Section 4
Health effects

Test Guideline No. 467

Defined Approaches for Serious Eye Damage and Eye Irritations

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OECD Guidelines for the Testing of Chemicals

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OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Defined Approaches (DAs) for Serious Eye Damage and Eye Irritation

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1 Introduction

1.1. General Introduction

1. The assessment of eye irritation/serious eye damage originally involved the use of albino rabbits according to the Draize eye test method (OECD Test Guideline 405) (1). The hazard potential of a test chemical was 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 the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (2). According to the UN GHS classification system, Category 1/serious damage (Cat. 1) is defined as causing irreversible effects (not fully reversible within 21 days) on the eye/serious damage to the eye. Category 2/irritation (Cat. 2) is defined as causing reversible effects (fully reversible within 21 days) on the eye/eye irritation. This category 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 test chemical does not require classification which corresponds to No Category (No Cat.).

2. In 2022, a stand-alone *in vitro* method (OECD TG 492B was adopted for the identification of test chemicals not requiring classification (UN GHS No Cat), requiring classification for eye irritation (UN GHS Cat 2) and requiring classification for serious eye damage (UN GHS Cat 1) (3). Furthermore, several Test Guidelines (TGs) on *in vitro* methods have been adopted for the identification of test chemicals inducing serious eye damage (UN GHS Cat. 1) or for the identification of test chemicals not requiring classification for eye irritation and serious eye damage hazards (UN GHS No Cat.), notably OECD TG 437, TG 438, TG 460, TG 491, TG 492, TG 494, and TG 496 (4, 5, 6, 7, 8, 9, 10). Data generated with these *in vitro* methods are proposed to be used together, as well as with information sources such as physicochemical properties, *in silico* and read-across predictions from chemical analogues, within integrated approaches to testing and assessment (IATA) or defined approaches (DAs) (11). Results from the individual information sources cannot be used in DAs if the chemicals are known to clearly fall outside the applicability domains of the methods, as may be detailed in the respective assay TGs. The prediction from a DA may be used alone or along with further information as part of an IATA (11) or according to the applicable legal criteria.

3. The major difficulty for a single *in vitro* test method to fully replace the *in vivo* rabbit eye test (TG 405) is to predict the middle category (UN GHS Cat. 2) and it is therefore recommended to make use of testing strategies (e.g., Top-Down or Bottom-Up approach) that combine the strengths of individual *in vitro* test methods to address the required ranges of irritation potential (12). The determination of the most relevant *in vivo* endpoint(s), in particular the effects on cornea, iris or conjunctiva, is important for the development of adequate *in vitro* methods as it allows to better understand the relationship between the *in vitro* and the *in vivo* data (13, 14). For this reason, it is recommended to take into consideration the most important drivers for Cat. 1 and Cat. 2 classifications as well as the

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distribution of *in vivo* effects for chemicals not requiring classification when selecting reference chemicals for the development, evaluation and/or validation of alternative methods and/or strategies for serious eye damage and eye irritation testing (11) (see "*In vivo* reference data (Draize eye test)" in the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation (15)*).

4. Modes of action for eye irritation are unknowable for the majority of chemicals and do not provide additional insight in evaluation of the test methods and DAs, and thus they are not considered for the analysis of the test chemicals for the DAs in the current Guideline (see paragraph 27 of the SD for more information).

5. Results from multiple information sources can be used together in DAs to predict the eye hazard potential of test chemicals. A DA consists of a fixed data interpretation procedure (DIP) (*i.e.* a mathematical model, a rule-based approach) applied to data (*e.g in silico* predictions, *in chemico*, *in vitro* data) generated with a defined set of information sources to derive a prediction without the need for expert judgment. The DAs use method combinations intended to overcome some of the limitations of the individual, stand-alone methods in order to provide increased confidence in the overall obtained result. The DAs provide information that can be used for eye hazard identification.

6. Testing laboratories should consider all relevant available information on the test chemical prior to conducting the studies according to a DA. Such information could include, for example, the identity and chemical structure of the test chemical and its physicochemical properties. Such information should be considered in order to determine whether the individual OECD TG methods under a specific DA are applicable for the test chemical.

7. When performing a hazard evaluation based on the output from the *in vivo* Draize eye test, from an *in chemico* test, from an *in vitro* test, from an *in silico* approach, from a DA, and any combination thereof, the same principles always apply, *i.e.* all available information relevant to the chemical in question should be taken into consideration as well as toxicological data on structurally related test chemicals, if available. However, specific regulatory requirements in the applicable legislation should be applied.

8. Two rule-based DAs for non-surfactant liquids and one rule-based DA for neat solids are included in this GL, and are described with respect to their intended regulatory purpose: hazard identification, *i.e.* discrimination between three UN GHS categories i.e., Category 1 (Cat. 1) on "serious eye damage"; Category 2 (Cat. 2) on "eye irritation" and No Category (No Cat.) for chemicals "not requiring classification and labelling" for eye irritation or serious eye damage (2). The evaluation and review of the DAs are described in detail in the *Supporting Document for Evaluation and Review of TG 467 on DAs for Serious Eye Damage / Eye Irritation (15).* For the non-surfactant liquids, a dataset of at least 86 chemicals with DA predictions, data on individual information sources, highly curated Draize eye test data, and physicochemical properties, was compiled and is attached as **Annex B (spreadsheets)** to the *Supporting Document for Evaluation and Review of TG 467 on DAs for Serious Eye Damage / Eye Irritation (15)*. A list of 109 solids with DA predictions, data on individual information sources, and highly curated Draize eye test data was compiled and is attached as **Annex A (spreadsheets)** to the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15)*. The list of chemicals was used to evaluate the performance of the DAs. The set of liquids and solids covers a broad range of uses and chemicals classes, with a wide range of organic functional groups (79 different OFGs for the liquids and 111 different OFG for the solids) defined according to OECD QSAR Toolbox analysis (version 3.2; https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

9. The dataset is chemically diverse as shown by the physicochemical properties covered by these chemicals: it contains small and large molecules, as well as hydrophobic

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and hydrophilic substances. Further details on the chemical characterization of the reference database are available in **Section 5.1.2.** of the *Supporting Document for Evaluation and Review of TG 467 on DAs for Serious Eye Damage / Eye Irritation (15)*.

10. Other DAs may be included in this GL following future review and approval.

1.2. DAs and Use Scenarios included in the Guideline

11. The DAs currently described in this GL are:

- **Part I** - Defined Approaches 1 for Eye hazard identification based on physicochemical properties and *in vitro* data (16), for neat non-surfactant liquids (DAL-1).
- **Part II** - Defined Approaches 2 for Eye hazard identification based on *in vitro* data (17) for non-surfactant neat liquids, liquids and solids dissolved in water (DAL-2).
- **Part III** - Defined Approach (DAS) for Eye hazard identification based on *in vitro* data for neat solids (DAS).

12. The DAL-1 described in this GL is based on the use of a combination of test methods described in OECD TG 437 and TG 492 as well as the physicochemical properties (PCP) of the test chemical. DAL-2 in contrast, is based on the use of a combination of test methods described in the OECD TG 437 and TG 491. The methods used in DAL-1 and DAL-2 encompass the following validated test methods: the Bovine Corneal Opacity and Permeability (BCOP) using the laser light-based opacitometer (LLBO)[1] according to the OECD TG 437, the Reconstructed human Cornea-like Epithelium (RhCE) (EpiOcular™ Eye Irritation Test or SkinEthic™ Human Corneal Epithelium (HCE) EIT) according to the OECD TG 492[2] and the Short Time Exposure in vitro (STE) according to the OECD TG 491. The DALs are hereafter referred to as DAL-1 PCP/EpiOcular/LLBO, DAL-1 PCP/SkinEthic/LLBO, and DAL-2 STE/LLBO. The DAS is based on the use of combination of test methods described in the OECD TG 437 and TG 492. The methods used in DAS encompass the following validated test methods: the BCOP LLBO[3] according to the OECD TG 437 and the SkinEthic™ HCE EIT according to the OECD TG 492[4] and is hereafter referred to as DAS SkinEthic/LLBO. Transferability, within- and between-laboratory reproducibility of these individual test methods have been assessed during their respective validation studies (18, 19, 20, 21, 22).

13. The DAs described in this GL can each be used to address countries' requirements for identifying chemicals causing serious eye damage (*i.e.* UN GHS Category 1), eye irritation (*i.e.* UN GHS Category 2), and test chemicals not requiring classification (*i.e.* UN GHS No Category), though they do so with different performance (detailed in the respective descriptions of each DA).

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[1] DIP with the BCOP OP-KIT test method did not meet the acceptance criteria for the current GL and thus is not used for DAL-1 or DAL-2 (see Annex A of the supporting document).

[2] Other similar methods from OECD TG 492 were not used for the DAL-1 analysis due to insufficient availability in data for those methods.

[3] DIP with the BCOP OP-KIT test method did not meet the acceptance criteria for the current GL and thus is not used for DAS.

[4] DIP with the EpiOcular™ EIT test method did not meet the acceptance criteria for the current GL and thus is not used for DAS.

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14. The DAs described in this GL are not designed to distinguish between Categories 2A and 2B.

15. DAL-1 and DAL-2 are applicable to liquids (i.e., pipettable test substances) and DAS is applicable to solids (i.e., not pipettable test substances). For additional details see **Section 2** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15)*.

16. The performance of DAL-1 PCP/EpiOcular/LLBO described in this GL for discriminating between the three UN GHS categories was evaluated using 94 non-surfactant liquids (17 Cat. 1, 22 Cat. 2, and 55 No Cat.) for which physicochemical properties, EpiOcular™ EIT predictions, BCOP LLBO predictions (available for all *in vivo* classified results but missing for 14/55 *in vivo* No Cat. substances), and classifications based on Draize Eye test data are available (for additional details see **Section 2** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15))*.

17. The performance of DAL-1 PCP/SkinEthic/LLBO described in this GL for discriminating between the three UN GHS categories was evaluated using 86 non-surfactant liquids (17 Cat. 1, 23 Cat. 2, and 46 No Cat.) for which physicochemical properties, SkinEthic™ HCE EIT predictions, BCOP LLBO predictions (available for all *in vivo* classified results but missing for 11/46 *in vivo* No Cat. substances), and classifications based on Draize Eye test data are available (for additional details see **Section 2** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15))*.

18. The performance of the DAL-2 STE/LLBO described in this GL for discriminating between the three UN GHS categories was evaluated using 164 non-surfactant liquids (17 Cat. 1, 24 Cat. 2, and 123 No Cat.) for which STE predictions, BCOP LLBO predictions (available for all *in vivo* classified results but missing for 67/123 *in vivo* No Cat. substances), and classifications based on Draize Eye test data are available (for additional details see **Section 2** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (15)*.

19. The performance of the DAS SkinEthic/LLBO described in this GL for discriminating between the three UN GHS categories was evaluated using 109 solids (31 Cat. 1, 18 Cat. 2, and 60 No Cat.) for which SkinEthic™ HCE EIT predictions, BCOP LLBO predictions (available for all *in vivo* classified results but missing for 5/60 *in vivo* No Cat. substances), and classifications based on Draize Eye test data are available (for additional details see Section 2 and Annex B.3 of the *Supporting Document to the GL on DAs for Serious Eye Damage/Eye Irritation (15).*

1.3. Performance and Applicability

1.3.1. Performance of the DAs

20. Table 1.1. Summary of the DAs included in this Guideline – Eye hazard identification**.1** provides an overview of the DAs included in this Guideline (GL), their information sources used, and summarises their performance against the Draize Eye reference data. More details are provided in Part I, Part II, and Part III of this GL, as well as in the *Supporting documents to the GL on DAs for Serious Eye Damage and Eye Irritation (15)*.

21. The performance of the DAs for UN GHS classification (Cat. 1, Cat. 2, and No Cat) when compared to the Draize Eye test reference data yielded balanced accuracies of 69.2% (DAL-1 PCP/EpiOcular/LLBO), 75.2% (DAL-1 PCP/SkinEthic/LLBO), 74.3% (DAL-

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2 STE/LLBO), and 66.7% (DAS SkinEthic/LLBO). Note that there is a raised degree of uncertainty relating to the derived Cat. 1 and Cat. 2 accuracies (correct predictions), as compared with the No Cat. accuracies due to the lower number of reference chemicals within these categories. It was however not possible to increase the number of chemicals because of the limited number of available Draize Eye test results with a Cat. 1 or Cat. 2 classification. Detailed performance statistics are reported in Part I, Part II, and Part III and in **Section 5** of the *Supporting documents to the GL on DAs for Serious Eye Damage and Eye Irritation (15).*

Table 1.1. Summary of the DAs included in this Guideline – Eye hazard identification

DA	DAL-1 PCP/EpiOcular/LLBO (N=94)	DAL-1 PCP/SkinEthic/LLBO(N=86)	DAL-2 STE/LLBO (N=164)	DAS Skinethic/LLBO (N=109)
Information Sources	Physicochemical properties, EpiOcular™ EITL (TG 492), BCOP LLBO (TG 437)	Physicochemical properties, SkinEthic™ HCE EITL (TG 492), BCOP LLBO[a] (TG 437)	STE (TG 491), BCOP LLBO (TG 437)	SkinEthic™ HCE EITS (TG 492), BCOP LLBO[b] (TG 437)
Applicable	Non-surfactant neat liquids	Non-surfactant neat liquids	Non-surfactant neat liquids, liquids and solids dissolved in water	Non-surfactant neat solids
Performance vs. Draize Eye test (Correct classification)	70.5% No Cat. (N=55) 59.1% Cat. 2 (N=22) 76.5% Cat. 1 (N=17)	79.7% No Cat. (N=46) 68.7% Cat. 2 (N=23) 76.5% Cat. 1 (N=17)	85.3% No Cat. (N=123) 56.3% Cat. 2 (N=24) 81.2% Cat. 1 (N=17)	70.0% No Cat. (N=60) 52.3% Cat. 2 (N=18) 77.4% Cat. 1 (N=31)

[a] BCOP LLBO based on opacity only; [b] BCOP LLBO based on opacity and/or permeability
Note: For performance, accuracy reflects correct classification rate within each UN GHS category.
EITL: Eye Irritation Test protocol for liquids and EITS protocol for solids.
Solid: non-pippetable neat substance.

1.3.2. Applicability domain of the DAs and of the individual components of the DAs

22. DAL-1 PCP/EpiOcular/LLBO and DAL-1 PCP/SkinEthic/LLBO are not applicable for surfactants and solids. Both DAs are applicable to neat liquids, excluding mixtures, UVCBs and multi-constituent substances. For impurities with concentration > 5% and < 20%, the physicochemical properties of the impurities also need to be determined, and only when all components meet the exclusion criteria, the liquid is predicted No Cat., in all other cases, proceed with an RhCE test method.

23. DAL-2 STE/LLBO is not applicable for surfactants and solids dispersed in water. The DAL-2 STE/LLBO is applicable to non-surfactant neat liquids, liquids and solids dissolved in water.

24. DAS SkinEthic/LLBO is not applicable to liquids. The DAS SkinEthic/LLBO is applicable to non-surfactant neat solids (i.e., not pipettable test substances).

25. Users should refer to the limitations of the individual *in vitro* test methods as specified in their respective TGs, which are revised as new data become available and should be consulted regularly. The most up-to-date published version of the respective TGs should always be used. Users should also refer to the limitations of the individual methods for measuring the physicochemical properties as specified in their respective GLs.

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1.3.3. Uncertainty of DAs

26. Details on accepting the results of individual information sources to determine confidence in DA predictions are provided in **Sections 2.1.4**, **3.1.4**, and **4.1.4** and in the respective TGs (TG 437; TG 491; TG 492) (4, 7, 8).

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2 PART I - Defined Approaches 1 (DAL-1), based on physicochemical properties and *in vitro* data, for neat non-surfactant[5] liquids

27. Part I of this GL applies to DAL-1 that is intended for hazard identification, *i.e.* distinguishing between serious eye damage and eye irritation potential of test chemicals (or the absence thereof), specifically for neat non-surfactant liquids based on physicochemical properties and *in vitro* data. A summary of the DAL-1 for hazard identification is provided below; additional detailed information can be found in the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation.*

2.1. DAL-1

2.1.1. Summary

28. The DAL-1 is intended for the identification of the eye irritation hazard of a test chemical without the use of animal testing, *i.e.* UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat. The data interpretation procedure (DIP) is not designed to provide information on sub-categorisation of Cat. 2 into 2A and 2B.

29. The DAL-1 presented in this GL describes the combination of one and/or three physicochemical properties with the results of two *in vitro* test methods (RhCE and BCOP LLBO) for the identification of the eye hazard potential of non-surfactant liquid substances primarily for the purposes of classification and labelling without the use of animal testing (1). The physicochemical properties can be retrieved from publicly available databases, can be determined by new experimental studies, or may be predicted using computational methods (e.g. Quantitative Structure-Activity Relationships ((Q)SAR)). The RhCE models that are part of DAL-1 are the EpiOcular™ EITL and the SkinEthic™ HCE EITL (OECD TG 492) (2). Furthermore, the Bovine Corneal Opacity and Permeability (BCOP) test method with the laser light-based opacitometer (LLBO) is used (OECD TG 437) (3).

30. The DAL-1 PCP/EpiOcular/LLBO was compared to 94 chemicals with curated Draize Eye test reference data and demonstrated a balanced accuracy of 68.7% (see Table 2.1). The DAL-1 PCP/SkinEthic/LLBO was compared to 86 chemicals with curated

[5] Surfactant, also called surface-active agent, this is a substance, such as a detergent, that can reduce the surface tension of a water and thus allow it to foam or penetrate solids; it is also known as a wetting agent.

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Draize Eye test reference data and demonstrated a balanced accuracy of 75.0% (see Table 2.2).

2.1.2. Data interpretation procedure

31. The data interpretation procedure (DIP) applied uses the readout of the prediction models of each of the individual test method as defined by the TGs and/or information on the physicochemical properties. A scheme of DAL-1 is presented in Figure 2.1. Physicochemical property exclusion rules based on water solubility (WS) or a combination of octanol-water partition coefficient (LogP), vapour pressure (VP) and surface tension (ST) of the neat liquid are used in a first step to identify liquid chemicals with no serious eye damage or eye irritation potential (details are provided in section 5.1.2. of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation*). Liquids that are not identified as No Cat. according to the physicochemical property-based exclusion rules, are then evaluated based on a RhCE test method (EpiOcular™ EIT or SkinEthic™ HCE EIT) in Step 2. Liquids that result in a tissue viability > 60% are classified No Cat. Liquids that result in a tissue viability ≤ 60% are then evaluated based on the BCOP LLBO test method in a third step. Liquids that result in an opacity > 145 are predicted Cat. 1 and the remaining liquids are assigned Cat. 2. Note that it is also possible to start with a RhCE method, followed by the physicochemical property exclusion rules in case the tissue viability measured with EpiOcular™ EIT or SkinEthic™ HCE EIT > 60% (Figure 2.2). Furthermore, when a RhCE method is used as a first step and if the tissue viability > 60%, the prediction is based on the stand-alone method.

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Figure 2.1. Scheme of the DAL-1 option 1; step 1 physicochemical exclusion rules (WS: water solubility in mg/mL; or LogP: octanol-water partition coefficient / VP: vapour pressure in mm Hg / ST: surface tension of the neat liquid in dyne/cm) to identify No Cat., step 2 RhCE EITL test method used to identify No Cat., and step 3 BCOP LLBO used to identify Cat. 1

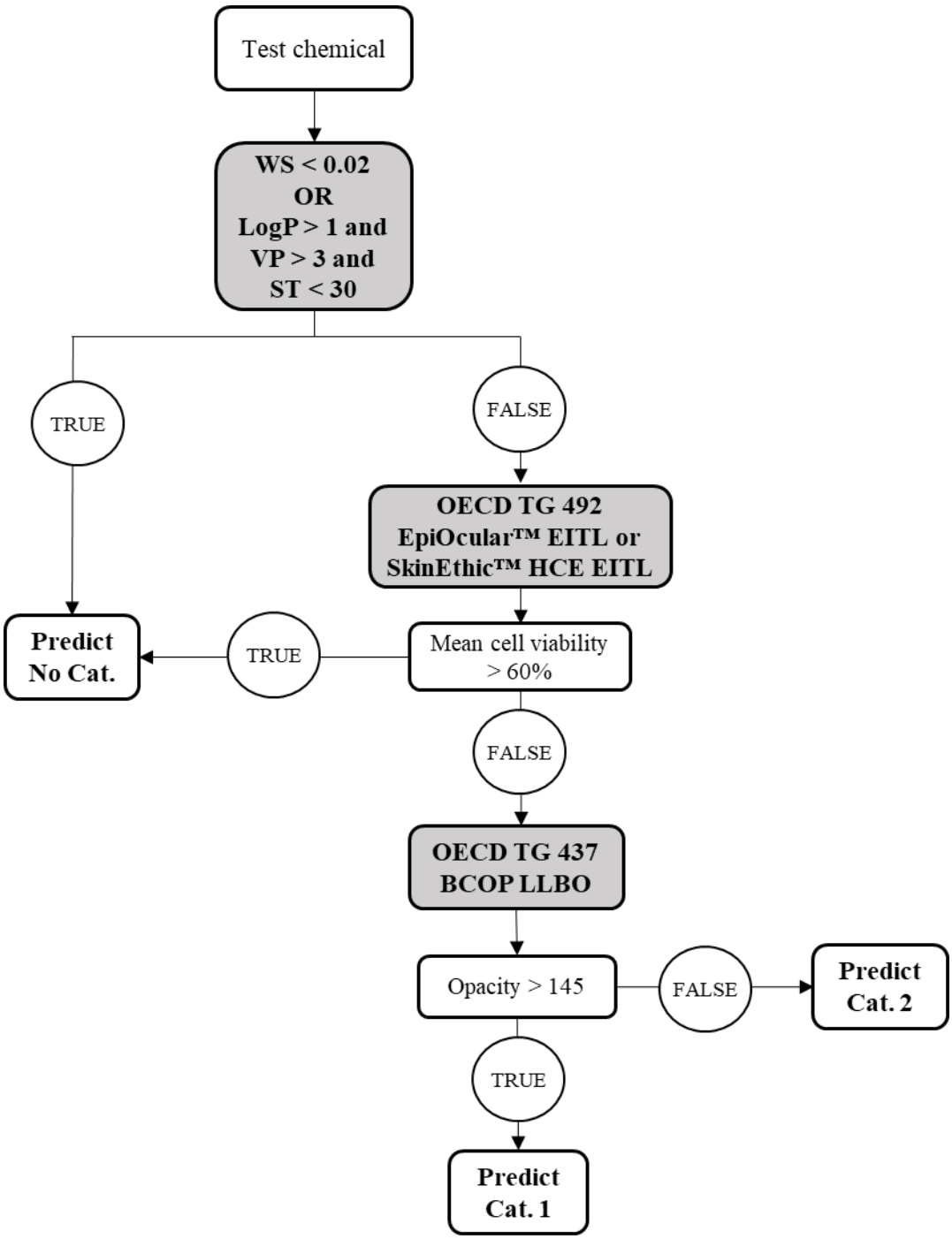

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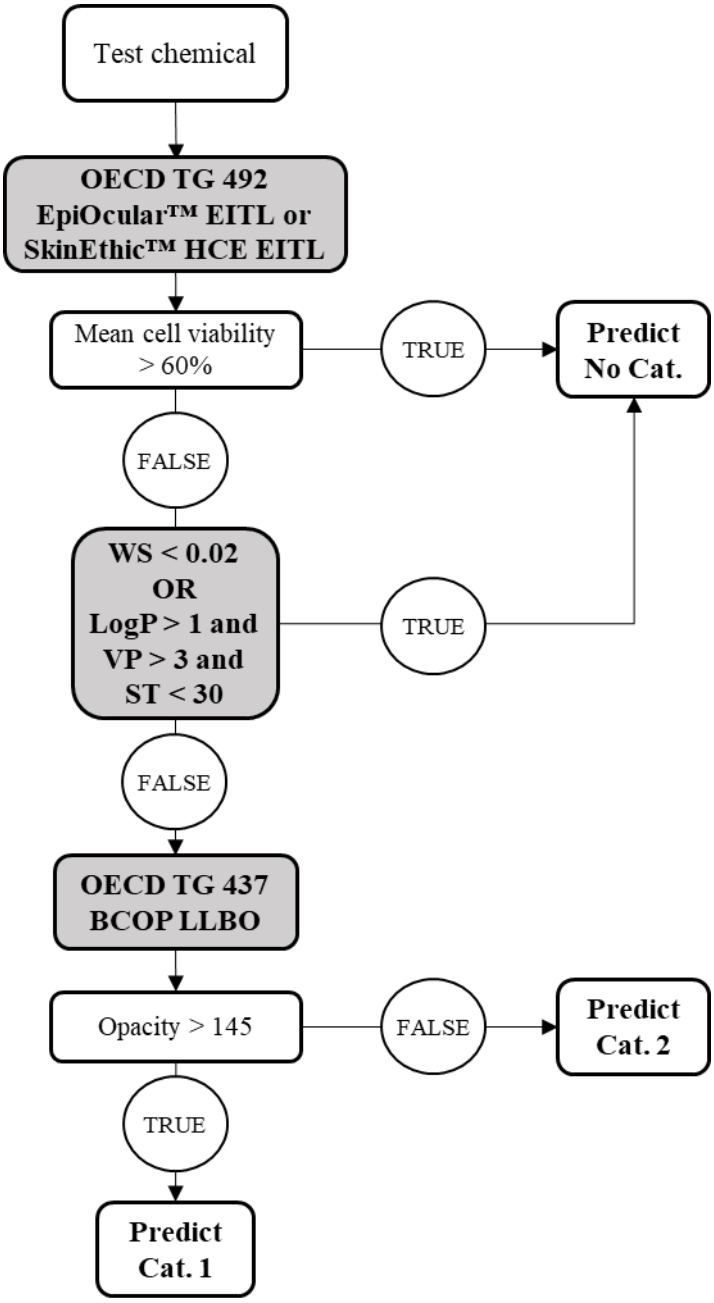
Figure 2.2. Scheme of the DAL-1 option 2; step 1 RhCE EITL test method used to identify No Cat., step 2: physicochemical exclusion rules (WS: water solubility in mg/mL; or LogP: octanol-water partition coefficient / VP: vapour pressure in mm Hg / ST: surface tension of the neat liquid in dyne/cm) to identify No Cat., and step 3 BCOP LLBO used to identify Cat. 1



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2.1.3. Description and limitations of the individual information sources

32. The individual information sources in the DA are the physicochemical properties and test methods included in OECD TG for serious eye damage/eye irritation or the absence thereof (OECD TG 437, 492) (2, 3), and the protocols are detailed therein.

33. The following *in vitro* test methods from those TGs have been characterised and included in the DAL-1.

- The RhCE EITL test methods: the methods measure the ability to induce cytotoxicity. In case borderline results are obtained, additional testing should be conducted, as specified in OECD TG 492 (2).
- BCOP LLBO test method: the eye hazard potential of a test chemical is measured by its ability to induce opacity and permeability in an isolated bovine cornea. Note that only opacity measurement is considered in the DAs. In case borderline results are obtained for opacity measurements, additional testing should be conducted, as specified in OECD TG 437 (3).

34. Any restriction regarding the applicability domain identified in the respective test method TGs (TG 437, TG 492) and analytical methods for measuring the physicochemical properties (GL 104, GL 105, GL 107, GL 115, GL 117, GL 123) is applicable to this GL (2, 3).

35. Measurements of physicochemical properties should be performed according to the OECD Guidelines (GL) and test reports are required corresponding with the information requested on data and reporting in each specific OECD GL (see Annex E). Prediction of physicochemical properties should use models that are based on the 5 OECD principles for QSAR models and that have a QMRF (QSAR Model Reporting Format).

2.1.4. Procedure for dealing with borderline result in test guidelines relevant to DAL-1

36. The first decision on whether each information element can be used is dictated by the applicability domain as described in the TGs of the respective *in vitro* methods (TG 437, TG 492) (2, 3). Even for within-domain substances, test results are inherently subject to variation and these variations increase the uncertainty of a test result, especially when close to a (classification) cut-off threshold, i.e. in the borderline range. The following procedures are in place to control the degree of uncertainty are described within the TGs of the respective information sources.

- TG 492 (RhCE EITL): A single test composed of at least two tissue replicates should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 60±5% a second test should be considered, as well as a third one in case of discordant results between the first two tests.
- TG 437 (BCOP LLBO): UN GHS Cat. 1 prediction based on opacity (Lux/7, mean opacity > 145), but 1 of 3 corneas with opacity (Lux/7) < 130; in cases of borderline results in the first testing run, a second testing run should be considered, as well as a third one in case of discordant predictions between the first two testing runs.

2.1.5. Predictive capacity of the DAL-1 PCP/EpiOcular/LLBO vs. the Draize Eye test

37. The predictive capacity of DAL-1 PCP/EpiOcular/LLBO is reported based on data generated by the Draize eye test (see **Table 2.1**) (see **Section 2.1** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation)*.

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Performance statistics are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 5** and **Annex B.2** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation for liquids (5).*

Table 2.1. Performance of DAL-1 PCP/EpiOcular/LLBO in comparison to Draize Eye reference data

UN GHS	Prediction using DAL-1 PCP/EpiOcular/LLBO[b]		
	Cat 1	Cat 2	No Cat
Cat. 1 (N='17),' %[a] (n/N)	**76.5%** **(13.0/17.0)**	23.5% (4.0/17.0)	0.0% (0.0/17.0)
Cat. 2 (N='22)' , %[a] (n/N)	27.3% (6.0/22.0)	**59.1%** **(13.0/22.0)**	13.6% (3.0/22.0)
No Cat. (N='55)' , %[a] (n/N)	5.5% (3.0/55.0)	24.0% (13.2/55.0)	**70.5%** **(38.8/55.0)**
68.7% balanced accuracy overall			

[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] EpiOcular™ EITL protocol for liquids.
Note 1: The performance is the same for the two versions of the DIP (Fig. 2.1 and Fig 2.2).

2.1.6. Predictive capacity of the DAL-1 PCP/SkinEthic/LLBO vs. the Draize Eye test

38. The predictive capacity of DAL-1 PCP/SkinEthic/LLBO is reported based on data generated by the Draize eye test (see **Table 2.2**) (see **Section 2.1** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation)*. Performance statistics are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 5** and **Annex B.2** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation for liquids (5).*

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Table 2.2. Performance of DAL-1 PCP/SkinEthic/LLBO in comparison to Draize Eye reference data

UN GHS	Prediction using DAL-1 PCP/SkinEthic/LLBO [b]		
	Cat 1	Cat 2	No Cat
Cat. 1 (N='17),' %[a] (n/N)	**76.5%** **(13.0/17.0)**	23.5% (4.0/17.0)	0.0% (0.0/17.0)
Cat. 2 (N='23),' %[a] (n/N)	30.4% (7.0/23.0)	**68.7%** **(15.8/23.0)**	0.9% (0.2/23.0)
No Cat. (N='46),' %[a] (n/N)	3.1% (1.4/46.0)	17.2% (7.9/46.0)	**79.7%** **(36.7/46.0)**
75.0% balanced accuracy overall			

[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] SkinEthic™ HCE EITL protocol for liquids
Note 1: The performance is the same for the two versions of the DIP (Fig. 2.1 and Fig 2.2).

2.1.7. Demonstration of Proficiency

39. The DAL-1 relies on a simple, rule-based data interpretation procedure and requires no expert judgment. Proficiency chemicals for the individual information sources are defined in the respective TGs (2, 3). Proficiency for the individual information sources demonstrates proficiency for the DA.

2.1.8. Reporting of the DA

40. The reporting of the DA application should include at a minimum the following elements:

- Test chemical identification (*e.g.,* chemical name, structural formula, composition, isomers, purity, chemical identity of impurities including their quantities as available, CAS number, batch and lot number, and other relevant identifiers).
- The DAL-1 option used, and the RhCE method used.
- Individual test reports performed per corresponding TGs (OECD TG 437, TG 492). Note that the chemical identity for each test report should match that above.
- Individual test reports on physicochemical properties corresponding with the information requested on data and reporting in each specific OECD GL (Annex E).
- Discussions on any uncertainties in the data with the *in vitro* methods and physicochemical properties applied in the DA that was used.
- Outcome of the DA application, including discussion of any uncertainties in the applied DA, as well as their predicted impact (e.g., over- or under-classification).
- Any deviation from or adaptation of the DA.
- Conclusion

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2.2. References

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

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3 PART II – Defined Approaches 2 (DAL-2), based on *in vitro* data, for non-surfactant[6] neat liquids, liquids and solids dissolved in water

41. Part II of this GL applies to DAL-2 STE/LLBO that is intended for hazard identification, *i.e.* distinguishing between serious eye damage and eye irritation potential of test chemicals (or the absence thereof), specifically for non-surfactant neat liquids, liquids and solids dissolved in water based on *in vitro* data. A summary of the DAL-2 for hazard identification is provided below; additional detailed information can be found in the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation.*

3.1. DAL-2

3.1.1. Summary

42. The DAL-2 STE/LLBO is intended for the identification of the eye irritation hazard of a test chemical without the use of animal testing, *i.e.* UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat. The data interpretation procedure (DIP) is not designed to provide information on sub-categorisation of Cat. 2 into 2A and 2B.

43. The DAL-2 STE/LLBO presented in this GL describes the combination of two *in vitro* test methods (STE: OECD TG 491 and BCOP LLBO: OECD TG 437) for the identification of the eye hazard potential of non-surfactant neat liquids, liquids and solids dissolved in water primarily for the purposes of classification and labelling without the use of animal testing (1, 2, 3).

44. The DAL-2 STE/LLBO was compared to 164 chemicals with curated Draize Eye test reference data and demonstrated a balanced accuracy of 74.3% (see **Table 3.1**).

3.1.2. Data interpretation procedure

45. The DIP applied uses the readout of the prediction models of each of the individual test methods as defined by the TGs (OECD 437, OECD 491) (1, 2). A scheme of DAL-2 STE/LLBO is presented in Figure 3.1. Scheme of the DAL-2 STE/LLBO option 1: start with

[6] Surfactant, also called surface-active agent, this is a substance, such as a detergent, that can reduce the surface tension of a water and thus allow it to foam or penetrate solids; it is also known as a wetting agent.

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the STE test method followed by the BCOP LLBO test method. The STE test method is used to identify liquid chemicals with no serious eye damage or eye irritation potential (No Cat.: liquids that result in a mean cell viability > 70% at a 5% and 0.05% concentration) or to identify liquids that cause serious eye damage/eye irritation (Cat. 1: liquids that result in a mean cell viability ≤ 70% at a 5% and 0.05% concentration). For liquids that result in a mean cell viability ≤ 70% at 5% concentration but > 70% at 0.05%, the BCOP LLBO is needed. Liquids that result in an opacity > 145 are predicted as Cat. 1 and the remaining liquids are assigned to Cat. 2. Note that it is also possible to start with the BCOP LLBO followed by the STE test method, this scheme of DAL-2 is presented in Figure 3.2.

Figure 3.1. Scheme of the DAL-2 STE/LLBO option 1: start with the STE test method followed by the BCOP LLBO test method

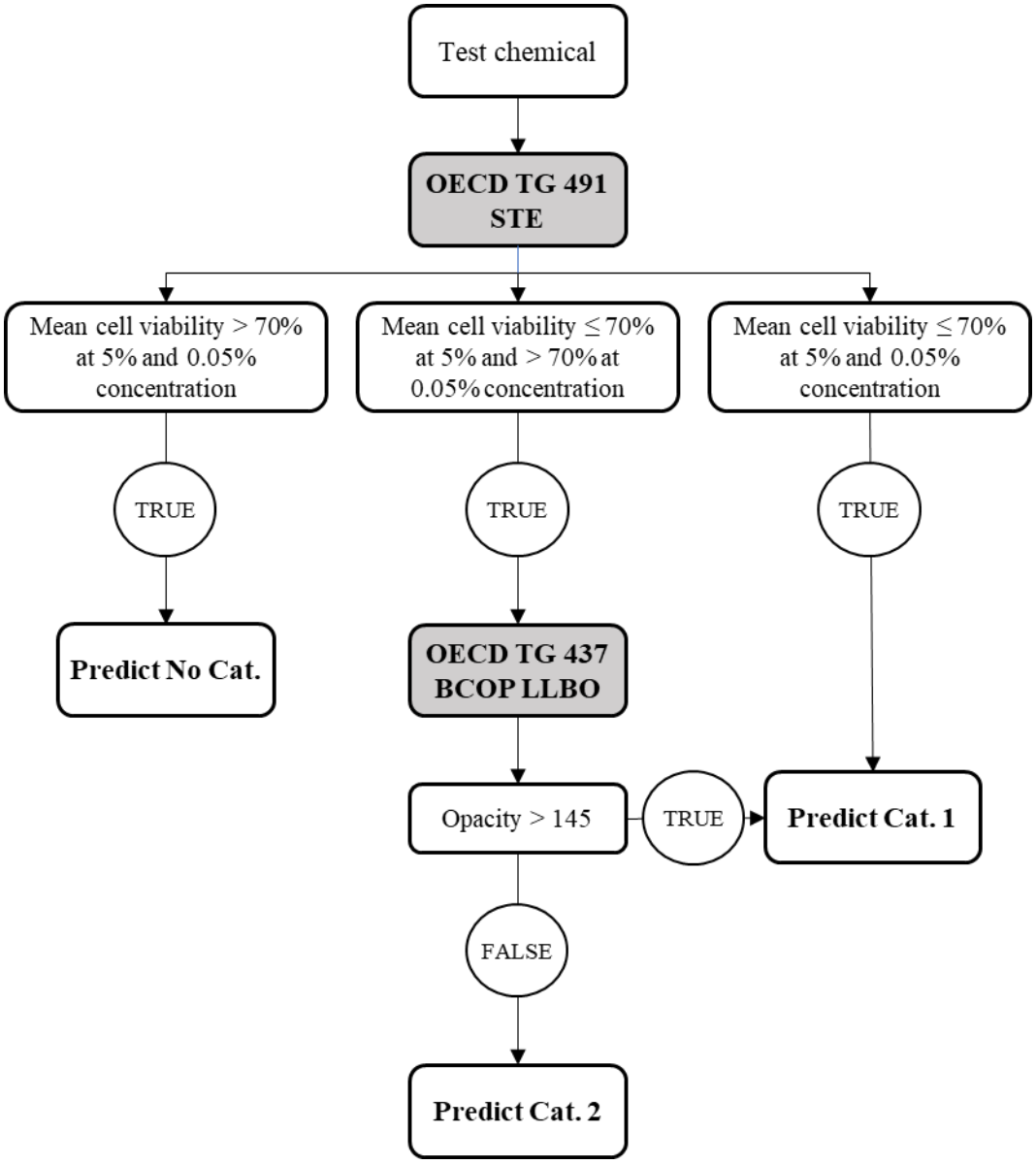

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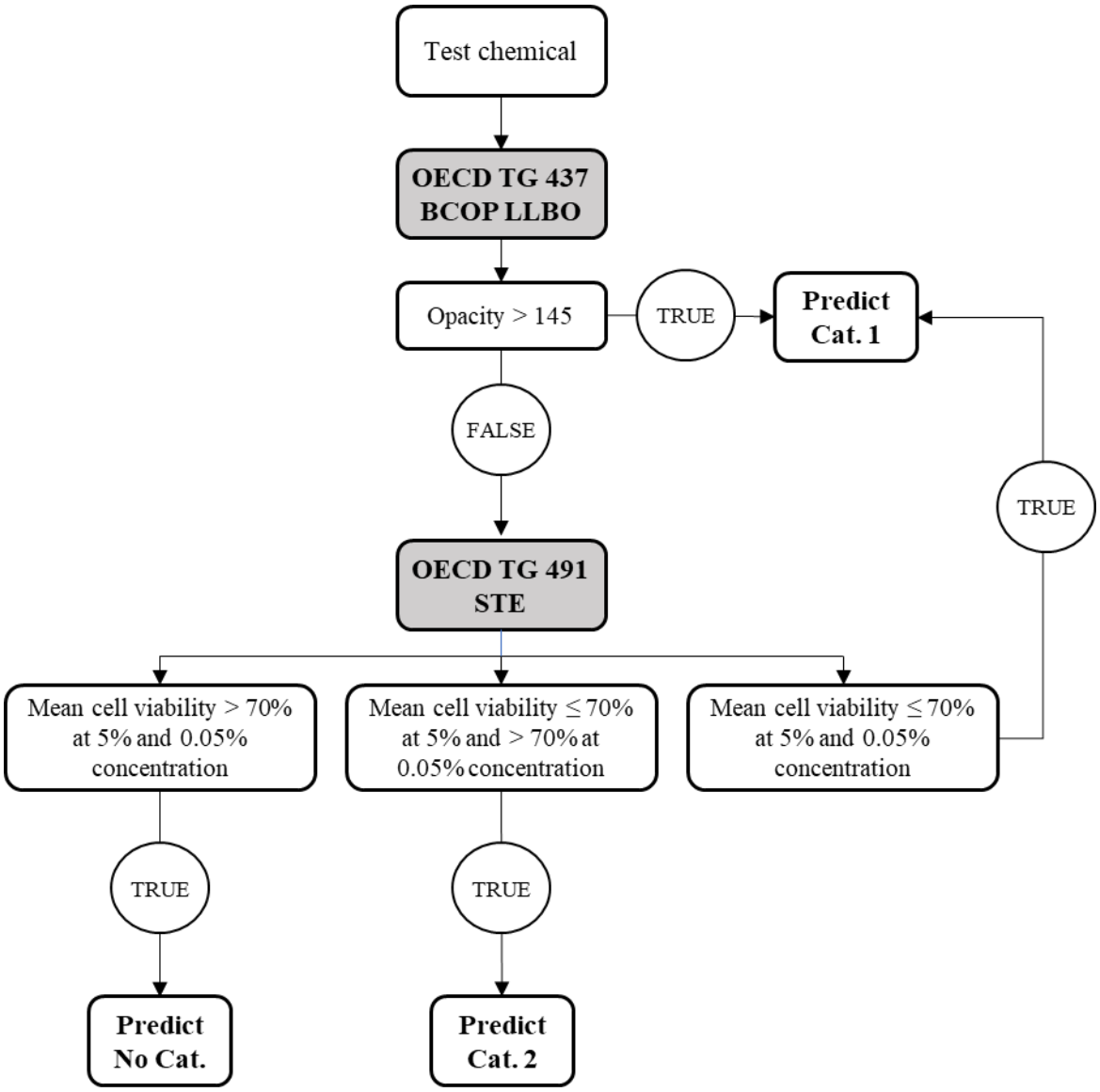
Figure 3.2. Scheme of the DAL-2 STE/LLBO option 2: start with the BCOP LLBO test method followed by the STE test method.



3.1.3. Description and limitations of the individual information sources

46. The individual information sources in the DA are test methods included in OECD TGs (OECD TG 437, 491) for serious eye damage/eye irritation or the absence thereof (1, 2), and the protocols are detailed therein.

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47. The following test methods from those TGs have been characterised and included in the DAL-2 STE/LLBO.

- BCOP LLBO test method: the eye hazard potential of a test chemical is measured by its ability to induce opacity and permeability in an isolated bovine cornea. Note that only opacity measurement is considered in the DAL-2. In case borderline results are obtained for opacity measurements, additional testing should be conducted, as specified in OECD TG 437 (1).
- STE test method: the eye hazard potential of a test chemical is assessed based on its ability to induce cytotoxicity on a confluent monolayer of Statens Seruminstitut Rabbit Cornea (SIRC) cells.

48. Any restrictions regarding the applicability domain identified in the respective TGs (TG 437, TG 491) are applicable to this GL (1, 2).

3.1.4. Procedures for dealing with borderline results in the test guidelines relevant to DAL-2 STE/LLBO predictions

49. The first decision on whether each information element can be used is dictated by the practical limitations as described in the TGs of the respective *in vitro* methods (TG 437, TG 491) (1, 2). Even for within-domain substances, test results are inherently subject to variation and these variations increase the uncertainty of a test result, especially when close to a (classification) cut-off threshold, i.e. in the borderline range. The following procedures to control the degree of uncertainty are described with the TG of the respective information sources.

- TG 437 (BCOP LLBO): UN GHS Cat. 1 prediction based on opacity (Lux/7, mean opacity > 145), but 1 of 3 corneas with opacity (Lux/7) < 130; in cases of borderline results in the first testing run, a second testing run should be considered, as well as a third one in case of discordant predictions between the first two testing runs.
- TG 491 (STE): Standard deviation of the final cell viability derived from three independent repetitions should be less than 15% for both 5% and 0.05% concentrations of the test chemical. If the standard deviation is greater than or equal to 15%, the results should not be used and three more repetitions should be performed.

3.1.5. Predictive capacity of the DAL-2 STE/LLBO vs. the Draize Eye test

50. The predictive capacity of DAL-2 STE/LLBO is reported based on data generated by the Draize eye test (see Table 3.1) (see **Section 2.1** and **Annex B.3** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation)*. Performance statistics are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 5** and **Annex B.2** of the *Supporting document to the GL on DAs for Serious Eye Damage/Eye Irritation.*

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Table 3.1. Performance of DAL-2 STE/LLBO in comparison to Draize Eye reference data

UN GHS	Prediction using DAL-2 STE/LLBO		
	Cat 1	Cat 2	No Cat
Cat. 1 (N=17), % [a] (n/N)	**81.2% (13.8/17.0)**	17.6% (3.0/17.0)	1.2% (0.2/17.0)
Cat. 2 (N=24), % [a] (n/N)	30.2% (7.2/24.0)	**56.3% (13.5/24.0)**	13.5% (3.2/24.0)
No Cat. (N=123), % [a] (n/N)	4.1% (5.1/123.0)	10.6% (13.0/123.0)	**85.3% (104.9/123.0)**
74.3% balanced accuracy overall			

[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.
Note 1: The performance was obtained using the version of the DIP provided in Fig 3.1.

3.1.6. Proficiency chemicals

51. The DAL-2 STE/LLBO relies on a simple, rule-based data interpretation procedure and requires no expert judgment. Proficiency chemicals for the individual information sources are defined in the respective TGs (1, 2). Proficiency for the individual information sources demonstrates proficiency for the DAL-2 STE/LLBO.

3.1.7. Reporting of the DA

52. The reporting of the DA application should include at a minimum the following elements:

- Test chemical identification (*e.g.,* chemical name, structural formula, composition, isomers, purity, chemical identity of impurities including their quantities as available, CAS number, batch and lot number, and other relevant identifiers).
- Describe the DAL-2 option used.
- Individual test reports performed per corresponding TGs (OECD TG 437, TG 491). Note that the chemical identity for each test report should match that above.
- Discussions on any uncertainties in the data with the *in vitro* methods applied in the DA that was used.
- Outcome of the DA application, including discussion of any uncertainties in the applied DA, as well as their predicted impact (e.g., over- or under-classification)
- Any deviation from or adaptation of the DA.
- Conclusion.

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3.2. References

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

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4 PART III – Defined Approaches (DAS), based on *in vitro* data, for neat solids[7]

54. Part III of this GL applies to DAS SkinEthic/LLBO that is intended for hazard identification, i.e., distinguishing between serious eye damage and eye irritation potential of test chemicals (or the absence thereof), specifically for neat solids based on *in vitro* data. A summary of the DAS for hazard identification is provided below; additional detailed information can be found in the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation (3)*.

4.1. DAS SkinEthic/LLBO

4.1.1. Summary

55. The DAS SkinEthic/LLBO is intended for the identification of the eye irritation hazard of a test chemical without the use of animal testing, i.e., UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat. The data interpretation procedure (DIP) is not designed to provide information on sub-categorisation of Cat. 2 into 2A and 2B.

56. The DAS SkinEthic/LLBO presented in this GL describes the combination of two *in vitro* test methods (SkinEthic™ HCE EITS: OECD TG 492 and BCOP LLBO: OECD TG 437) for the identification of the eye hazard potential of neat solids primarily for the purposes of classification and labelling without the use of animal testing (1, 2).

57. The DAS SkinEthic/LLBO was compared to 109 chemicals with curated Draize Eye test reference data and demonstrated a balanced accuracy of 66.7% (see Table 4.1. Performance of DAS SkinEthic/LLBO in comparison to Draize Eye reference data).

4.1.2. Data interpretation procedure

58. The DIP uses the readout of the prediction models of each of the individual test methods as defined by the TGs (OECD 437, OECD 492) (1, 2). A scheme of DAS SkinEthic/LLBO is presented in Figure 4.1. The SkinEthic™ HCE EITS test method is used to identify solid chemicals with no serious eye damage or eye irritation potential (No Cat.: solids that result in a mean tissue viability > 50%). For solids that result in a mean tissue viability ≤ 50%, the BCOP LLBO is needed. Solids that result in an opacity > 145 or OD > 2.5, or both opacity > 145 and OD > 2.5 are predicted as Cat. 1 and the remaining solids are assigned to Cat. 2.

[7] A solid is a non-pipettable test substance.

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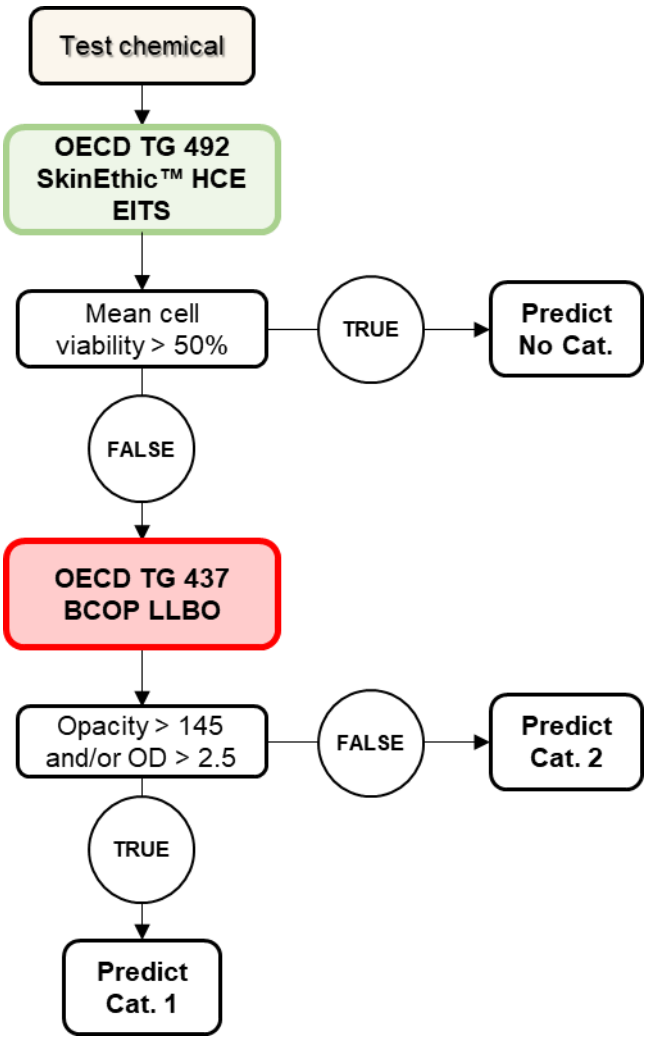
Figure 4.1. Scheme of the DAS SkinEthic/LLBO: start with the SkinEthic™ HCE EITS test method followed by the BCOP LLBO test



4.1.3. Description and limitations of the individual information sources

59. The individual information sources in the DA are test methods included in OECD TGs (OECD TG 492, 437) for serious eye damage/eye irritation or the absence thereof (1, 2), and the protocols are detailed therein.

60. The following test methods from those TGs have been characterised and included in the DAS SkinEthic/LLBO.

- SkinEthic™ HCE EITS test method: the method measures the ability to induce cytotoxicity. In case borderline results are obtained, additional testing should be conducted, as specified in OECD TG 492 (1).
- BCOP LLBO test method: the eye hazard potential of a test chemical is measured by its ability to induce opacity and permeability in an isolated bovine cornea. In case borderline results are obtained for opacity or permeability measurements, additional testing should be conducted, as specified in OECD TG 437 (2).

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61. Any restrictions regarding the applicability domain identified in the respective TGs (TG 437, TG 492) are applicable to this GL (1, 2).

4.1.4. Procedures for dealing with borderline results in the test guidelines relevant to DAS SkinEthic/LLBO predictions

62. The first decision on whether each information element can be used is dictated by the practical limitations as described in the TGs of the respective *in vitro* methods (TG 437, TG 492) (1, 2). Even for within-domain substances, test results are inherently subject to variation and these variations increase the uncertainty of a test result, especially when close to a (classification) cut-off threshold, i.e., in the borderline range. The following procedures to control the degree of uncertainty are described within the respective TG.

- TG 492 (SkinEthic™ HCE EITS): A single test composed of at least two tissue replicates should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 50±5% a second test should be considered, as well as a third one in case of discordant results between the first two tests.
- TG 437 (BCOP LLBO): UN GHS Cat. 1 prediction based on (i) opacity (Lux/7, mean opacity > 145), but 1 of 3 corneas with opacity (Lux/7) < 130 or (ii) OD (mean OD > 2.5), but 1 of 3 corneas with OD < 2.0; in cases of borderline results in the first testing run, a second testing run should be considered, as well as a third one in case of discordant predictions between the first two testing runs.

4.1.5. Predictive capacity of the DAS SkinEthic/LLBO vs. the Draize Eye test

63. The predictive capacity of DAS SkinEthic/LLBO is reported based on data generated by the Draize eye test (see Table 4.1. Performance of DAS SkinEthic/LLBO in comparison to Draize Eye reference data) (see **Section 5.1** and **Annex A.2** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation for neat solids (3))*. Performance statistics are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 7** and **Annex A.2** of the *Supporting document to the GL on DAs for Serious Eye Damage and Eye Irritation for neat solids (3).*

Table 4.1. Performance of DAS SkinEthic/LLBO in comparison to Draize Eye reference data

UN GHS	Prediction using DAS SkinEthic/LLBO		
	Cat 1	Cat 2	No Cat
Cat. 1 (N=31), % [a] (n/N)	**77.4%** **(24.0/31.0)**	22.6% (7.0/31.0)	0.0% (0.0/31.0)
Cat. 2 (N=18), % [a] (n/N)	29.5% (5.3/18.0)	**52.3%** **(9.4/18.0)**	18.2% (3.3/18.0)
No Cat. (N=60), % [a] (n/N)	1.7% (1.0/60.0)	28.3 (17.0/60.0)	**70.0%** **(42.0/60.0)**
66.7% balanced accuracy overall			

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[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.

4.1.6. Proficiency chemicals

64. The DAS SkinEthic/LLBO relies on a simple, rule-based data interpretation procedure and requires no expert judgment. Proficiency chemicals for the individual information sources are defined in the respective TGs (1, 2). Proficiency for the individual information sources demonstrates proficiency for the DAS.

4.1.7. Reporting of the DA

65. The reporting of the DA application should include at a minimum the following elements:

- Test chemical identification (*e.g.,* chemical name, structural formula, composition, isomers, purity, chemical identity of impurities including their quantities as available, CAS number, batch and lot number, and other relevant identifiers).
- Individual test reports performed per corresponding TGs (OECD TG 437, TG 492). Note that the chemical identity for each test report should match that above.
- Discussions on any uncertainties in the data with the *in vitro* methods applied in the DA that was used.
- Outcome of the DA application, including discussion of any uncertainties in the applied DA, as well as their predicted impact (e.g., over- or under-classification).
- Any deviation from or adaptation of the DA.
- Conclusion.

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4.2. References

(1) OECD (2023), OECD Guidelines for the Testing of Chemicals 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.

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

(3) OECD (2024), Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage and Eye Irritation(Second Edition). Series on Testing and Assessment No. 354. Organisation for Economic Cooperation and Development, Paris. Available at: [https://www.oecd.org/chemicalsafety/testing /series-testing-assessment-publications-number.htm]

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5 PART IV – Defined Approach (DA), based on in vitro data, for surfactants (DASF)

67. Part IV of this GL applies to DASF that is intended for hazard identification, *i.e.* distinguishing between serious eye damage and eye irritation potential of test chemicals (or the absence thereof), for surfactants[8] (neat and in dilution in deionized water) based on *in vitro* data. A summary of the DASF for hazard identification is provided below; additional detailed information can be found in the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation.*

5.1. DASF

5.1.1. Summary

68. The DASF, which was develop specifically for surfactants, is intended for the identification of the eye irritation hazard of a test chemical without the use of animal testing, *i.e.* UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat. The data interpretation procedure (DIP) is not designed to provide information on sub-categorisation of Cat. 2 into 2A and 2B. DASF fills an important gap in this Test Guideline, however, due to the currently small dataset for Cat.2 (n=10), if a surfactant is predicted as Cat.2, additional information may be required, taking into account that the prospective use of animals is only to be used as a last resort.

69. The DASF presented in this GL describes the combination of two *in vitro* test methods (EpiOcular™ Eye Irritation Test (EIT) or SkinEthic™ HCE EIT: OECD TG 492 and $STE^{0.5}$ for surfactants, as part of OECD TG 491) for the identification of the eye hazard potential of surfactants (liquids and solids) primarily for the purposes of classification and labelling without the use of animal testing (1, 2).

70. The performance of the DASF was compared to 50 surfactants (45 liquids and 5 solids) with curated Draize eye test reference data and demonstrated a balanced accuracy of 79.1% (see Table 5.1. Performance of DASF in comparison to Draize Eye reference data). No *in vivo* data on neat Cat. 2 surfactants were found to assess the performance of the DASF, therefore when the DASF predicts a Cat.2 for a neat surfactant, additional information may be required,-taking into account that the prospective use of animals is only

[8] 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.

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to be used as a last resort. Note that caution should be exercised when interpreting the DASF results for anionic surfactants as this class has a tendency to overpredict.

5.1.2. Data interpretation procedure

71. The DIP applied uses the readout of the prediction models of each of the individual test methods as defined by the OECD TG 492 and $STE^{0.5}$ for surfactants (1, 2). A schematic representation of DASF is provided in Figure 5.1. 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.

(bottom-up approach) and Figure 5.2. 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.

72. (top-down approach). Depending on the available data, the most relevant approach will be selected: if the test substance is most likely classified as UN GHS Cat. 1, or if there is high certainty that no classification is needed (UN GHS No Cat), either a Top-Down or Bottom-Up approach will be considered, respectively (3). The EpiOcular™ EIT or SkinEthic™ HCE EIT test method are used to identify surfactants with no serious eye damage or eye irritation potential. Surfactants predicted as No Cat. are those that result in a mean tissue viability > 60% for EpiOcular™ EIT (liquid or solid) or > 60% (liquid) or > 50% (solid) for SkinEthic™ HCE EIT, respectively. For surfactants that result in a mean tissue viability below the threshold value, the $STE^{0.5}$ for surfactants is needed. Surfactants (liquid or solid) that result in a mean cell viability ≤ 20% at a 0.5% test concentration are predicted as Cat. 1 and the remaining surfactants are assigned to Cat. 2.

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Figure 5.1. 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.

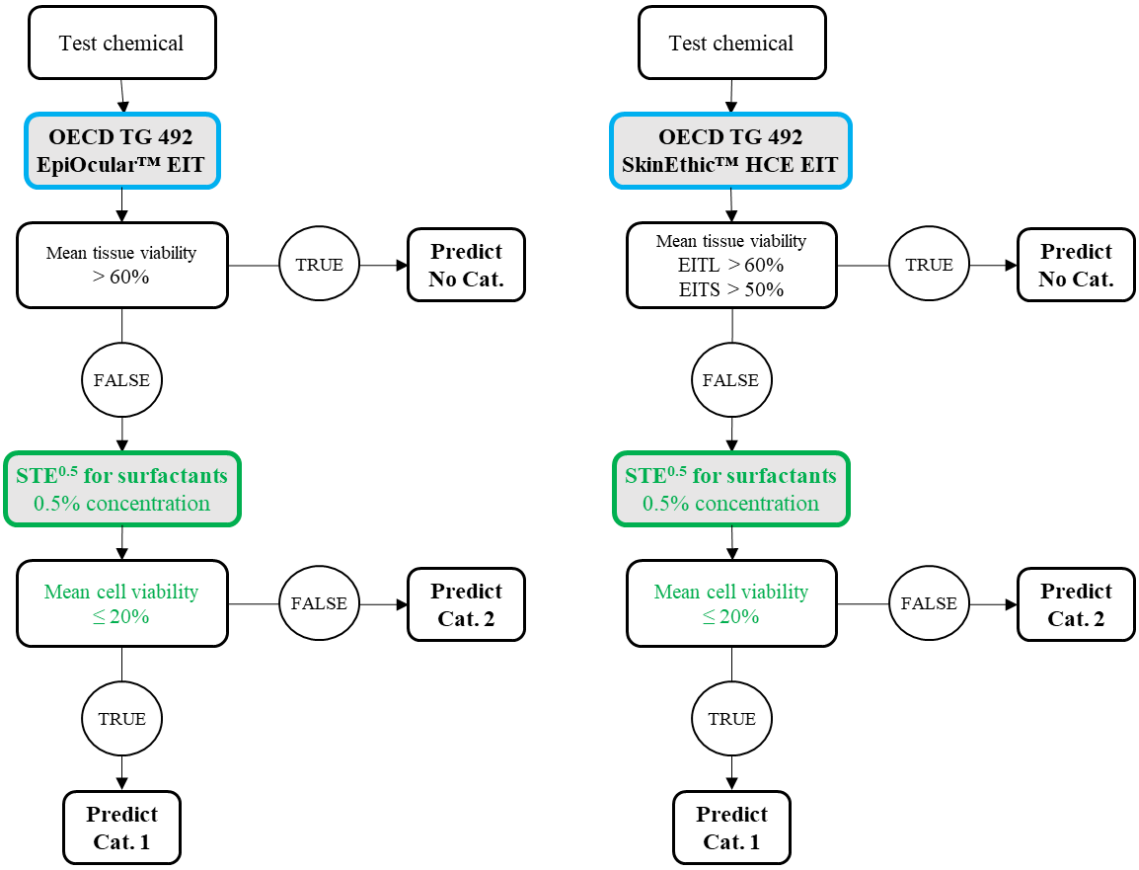

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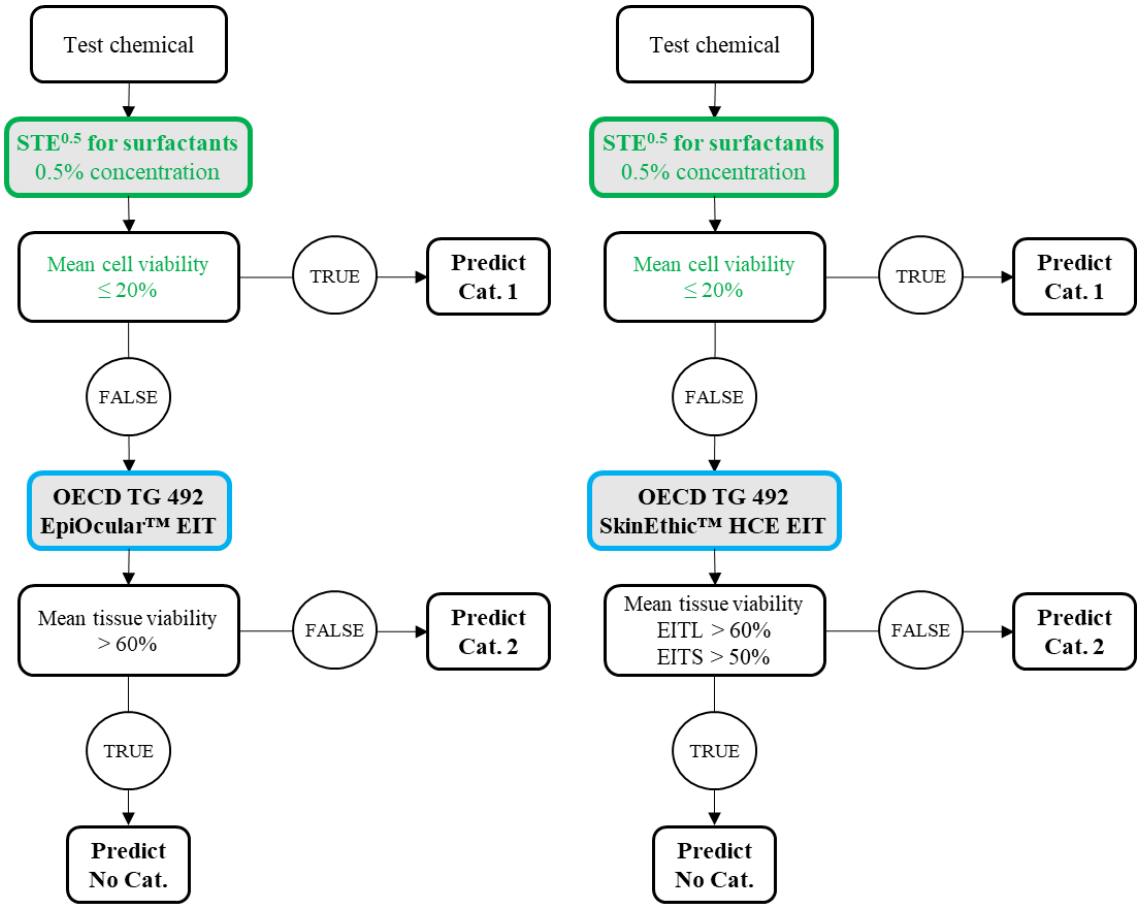
Figure 5.2. Top-down approach of the DASF; step 1 STE$^{0.5}$ for surfactants used to identify Cat. 1, and step 2 EpiOcular™ EITL / EITS or SkinEthic™ HCE EITL / EITS test method used to identify No Cat.



5.1.3. Description and limitations of the individual information sources

73. The individual information sources in the DA are test methods included in OECD TGs (OECD TG 492, 491) for serious eye damage/eye irritation or the absence thereof (1, 4), and the protocols are detailed therein.

74. The following test methods from those TGs have been characterised and included in the DASF.

- The RhCE (EpiOcular™ EIT and SkinEthic™ HCE EIT) test methods: the methods measures the ability to induce cytotoxicity. In case borderline results are obtained, additional testing should be conducted, as specified in OECD TG 492 (1).
- STE test method: the eye hazard potential of a test chemical is assessed based on its ability to induce cytotoxicity on a confluent monolayer of Statens Seruminstitut Rabbit Cornea (SIRC) cells. The STE$^{0.5}$ for surfactants differs from the procedure described in OECD TG 491 in that cell viability in SIRC 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 (2, 4), instead of measuring cell viability after a 5

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minutes exposure to a 5% and 0.05% concentration and using a cut-off value of 70% cell viability for classification (4).

75. Any restrictions regarding the applicability domain identified in the respective TGs (TG 492, TG 491) are applicable to this GL (1, 4) as well.

76. No *in vivo* data was found to assess the performance of DASF for neat Cat. 2 surfactants. When the DASF predicts a Cat.2 for a neat surfactant, additional information may be required, taking into account that the prospective use of animals is only to be used as a last resort. Furthermore, caution should be exercised when interpreting the DASF results for anionic surfactants as this class has a tendency to overpredict (see **Section 7.4** and **Table 7-10** of the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation)*.

5.1.4. Procedures for dealing with borderline results in the test guidelines relevant to DASF prediction

77. The first decision on whether each information element can be used is dictated by the practical limitations as described in the TGs of the respective *in vitro* methods (TG 492, TG 491) (1, 4). Even for within-domain substances, test results are inherently subject to variation and these variations increase the uncertainty of a test result, especially when close to a (classification) cut-off threshold, i.e. in the borderline range. The following procedures to control the degree of uncertainty are described with the TG of the respective information sources.

- TG 492 (EpiOcular™ EIT): A single test composed of at least two tissue replicates should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 60±5% (EITL and EITS) a second test should be considered, as well as a third one in case of discordant results between the first two tests.
- TG 492 (SkinEthic™ HCE EIT): A single test composed of at least two tissue replicates should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent tissue viability equal to 60±5% (EITL) or to 50±5% (EITS) a second test should be considered, as well as a third one in case of discordant results between the first two tests.
- TG 491 ($STE^{0.5}$ for surfactants): A single test composed of at least three replicate wells should be sufficient for a test chemical when the result is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean percent viability equal to 20±5%, a second test should be considered, as well as a third one in case of discordant results between the first two tests.

5.1.5. Predictive capacity of the DASF vs. the Draize Eye test

78. The predictive capacity of DASF is reported based on data generated by the Draize eye test (see Table 5.1. Performance of DASF in comparison to Draize Eye reference data) (see **Section 2.1** and **Annex B.2** of the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation)*. Performance statistics

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are reported for weighted predictions as compared to Draize eye test reference data. DA predictions for specific chemicals and further details are available in **Section 5** and **Annex A.B** of the *Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Serious Eye Damage/Eye Irritation.*

Table 5.1. Performance of DASF in comparison to Draize Eye reference data

UN GHS	Prediction using DASF		
	Cat 1	Cat 2	No Cat
Cat. 1 (N=23), % [a] (n/N)	**91.3% (21.0/23.0)**	8.7% (2.0/23.0)	0.0% (0.0/23.0)
Cat. 2 (N=10), % [a] (n/N)	30.0% (3.0/10.0)	**70.0% (7.0/10.0)**	0.0% (0.0/10.0)
No Cat. (N=17), % [a] (n/N)	5.9% (1.0/17.0)	18.1 (3.1/17.0)	**76.0% (12.9/17.0)**
79.1% balanced accuracy overall			

[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 (see Annex D of OECD SD 354). 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.
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, taking into account that the prospective use of animals is only to be used as a last resort.. Furthermore, caution should be exercised when interpreting the DASF results for anionic surfactants as this class has a tendency to overpredict.

5.1.6. Demonstration of Proficiency

79. The DASF relies on a simple, rule-based data interpretation procedure and requires no expert judgement. Proficiency chemicals for the individual information sources are defined in the respective TGs (1,4). Proficiency for the individual information sources demonstrates proficiency for the DAs (e.g., para 39 for DAL-1).

5.1.7. Reporting of the DA

80. The reporting of the DA application should include at a minimum the following elements:

- Test chemical identification (*e.g.* chemical name, structural formula, composition, isomers, purity, chemical identity of impurities including their quantities as available, CAS number, batch and lot number, and other relevant identifiers)
- Individual test reports performed per corresponding TGs (OECD TG 492, TG 491), including the elements described therein. Note that the chemical identity for each test report should match that above.
- Discussions on any uncertainties in the data on *in vitro* methods applied in the DA used.
- Outcome of the DA application, including discussion of any uncertainties in the applied DA, as well as their predicted impact (e.g., over- or under-classification)

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- Any deviation from or adaptation of the DA
- Conclusion

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5.2. References

1. OECD (2019), OECD Guidelines for the Testing of Chemicals 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.
2. 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
3. OECD (2023), OECD Guidelines for the Testing of Chemicals 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.
4. Alépée N, Mewes KR, Abo T, Cavarzan A, O'Driscoll A, Adriaens E (2024). Performance of the DASF compared to other combinations of OECD NAMs for eye hazard identification of surfactants. ALTEX. 2024 Aug 28. doi: 10.14573/altex.2406031. Epub ahead of print. PMID: 39228327.

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