50.0
CO > 0	2	100
CO = 0	11	100
Accuracy	**46**	90.6

[a] The proportion in the tables are based on weighted calculation. For each chemical, all results were taken into account and a correction factor was applied so that all chemicals had the same weight (weight of 1).

** Indicates at least one animal with a mean score of days 1–3 above the classification cut-off for at least one endpoint.

57. The performance by driver of classification (Cat. 1 and Cat. 2) or by subgroup (No Cat.) with the DASF is shown in

58. Table 7.8. The UP rate for the Cat. 1 driver of classification CO mean ≥ 3 and CO pers D21 was low, 14.3% and 8.3%, respectively. Cat. 2 surfactants that were classified based on CO mean ≥ 1 resulted in a higher over-prediction rate in comparison with those that were classified based on Conj mean ≥ 2. The FP rate surfactants from the subgroup CO = 0 was low in comparison to the FP rates that were observed for the subgroups CO > 0 ** and CO > 0.

Table 7.8. Predictive performance considering the three UN GHS categories (Cat. 1, Cat. 2, No Cat.) of DASF

	DASF prediction	CO mean ≥ 3	CO pers D21	CO=4	other	**All**
N		**7 (3N, 4D)**	**12 (2 N, 10D)**	**2 (1N, 1D)**	**2 (2D)**	**31**
Reference classification UN GHS Cat. 1	Cat. 1 (%) [a]	85.7	91.7	100	100	**91.3**
	Cat. 2 (%) [a]	14.3 (1N)	8.3 (1D)	0.0	0.0	**8.7**
	No Cat. (%) [a]	0.0	0.0	0.0	0.0	**0.0**

	DASF prediction	CO mean ≥ 1	Conj mean ≥ 2	**All**
N		**7 (7D)**	**3 (3D)**	**10**
Reference classification UN GHS Cat. 2	Cat. 1 (%) [a]	43.0	0.0	**30.0**
	Cat. 2 (%) [a]	57.1	100	**70.0**
	No Cat. (%) [a]	0.0	0.0	**0.0**

	DASF prediction	CO > 0 **	CO > 0	CO = 0	**All**

N		**2 (2D)**	**2 (2D)**	**13 (6N, 7D)**	**17**
Reference classification UN GHS No Cat.	Cat. 1 (%) [a]	50.0	0	0.0	**5.9**
	Cat. 2 (%) [a]	50.0	50.0	8.3 (1D)	**18.1**
	No Cat. (%) [a]	0.0	50.0	91.7	**76.0**

N: surfactant tested neat; D: surfactant tested in dilution

[a] The proportion in the tables are based on weighted calculation. For each chemical, all results were taken into account and a correction factor was applied so that all chemicals had the same weight (weight of 1). To improve the readability of the numbers in the table, the numbers n/N have been rounded, so they may deviate slightly from the percentage corresponding to the weighted calculation.

** Indicates at least one animal with a mean score of days 1–3 above the classification cut-off for at least one endpoint.

Analysis of the performance for surfactant classes with DASF

59. This section focuses on the performance of the DASF by surfactant class. The results of the DASF are presented in tables summarising concordant and discordant predictions as compared to the Draize eye test benchmark data.

60. The distribution according to the UN GHS category is shown in Table 7.9.

Table 7.9. Number of surfactants per class according to the UN GHS category of the reference classification

Class	**% of total N (=50)**	**n**	**UN GHS (n)**		
			Cat. 1	Cat. 2	No Cat.
Cationic	44.0	22	14	5	3
Anionic	20.0	10	4	2	4
Nonionic	32.0	16	4	2	10
Amphoteric	4.0	2	1	1	-

61. The performance of the DASF by surfactant class is shown in Table 7.10. A concordance of 90.5% (N=22) with the reference classification was observed for cationic surfactants. Only two UN GHS Cat. 1 cationic surfactants (Distearyldimethylammonium chloride CASRN 107-64-2 and Benzalkonium chloride 1% CASRN 6 63449-41-2) were predicted Cat. 2 with DASF. For nonionic surfactants, the concordance with the reference classification was of 87.5% (N=16), discordant predictions correspond with over-predictions. A concordance of 50% (N=10) was observed for anionic surfactants and discordant predictions correspond with over-predictions. Note that the three UN GHS No Cat. anionic surfactants that were over-predicted with the DASF, belong to the CO > 0 or CO > 0 ** group, indicating that corneal opacity was observed in the rabbit eye (see footnote Table 7.4). The UN GHS No Cat. anionic surfactant that was concordant with the reference classification belongs to the CO = 0 group, so no corneal opacity was observed in the rabbit eye throughout the duration of the study. The set contained only two amphoteric surfactants, both resulted in a concordant prediction with the reference classification.

Table 7.10. Predictive performance considering the three UN GHS categories (Cat. 1, Cat. 2, No Cat.) of DASF with EpiOcular™ EIT or SkinEthic™ HCE EIT and STE[0.5] for surfactants

		Surfactant class			
UN GHS based on Draize eye test	DASF Predicted class	Cationic N = 22	Nonionic N = 16	Anionic N = 10	Amphoteric N = 2
Cat. 1	**Cat. 1**	12.0/14	4.0/4	4.0/4	1.0/1
	Cat. 2	2.0/14	0.0/4	0.0/4	0.0/1
	No Cat.	0.0/14	0.0/4	0.0/4	0.0/1
Cat. 2	Cat. 1	0.0/5	1.0/2	2.0/2	0.0/1
	Cat. 2	5.0/5	1.0/2	0.0/2	1.0/1
	No Cat.	0.0/5	0.0/2	0.0/2	0.0/1
No Cat.	Cat. 1	0.0/3	0.0/10	1.0/4	NA
	Cat. 2	0.08/3	1.0/10	2.0/4	NA
	No Cat.	2.92/3	9.0/10	1.0/4	NA

Analysis of the performance for neat and diluted surfactants with DASF

62. The performance of the DASF by surfactant dilution (neat or diluted) is shown in Table 7.11. The set of reference substances contained 12 neat surfactants, a concordance of 91.7% with the reference classification was observed. The concordance of the 38 reference surfactants that were tested in dilution was 78.9%.

63. Note that no neat UN GHS Cat. 2 reference surfactant could be identified, which prevents establishing confidence in the DASF for this category. As a result, when the DASF predicts a Cat. 2 for a neat surfactant, additional information may be required to confirm the result, taking into account that the prospective use of animals is only to be used as a last resort.

Table 7.11. Predictive performance considering the three UN GHS categories (Cat. 1, Cat. 2, No Cat.) of DASF with EpiOcular™ EIT or SkinEthic™ HCE EIT and STE[0.5] for surfactants

UN GHS based on Draize eye test	DASF Predicted class	Neat surfactant N = 12	Diluted surfactant N=38
Cat. 1	**Cat. 1**	5.0/6	16.0/17
	Cat. 2	1.0/6	1.0/17
	No Cat.	0.0/6	0.0/17
Cat. 2	Cat. 1	NA	3.0/10
	Cat. 2	NA	7.0/10
	No Cat.	NA	0.0/10
No Cat.	Cat. 1	0.0/6	1.0/11
	Cat. 2	0.0/6	3.0/11
	No Cat.	6.0/6	7.0/11

Annex A.

Performance of the testing strategy with the in vitro test methods: EpiOcular™ EIT or SkinEthic™ HCE EIT and STE

64. The performance of the testing strategy that combines EpiOcular™ EIT or SkinEthic™ HCE EIT (OECD TG 492, 2024) and STE (OECD TG 491, 2023) for the same set of reference surfactants is shown in Table A A.1. In the STE, the cell viability in corneal epithelial cells is measured after a 5-minute exposure to both a 5% and a 0.05% concentration, using a cut-off value of 70%. Substances are classified Cat. 1 if the relative cell viability ≤ 70% and do not require classification if the relative cell viability is > 70% at both test concentrations. The main difference is in terms of concordant Cat. 1 predictions for the same set of surfactants, i.e. 56.5% (n=23, Table A A.1) and 91.3% (n=23, Table 5.3), were correctly identified with the STE and $STE^{0.5}$ for surfactants, respectively. Therefore, the performance criteria for at least 75% correct Cat. 1 identification were not met for the variation of the DIP and therefore this does not fall under this GL.

Table A A.1. Performance of the DA based on EpiOcular™ EIT or SkinEthic™ HCE EIT and STE (N = 50 surfactants)

UN GHS	DAS		
	Cat 1	Cat 2	No Cat
Cat. 1 (N=23), % [a] (n/N)	*≥ 75%* [b] **56.5% (13.0/23.0)**	*≤ 25%* [b] 43.5% (10.0/23.0)	*≤ 5%* [b] 0.0% (0.0/23.0)
Cat. 2 (N=10), % [a] (n/N)	*≤ 30%* [b] 10.0% (1/10)	*≥ 50%* [b] **90.0% (9/10)**	*≤ 30%* [b] 0.0% (0/10)
No Cat. (N=17), % [a] (n/N)	*≤ 5%* [b] 0.0% (0/17.0)	*≤ 30%* [b] 24.0% (3.1/17.0)	*≥ 70%* [b] **76.0% (12.9/17.0)**

74.2 % balanced accuracy

[a] 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 applying a correction factor so that all chemicals have a weight of 1. To improve the readability of the numbers in the table, the numbers n/N have been rounded, so they may deviate slightly from the percentage corresponding to the weighted calculation.
[b] Performance metrics for assessment of the predictivity of a DA for eye hazard identification

Annex B. Spreadsheets

Table A B.1. List of unique surfactants that were used for DASF.

Name	CAS RN	Physical form test items	Type	Class	Family	Concentrations tested and their corresponding UN GHS classification based on the Draize eye test		
						Cat. 1	Cat. 2	No Cat.
Cetyltrimethyl ammonium bromide	57-09-0	Liquid	mono-constituent	Cationic	Quaternary ammonium compound	10%		
Stearyltrimethylammonium chloride	112-03-8	Liquid	mono-constituent	Cationic	Quaternary ammonium compound	10%		
Didecyl dimethyl ammonium chloride	7173-51-5	Liquid	mono-constituent	Cationic	Quaternary ammonium compound	1%		
Benzalkonium chloride	63449-41-2	Liquid	UVCB	Cationic	Quaternary ammonium compound	10%, 5%, 1%		
Domiphen bromide	538-71-6	Liquid	mono-constituent	Cationic	Quaternary ammonium compound	10%		
Di(2-ethylhexyl)sodium sulphosuccinate	577-11-7	Liquid	mono-constituent	Anionic	Sulphosuccinates	10%		
Coco amidopropyl betaine	61789-40-0	Liquid	UVCB	Amphoteric	Betaines	25%		
Cetylpyridinium chloride	6004-24-6	Liquid	mono-constituent	Cationic	Alkyl pyridinium	10%		0.10%
Sodium lauryl sulphate	151-21-3	Liquid	mono-constituent	Anionic	Alkyl sulfates	15%		3%, 1%
Ethylhexyl acid phosphate ester	12645-31-7	Liquid	multi-constituent	Anionic	Fatty alcohol phosphoric acid esters	Neat		
Distearyldimethylammonium chloride	107-64-2	Solid	mono-constituent	Cationic	Quaternary ammonium compound	Neat		
Ethyl lauroyl arginate HCl	60372-77-2	Solid	mono-constituent	Cationic	Ammonium salt	Neat		
Surfonic HDL-1 (Chemical name: Nonyl phenol ethoxylate, branched)	9016-45-9	Liquid	UVCB	Nonionic	Nonylphenols, ethoxylated with triethanolamine	Neat		
Isotridecanol, ethoxylated (9 EO)	69011-36-5	Liquid	UVCB	Nonionic	Alcohol ethoxylate	Neat	10%	
Triton X-100	9002-93-1	Liquid	UVCB	Nonionic	Octylphenol, ethoxylated	Neat, 10%	5%	1%
1-Hexadecanaminium, N,N,N-trimethyl-, chloride	112-02-7	Liquid	mono-constituent	Cationic	Quaternary ammonium compound	25%	2%	
Cetyl pyridinium bromide	140-72-7	Liquid	mono-constituent	Cationic	Alkyl pyridinium	10%, 6%	1%	0.10%
Benzethonium chloride	121-54-0	Liquid	mono-constituent	Cationic	Quaternary ammonium compound	10%	1%	
N-Lauroyl sarcosine Na salt	137-16-6	Liquid	mono-constituent	Anionic	Amino acid surfactant	30%	10%	3%
Deoxycholic acid Na salt	302-95-4	Liquid	mono-constituent	Anionic	Steroid		10%	
Lauryl sulphobetaine	14933-08-5	Liquid	mono-constituent	Amphoteric	Sulphobetaine		10%	
Methyl N,N,N-trimethyl-4-[(4,7,7-trimethyl-3-oxobicyclo[2.2.1]hept-2-ylidene)methyl]anilinium sulphate	52793-97-2	Liquid	mono-constituent	Cationic	Quaternary ammonium compound		30%	
Behentrimonium chloride	17301-53-0	Liquid	mono-constituent	Cationic	Quaternary ammonium compound		5%	
Polyethylene glycol monolaurate (10EO)	9004-81-3	Liquid	UVCB	Nonionic				10%
Steareth-10 allyl ether/acrylates copolymer	109292-17-3	Liquid	multi-constituent	Anionic				40%
Polyoxyethylene 23 lauryl ether (Brij-35)	9002-92-0	Liquid	UVCB	Nonionic	Alcohol ethoxylate			10%
Polyoxyethylene 8-stearate (Myrj-45)	9004-99-3	Liquid	UVCB	Nonionic	Alcohol ethoxylate			10%
Tween 80	9005-65-6	Liquid	UVCB	Nonionic	Sorbitan ethoxylated fatty acid ester			Neat, 10%
Tween 20	9005-64-5	Liquid	UVCB	Nonionic	Sorbitan ethoxylated fatty acid ester			Neat
Polyglyceryl-3-diisooctadecanoate	63705-03-3	Liquid	UVCB	Nonionic				Neat
Polyethylene glycol (PEG-40) hydrogenated castor oil	61788-85-0	Solid	UVCB	Nonionic	Alkoxylated fatty acids			Neat
Cellulose,2-(2-hydroxy-3-(trimethylammonium)propoxy)ethyl ether chloride	68610-92-4	Solid		Cationic	polymeric quaternary ammonium salt of hydroxyethyl cellulose			Neat
Myristyl myristate	3234-85-3	Solid	mono-constituent	Nonionic				Neat

UVCB: unknown or variable composition, complex reaction products and biological materials; NA: Not available; EO: ethylene oxide

Table A B.2. Spreadsheet with the identification of the substances that were used in the training set and the test set of DASF and predictions of the individual test methods and DASF.

ID	Draize study source	DRD Study No.	Surfactant	CAS RN	Number of studies	UN GHS	Most Important Driver	Class	Set	EpiOcular™ EIT (TG 492)				SkinEthic™ HCE EIT (TG 492)				STE									Combination	DASF prediction			DASF	Agreement in prediction TP/BU
										N	Tissue viability	Prediction		N	Tissue viability	Prediction		N	Cell viability			OECD TG 491			STE$^{0.5}$ for surfactants		TG491 with TG492				Prediction	
											min - max	NPCN	No Cat			NPCN	No Cat		5%	0.5%	0.05%	Cat. 1	NPCN	No Cat	Cat. 1	Not Cat		Cat. 1	Cat. 2	No Cat		
1	DRD	10	Ethylhexyl acid phosphate ester	12645-31-7	1	Cat 1	CO mean ≥ 3	Anionic	Training	2	0	1.00	0.00	2	0	1.00	0.00	1	2.7	3.1	45.1	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
2	DRD	16, 145	Benzalkonium chloride (10%)	63449-41-2	2	Cat 1	CO mean ≥ 3 // CO=4	Cationic	Training	3	4.1 - 7.2	1.00	0.00	1	1.5	1.00	0.00	1	6.5	4.1	48.3	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
3	DRD	17	Benzethonium chloride (10%)	121-54-0	1	Cat 1	CO mean ≥ 3	Cationic	Test	1	3.9	1.00	0.00	9	1.4 - 2.5	1.00	0.00	1	6.2	1.9	58.3	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
4	DRD	18	Domiphen bromide (10%)	538-71-6	1	Cat 1	CO mean ≥ 3	Cationic	Training	3	5.0 - 6.6	1.00	0.00	11	1.7 - 3.7	1.00	0.00	1	5.2	2.9	51.6	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
5	DRD	28	Distearyldimethylammonium chloride	107-64-2	1	Cat 1	CO mean ≥ 3	Cationic	Training	2	9.0 - 31.1	1.00	0.00	2	2.0 - 3.2	1.00	0.00	2	19.2 - 57.6	27.4 - 49.0	78.6 - 101.7	0.000	1.000	0.000	0.000	1.000	Cat. 2	0.000	1.000	0.000	Cat. 2	Yes
6	DRD	29	Ethyl lauroyl arginate HCl	60372-77-2	1	Cat 1	CO mean ≥ 3	Cationic	Test	3	15.0 - 20.1	1.00	0.00	Not tested				1	6.3	6.7	5.4	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
7	DRD	40	Cetyl pyridinium bromide (10%)	140-72-7	1	Cat 1	CO mean ≥ 3	Cationic	Training	1	3.9	1.00	0.00	3	1.4 - 2.4	1.00	0.00	1	2.5	4.3	60.7	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
8	DRD	36, 37	Triton X-100	9002-93-1	2	Cat 1	IR mean > 1.5 // CO = 4	Nonionic	Training	1	1.5	1.00	0.00	1	0.8	1.00	0.00	3	-0.2 - 1.2	-0.2 - 0.7	0.1 - 3.4	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
9	DRD	39	Benzalkonium chloride (5%)	63449-41-2	1	Cat 1	CO = 4	Cationic	Training	2	3.0 - 4.0	1.00	0.00	1	1.9	1.00	0.00	1	8.9	10.9	80.7	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
10	DRD	54	Coco amidopropyl betaine (25%)	61789-40-0	1	Cat 1	CO pers D21	Amphoteric	Test	9	3.6 - 7.6	1.00	0.00	1	0.3	1.00	0.00	1	1.5	0.7	79.3	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
11	DRD	63	Surfonic HDL-1 (Nonyl phenol ethoxylate, branched)	9016-45-9	1	Cat 1	CO pers D21	Nonionic	Training	1	27.1	1.00	0.00	1	0.5	1.00	0.00	1	-0.3	1	3.4	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
12	DRD	69, 70	Cetyltrimethyl ammonium bromide (10%)	57-09-0	2	Cat 1	CO pers D21	Cationic	Training	3	6.0 - 17.3	1.00	0.00	11	1.6 - 4.2	1.00	0.00	1	2.5	5.9	69	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
13	DRD	66, 67	Benzalkonium chloride (1%)	63449-41-2	2	Cat 1	CO pers D21	Cationic	Training	1	2.4	1.00	0.00	9	1.7 - 3.1	1.00	0.00	1	4.2	61.6	92.5	0.000	1.000	0.000	0.000	1.000	Cat. 2	0.000	1.000	0.000	Cat. 2	Yes
14	DRD	71	Di(2-ethylhexyl)sodium sulphosuccinate (10%)	577-11-7	1	Cat 1	CO pers D21	Anionic	Test	1	4	1.00	0.00	9	2.5 - 4.7	1.00	0.00	1	9.6	5.0	93.4	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
15	DRD	68	Cetylpyridinium chloride (10%)	6004-24-6	1	Cat 1	CO pers D21	Cationic	Training	1	4.5	1.00	0.00	1	1.1	1.00	0.00	1	1.4	3.1	65.7	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
16	DRD	76	Sodium lauryl sulphate (15%)	151-21-3	1	Cat 1	CO pers D21	Anionic	Training	2	2.0	1.00	0.00	Not tested				1	0.1	0.8	65.7	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
17	DRD	77	Stearyltrimethylammonium chloride (10%)	112-03-8	1	Cat 1	CO pers D21	Cationic	Test	1	12.1	1.00	0.00	3	3.9 - 6.9	1.00	0.00	1	3.2	4.9	80.7	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
18	Blazka, 2000		Didecyl dimethyl ammonium chloride (1%)	7173-51-5	1	Cat 1	CO pers D21	Cationic	Test	1	33.1	1.00	0.00	1	21.6	1.00	0.00	1	2.6	18.9	92.9	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
19	Blazka, 2000		N-Lauroyl sarcosine Na salt (30%)	137-16-6	1	Cat 1	CO pers D21	Anionic	Training	1	Not published	1.00	0.00	1	0.5	1.00	0.00	2	-0.3 - 0.4	-0.3 - -0.6	83.5 - 93.5	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
20	Scheel et el., 2011		1-Hexadecanaminium, N,N,N-trimethyl-, chloride (25%)	112-02-7	1	Cat 1	CO pers D21	Cationic	Test	2	6.9 - 11.9	1.00	0.00	2	0.9 - 1.7	1.00	0.00	1	0.7	3	31.4	1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
21	Proprietary		Isotridecanol, ethoxylated (9 EO)	69011-36-5	1	Cat 1	CO pers D21	Nonionic	Test	1	Not published	1.00	0.00	Not tested				1	lot published			1.000	0.000	0.000	1.000	0.000	Cat. 1	1.000	0.000	0.000	Cat. 1	Yes
22	DRD	112	Triton X-100 (10%)	9002-93-1	1	Cat 1	Conj pers D21	Nonionic	Training	1	6.6	1.00	0.00	9	0.6 - 1.0	1.00	0.00	1	0.5	2	104.6	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
23	DRD	41	Cetyl pyridinium bromide (6%)	140-72-7	1	Cat 1	IR mean > 1.5	Cationic	Training	2	8.5 - 35.1	1.00	0.00	3	1.8 - 5.7	1.00	0.00	1	4.1	13.1	80.9	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
24	DRD	185	Cetyl pyridinium bromide (1%)	140-72-7	1	Cat 2	CO mean ≥ 1	Cationic	Training	1	49.6	1.00	0.00	3	9.5 - 40.4	1.00	0.00	2	3.7 - 7.3	71.9 - 78.0	92 - 100.6	0.00	1.00	0.00	0.00	1.00	Cat. 2	0.00	1.000	0.000	Cat. 2	Yes
25	DRD	186	Deoxycholic acid Na salt (10%)	302-95-4	1	Cat 2	CO mean ≥ 1	Anionic	Training	1	5.5	1.00	0.00	1	0.6	1.00	0.00	2	0.9	5.9 - 9.4	01.6 - 101.	0.00	1.00	0.00	1.000	0.000	Cat. 2	1.00	0.000	0.000	Cat. 1	Yes
26	DRD	187	Lauryl sulphobetaine (10%)	14933-08-5	1	Cat 2	CO mean ≥ 1	Amphoteric	Test	1	3	1.00	0.00	1	0.4	1.00	0.00	1	0.8	33.2	103.2	0.00	1.00	0.00	0.00	1.00	Cat. 2	0.00	1.000	0.000	Cat. 2	Yes
27	DRD	207, 647	Triton X-100 (5%)	9002-93-1	2	Cat 2	CO mean ≥ 1	Nonionic	Training	4	3.0 - 5.8	1.00	0.00	1	5	1.00	0.00	1	1.8	37.1	105.2	0.00	1.00	0.00	0.00	1.00	Cat. 2	0.00	1.000	0.000	Cat. 2	Yes
28	DRD	209	N-Lauroyl sarcosine Na salt (10%)	137-16-6	1	Cat 2	CO mean ≥ 1	Anionic	Training	1	Not published	1.00	0.00	1	1.8	1.00	0.00	1	0.1	2	101.1	0.00	1.00	0.00	1.000	0.000	Cat. 2	1.00	0.000	0.000	Cat. 1	Yes
29	Proprietary		Isotridecanol, ethoxylated (9 EO) (10%)	69011-36-5	1	Cat 2	CO mean ≥ 1	Nonionic	Test	1	Not published	1.00	0.00	Not tested				1	lot published			1.00	0.00	0.00	1.000	0.000	Cat. 1	1.00	0.000	0.000	Cat. 1	Yes
30	DRD	178, 205	Methyl N,N,N-trimethyl-4-[(4,7,7-trimethyl-3-oxobicyclo[2.2.1]hept-2-ylidene)methyl]anilinium sulphate (30%)	52793-97-2	2	Cat 2	CO mean ≥ 1 // Conj mean ≥ 2	Cationic	Training	9	9.9 - 17.9	1.00	0.00	1	4.6	1.00	0.00	1	29.2	100.7	103.2	0.00	1.00	0.00	0.00	1.00	Cat. 2	0.00	1.000	0.000	Cat. 2	Yes
31	Blazka, 2000	859	Benzethonium chloride (1%)	121-54-0	1	Cat 2	Conj mean ≥ 2	Cationic	Training	1	3.3	1.00	0.00	1	2	1.00	0.00	1	0.8	64.9	96.5	0.00	1.00	0.00	0.00	1.00	Cat. 2	0.00	1.000	0.000	Cat. 2	Yes
32	Scheel et el., 2011	Scheel 2%	1-Hexadecanaminium, N,N,N-trimethyl-, chloride (2%)	112-02-7	1	Cat 2	Conj mean ≥ 2	Cationic	Test	1	7.2	1.00	0.00	1	3.7	1.00	0.00	1	5.5	24.6	92	0.00	1.00	0.00	0.00	1.00	Cat. 2	0.00	1.000	0.000	Cat. 2	Yes
33	SCCP/0917/05		Behentrimonium chloride (5%)	17301-53-0	1	Cat 2	Conj mean ≥ 2	Cationic	Test	Not tested				1	0.7	1.00	0.00	1	20.14	58.66					0.00	1.00	Cat. 2	0.00	1.000	0.000	Cat. 2	Yes
34	DRD	265	N-Lauroyl sarcosine Na salt (3%)	137-16-6	1	No Cat	CO > 0 **	Anionic	Test	1	Not published	1.000	0.000	1	8.5	1.000	0.000	1	0.4	99	100.2	0.000	1.000	0.000	0.000	1.000	Cat. 2	0.000	1.000	0.000	Cat. 2	Yes
35	DRD	266	Sodium lauryl sulphate (3%)	151-21-3	1	No Cat	CO > 0 **	Anionic	Training	2	3.0 - 6.0	1.000	0.000	1	7.7	1.000	0.000	1	1.1	13.6	100.4	0.000	1.000	0.000	1.000	0.000	Cat. 2	1.000	0.000	0.000	Cat. 1	Yes
36	DRD	294	Cetyl pyridinium bromide (0.1%)	140-72-7	1	No Cat	CO > 0	Cationic	Training	1	80.9	0.000	1.000	3	88.8 - 99.0	0.000	1.000	1	80.2	96.4	103.4	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
37	DRD	296, 297	Sodium lauryl sulphate (1%)	151-21-3	2	No Cat	CO > 0	Anionic	Training	1	21.3	1.000	0.000	1	16.3	1.000	0.000	1	0.5	102.4	99.6	0.000	1.000	0.000	0.000	1.000	Cat. 2	0.000	1.000	0.000	Cat. 2	Yes
38	DRD	452	Polyethylene glycol monolaurate (10EO) (10%)	9004-81-3	1	No Cat	CO = 0	Nonionic	Test	1	89.9	0.000	1.000	1	84.1	0.000	1.000	1	106.7	105.6	100.8	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
39	DRD	453	Polyglyceryl-3-diisooctadecanoate	63705-03-3	1	No Cat	CO = 0	Nonionic	Test	9	86.9 - 103.9	0.000	1.000	1	91.4	0.000	1.000	1	105.8	100.8	100.8	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
40	DRD	463	Steareth-10 allyl ether/acrylates copolymer (30%)	109292-17-3	1	No Cat	CO = 0	Anionic	Test	9	69.6 - 112.3	0.000	1.000	Not tested				Not tested									No Cat.	0.000	0.000	1.000	No Cat.	no STE
41	DRD	475	Triton X-100 (1%)	9002-93-1	1	No Cat	CO = 0	Nonionic	Training	1	18	1.000	0.000	3	1.2 - 10.1	1.000	0.000	1	8.4	104.1	102.6	0.000	1.000	0.000	0.000	1.000	Cat. 2	0.000	1.000	0.000	Cat. 2	Yes
42	DRD	476	Tween 20	9005-64-5	1	No Cat	CO = 0	Nonionic	Training	5	79.8 - 102.8	0.000	1.000	9	75.1 - 90.8	0.000	1.000	4	21.1 - 82.9	95 - 105	91 - 104	0.000	0.750	0.250	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
43	DRD	478	Tween 80	9005-65-6	1	No Cat	CO = 0	Nonionic	Training	4	63.4 - 85.5	0.000	1.000	2	74.5 - 76.7	0.000	1.000	2	91 - 114	96 - 105	99 - 105	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
44	DRD	479	Tween 80 (10%)	9005-65-6	1	No Cat	CO = 0	Nonionic	Training	1	104.1	0.000	1.000	1	87.9	0.000	1.000	1	96.7	101	98.9	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
45	DRD	487	Polyoxyethylene 23 lauryl ether (Brij-35) (10%)	9002-92-0	1	No Cat	CO = 0	Nonionic	Training	Not tested				3	72.5 - 89.4	0.000	1.000	1	118	104.2	103.8	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
46	DRD	489	Cetylpyridinium chloride (0.1%)	6004-24-6	1	No Cat	CO = 0	Cationic	Training	1	83.2	0.000	1.000	1	87	0.000	1.000	1	65.4	100.2	102.4	0.000	1.000	0.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
47	DRD	491	Polyoxyethylene 8-stearate (Myrj-45) (10%)	9004-99-3	1	No Cat	CO = 0	Nonionic	Test	1	85.3	0.000	1.000	1	94.6	0.000	1.000	1	115	105.3	97.5	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
48	DRD	502	Polyethylene glycol (PEG-40) hydrogenated castor oil	61788-85-0	1	No Cat	CO = 0	Nonionic	Training	3	65.2 - 89.2	0.000	1.000	9	73.9 - 115.3	0.000	1.000	4	91 - 118	98 - 103	94 - 108	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes
49	DRD	546	Cellulose,2-(2-hydroxy-3-(trimethylammonium)propoxy)ethyl ether chloride	68610-92-4	1	No Cat	CO = 0	Cationic	Training	3	59.8 - 66	0.333	0.667	9	63.6 - 79.6	0.000	1.000	Issue [a]									No Cat.	0.000	0.083	0.917	No Cat.	no STE
50	DRD	563	Myristyl myristate	3234-85-3	1	No Cat	CO = 0	Nonionic	Test	3	98.6 - 136	0.000	1.000	9	37.9 - 112.8	0.000	1.000	1	103.4	102.1	102	0.000	0.000	1.000	0.000	1.000	No Cat.	0.000	0.000	1.000	No Cat.	Yes

[a] Test item could not be washed from the plate

Annex C. Background information

Annex C.1

Discussion within OECD Expert Group on Eye Irritation on reference database for the Defined Approaches for Surfactants.

Development of DASF was discussed in numerous teleconferences held for OECD Expert Group (EG) on Skin and Eye Irritation from 2022 – 2024. The main issue discussed within OECD EG was the number of surfactants in the reference database. The EG was divided on this issue, with two differing opinions:

- One view argued that the number of Cat. 2 surfactants (n=10) in the current reference database is insufficient to instil confidence in the DASF, particularly because the training set includes only 6 surfactants, and the testing set only 4. Due to small sample size, there is uncertainty whether the acceptance criterion of 50% correct prediction for Cat. 2 substance was met.
- The opposing view emphasized that (i) the DASF's applicability domain is limited to surfactants, so the database size is inherently smaller, (ii) all available *in vivo* data were carefully curated based on rigorous selection criteria and (iii) the DASF outperforms all other *in vitro* methods currently available in OECD Test Guidelines (TGs) for surfactants, and it has been tested on the largest number of surfactants. Experts supporting the development of DASF also argued that the chemistry underlying the classification of 'surfactants' likely gives them similar mechanisms for damaging membrane barriers and cell membranes.

Annex C.2

Development of a Defined Approach for Eye hazard identification of chemicals having surfactant properties according to the three UN GHS categories.

Alépée N, Adriaens E, Abo T, Magby J, Mewes KR, Giusti A. (2023) Toxicol In Vitro. 89, 105576. doi: 10.1016/j.tiv.2023.105576. Epub 2023 Feb 20. PMID: 36809832.

Abstract

The purpose of this study was to develop a defined approach (DA) for eye hazard identification according to the three UN GHS categories for surfactants (DASF). The DASF is based on a combination of Reconstructed human Cornea-like Epithelium test methods (OECD TG 492; EpiOcular™ EIT and SkinEthic™ HCE EIT) and the modified Short Time Exposure (STE) test method (0.5% concentration of the test substance after a 5-min exposure). DASF performance was assessed by comparing the prediction results with the historical in vivo data classification and against the criteria established by the OECD expert group on eye/skin. The DASF yielded a balanced accuracy of 80.5% and 90.9% of Cat. 1 (N = 22), 75.0% of Cat. 2 (N = 8), and 75.5% of No Cat. (N = 17) surfactants were correctly predicted. The percentage of mispredictions was below the established maximum values except for in vivo No Cat. surfactants that were over-predicted as Cat. 1 (5.6%, N = 17), with a maximum value set at 5%. The percentage of correct

predictions did meet the minimum performance values of 75% Cat. 1, 50% Cat. 2, and 70% No Cat. established by the OECD experts. The DASF has shown to be successful for eye hazard identification of surfactants.

Annex C.3

Performance of the DASF compared to other combinations of OECD NAMs for eye hazard identification of surfactants.

Alépée N, Mewes KR, Abo T, Cavarzan A, Odriscoll C, Adriaens E (2024) ALTEX https://doi.org/10.14573/altex.2406031

Abstract

Currently, the OECD has adopted three defined approaches (DAs) for eye hazard identification of non-surfactant liquids and solids (TG467) according to the three UN GHS categories (Cat.1, Cat.2, No Cat.). We are now expanding the applicability domain with a new DA for chemicals having surfactant (SF) properties (DASF). It is based on a combination of RhCE test methods (OECD TG492: EpiOcular™ EIT or SkinEthic™ HCE EIT) and a modification of the Short Time Exposure (STE, TG491) method. The aim of the current study was to compare the performance of the DASF with the performance of other NAMs currently included in the OECD TGs and with the classification based on the Draize eye test to identify potential additional DAs. The minimum performance criteria (75% Cat.1, 50% Cat.2, 70% No Cat.) used for the adoption of the DAs currently included in OECD TG467 were used for this purpose. The DASF identified 90.9% of Cat. 1 (N=23), 77.8% of Cat. 2 (N=9) and 76.0% of No Cat. (N=17) surfactants, meeting the minimum performance criteria. Some of the NAMs that are currently included in the OECD TGs seem promising methods to be part of a DA to identify Cat. 1 or No Cat. for eye hazard assessment of surfactants. However, the number of surfactants that have been tested to evaluate reliability and relevance was often too small. To date, the DASF is the only DA that has evaluated a sufficiently large number of surfactants and whose performance met the OECD acceptance criteria.