Committee of Experts on the Transport of Dangerous Goods and on the Globally Harmonized System of Classification and Labelling of Chemicals

Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals **16 November 2020**

Thirty-ninth session
Geneva, 8-10 July 2020
Item 3 (e) of the provisional agenda
Classification criteria and related hazard communication: use of non-animal testing methods for classification of health hazards

Use of non-animal testing methods for classification of health hazards: Status report

Transmitted by the experts from the United Kingdom and the Netherlands on behalf of the informal working group

Introduction

1. This informal document provides an update on the work performed by the Informal Working Group on "Use of non-animal testing methods for classification of health hazards" since the last update provided to the Sub-Committee in July 2020 (see informal document INF.12 (39th session)).

Background

2. The previous status update report in informal document INF.12 (39th session) provides:

(a) References to associated papers regarding the informal working groups:

(i) programme of work for the 2019-2020 biennium (see ST/SG/AC.10/C.4/72 and informal document INF.27/Rev.1 (36th session));

(ii) terms of reference (see ST/SG/AC.10/C.4/62 and informal document INF.27/Rev.2 (31th session)); and

(b) details of the progress of the work undertaken by the informal working group this biennium up until July 2020, including a summary of the main issues that had arisen during the groups consideration of Chapter 3.3 to integrate non animal test methods.

Status report

3. Since the last update to the GHS Sub-Committee in July 2020, the informal working group has continued to work hard and have undertaken detailed discussions on each successive version of the draft revised Chapter 3.3 at seven webinar meetings (16 June 2020; 14 and 22 July 2020; 12 August 2020; 2 September 2020; 6 October 2020; and 11 November 2020), with a further webinar planned in early December 2020. After each meeting the Netherlands and the United Kingdom, the joint leads, with the assistance of the Joint research Centre (JRC) have revised the draft text of Chapter 3.3, drafted detailed records of the meeting discussions and prepared papers on specific topics to take forward the discussions, taking into account written comments and information on specific topics provided by the participants.

4. The working draft (version 9) that was considered by the working group in October 2020 is provided in Annex I. Comments received from members during the November 2020 meeting have not yet been incorporated into this draft. New text relative to the 8th revised edition of the GHS is shown in blue; text on which there is on-going discussions is shown in red; for clarity deleted text is not shown. This is still a work in progress and the wording of some sections has not yet been finally discussed by the informal working group. This working draft is presented so the Sub-Committee can see what has been achieved so far, and steer the working group as it considers appropriate, in particular with a view to discussing whether and, if so, how the revised Chapter 3.3 should be processed further to achieve adoption by the Sub-Committee.

5. To provide the Sub-Committee with an indication of the nature of the work that has been undertaken, the issues that have been identified, and the progress that has been made since July 2020, paragraphs 6 to 14 below summarise the main issues that have arisen in the working groups consideration of Chapter 3.3.

Application of the pH rule within the GHS

6. As outlined in the July 2020 status report for the informal working group, the discussion on classification using the pH-rule, with or without acid/alkali reserve, has continued in this biennium.

7. Good progress was made and agreement was able to be reached in relation to the application of the pH rule to the classification of substances and mixtures. Conforming changes will be made in chapter 3.2.

Classification of mixtures

8. To assist the discussions on mixtures, the expert from Germany produced two thought starter papers to aid the discussions on the application of the pH rule, bridging principles and the weight of evidence to enable the classification of mixtures using these types of approaches.

9. Although members of the working group supported further exploration of the issue, there was concern that there might be implications for a number of other chapters within the GHS. The group considered that further discussion on this issue was beyond the mandate of the informal working group.

10. The issue was then taken outside the informal working group and was discussed separately on the 13 October 2020 at a meeting chaired by the German expert who has since indicated that they would submit a proposal for the consideration of the Sub-Committee at the December 2020 session on potential ways forward to address this issue next biennium.[1]

Technical and editorial corrections or editorial improvements

11. On a number of occasions during the discussions on the integration of non-animal test methods into Chapter 3.3, it has been raised that the chapter contains some technical or editorial errors that should be corrected, or that other editorial improvements to the text should be made to improve the readability of the chapter. These improvements were often supported by the informal working group from a technical perspective.

12. However, several questions were raised regarding whether the group could propose such changes as these amendments/corrections are not specifically mentioned under the current terms of reference.

13. The working groups project leads produced a thought starter on this issue, outlining the various types of errors and improvements, together with examples, to aid the discussion.

[1] **Note by the secretariat :** See informal document INF.23.

The group subsequently considered that it would be useful to identify technical errors and/or editorial improvements during the review of chapters that are not related to non-animal criteria and send them to the appropriate workgroup (e.g. PCI) for implementation or present them in a working paper directly to the Sub-Committee.

14. Consequently, the informal working group propose to amend their current terms of reference as detailed above and in informal document INF.26 that has been submitted for the consideration of the Sub-Committee at the thirty-ninth session.

On-going work

15. The informal working group will continue its work on the revision of Chapter 3.3 during its next webinar meeting in early December 2020 followed by further webinar meetings in early 2021. There is tentative hope that it will be possible to finalise the revision of Chapter 3.3 in time for adoption by the Sub-Committee early in the next biennium.

16. The Sub-Committee is invited to note the progress of the revision of Chapter 3.3 (a snapshot is provided in Annex I of this document) and the issues outlined in this informal document.

Annex

Working draft of Chapter 3.3 (Version 9; 21 October 2020)

Black text is from current GHS Chapter 3.3.
Blue text is new in this chapter.
Red text requires further discussion.

"CHAPTER 3.3
SERIOUS EYE DAMAGE/EYE IRRITATION

3.3.1 Definitions and general considerations

3.3.1.1 *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.

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.

3.3.1.2 To classify, all available and relevant information on serious eye damage/eye irritation is collected and its quality in terms of adequacy and reliability is assessed. Classification should be based on mutually acceptable data generated using methods/approaches that are validated according to international procedures, such as OECD Guidelines or equivalent methods/approaches (see 1.3.2.4.3). Sections 3.3.2.1 to 3.3.2.8 provide classification criteria for the different types of information that may be available.

3.3.1.3 A tiered approach (see 3.3.2.10) organises the available information into levels/tiers and provides for decision-making in a structured and sequential manner. Classification results directly when the information consistently satisfies the criteria. However, where the available information gives inconsistent and/or conflicting results within a tier, classification of a substance or a mixture is made on the basis of the weight of evidence within that tier. In some cases when information from different tiers gives inconsistent and/or conflicting results (see 3.3.2.10.3) or where data individually are insufficient to conclude on the classification, an overall weight of evidence ~~approach~~ assessment is used (see 1.3.2.4.9, 3.3.2.9 and 3.3.5.3.1).

3.3.1.4 Guidance on the interpretation of criteria and references to relevant guidance documents are provided in 3.3.5.3.

3.3.2 Classification criteria for substances

Substances are allocated to one of the categories within this hazard class, Category 1 (serious eye damage) or Category 2 (eye irritation), as follows:

(a) Category 1 (serious eye damage/irreversible effects on the eye):

substances that have the potential to seriously damage the eyes.

(b) Category 2 (eye irritation/reversible effects on the eye):

substances that have the potential to induce reversible eye irritation.

Those authorities desiring one category for classification of "eye irritation" may use the overall Category 2; others may want to distinguish between Category 2A and Category 2B.

3.3.2.1 *Classification based on human data (Tier 1 in Figure 3.3.1)*

Existing reliable and good quality human data on serious eye damage/eye irritation should be given high weight where relevant for classification (see 3.3.5.3.2) and should be the first line of evaluation, as this gives information directly relevant to effects on the eye. Existing human data could be derived from single or repeated exposure(s), for example in occupational, consumer, transport or emergency response scenarios and epidemiological and clinical studies

in well-documented case reports and observations (see 1.1.2.5 (c), 1.3.2.4.7 and 1.3.2.4.9). Although human data from accident or poison centre databases can provide evidence for classification, absence of incidents is not itself evidence for no classification, as exposures are generally unknown or uncertain.

3.3.2.2 ***Classification based on standard animal data (Tier 1 in Figure 3.3.1)***

OECD Test Guideline 405 is the currently available and internationally accepted animal test method for classification as serious eye damaging or eye irritant (see Tables 3.3.1 and 3.3.2, respectively) and is the standard animal test. The current version of OECD Test Guideline 405 uses a maximum of 3 animals. Results from animal studies conducted under previous versions of OECD Test Guideline 405 that used more than 3 animals are also considered standard animal tests when interpreted in accordance with 3.3.5.3.3.

3.3.2.2.1 *Serious eye damage (Category 1)/irreversible effects on the eye*

A single hazard category (Category 1) is adopted for substances that have the potential to seriously damage the eyes. This hazard category includes as criteria the observations listed in Table 3.3.1. These observations include animals with grade 4 cornea lesions and other severe reactions (e.g. destruction of cornea) observed at any time during the test, as well as persistent corneal opacity, discoloration of the cornea by a dye substance, adhesion, pannus, and interference with the function of the iris or other effects that impair sight. In this context, persistent lesions are considered those which are not fully reversible within an observation period of normally 21 days. Hazard classification as Category 1 also contains substances fulfilling the criteria of corneal opacity ≥ 3 or iritis > 1.5 observed in at least 2 of 3 tested animals, because severe lesions like these usually do not reverse within a 21 days observation period.

Table 3.3.1: Serious eye damage/Irreversible effects on the eye category[a, b]

	Criteria
Category 1: **Serious eye damage/Irreversible effects on the eye**	A substance that produces: (a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or (b) in at least 2 of 3 tested animals, a positive response of: (i) corneal opacity ≥ 3; and/or (ii) iritis > 1.5; calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material.

[a] *Grading criteria are understood as described in OECD Test Guideline 405.*

[b] *Evaluation of a 4, 5 or 6-animal study should follow the criteria given in 3.3.5.3.3.*

3.3.2.2.2 *Eye irritation (Category 2)/Reversible effects on the eye*

3.3.2.2.2.1 Substances that have the potential to induce reversible eye irritation should be classified in Category 2 where further categorisation into Category 2A and Category 2B is not required by a competent authority or where data are not sufficient for further categorisation. When a substance is classified as Category 2, without further categorisation, the classification criteria are the same as those for Category 2A.

3.3.2.2.2.2 For those authorities wanting more than one designation for reversible eye irritation, Categories 2A and 2B are provided:

(a) When data are sufficient and where required by a competent authority substances may be classified in Category 2A or 2B in accordance with the criteria in Table 3.3.2;

(b) For substances inducing eye irritant effects reversing within an observation time of normally 21 days, Category 2A applies. For substances inducing eye irritant effects reversing within an observation time of 7 days, Category 2B applies.

3.3.2.2.2.3 For those substances where there is pronounced variability among animal responses, this information may be taken into account in determining the classification.

Table 3.3.2: Reversible effects on the eye categories[a, b]

	Criteria
	Substances that have the potential to induce reversible eye irritation
Category 2/2A	Substances that produce in at least 2 of 3 tested animals a positive response of: (a) corneal opacity ≥ 1; and/or (b) iritis ≥ 1; and/or (c) conjunctival redness ≥ 2; and/or (d) conjunctival oedema (chemosis) ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material, and which fully reverses within an observation period of normally 21 days.
Category 2B	Within Category 2A an eye irritant is considered mildly irritating to eyes (Category 2B) when the effects listed above are fully reversible within 7 days of observation.

[a] *Grading criteria are understood as described in OECD Test Guideline 405.*

[b] *Evaluation of a 4, 5 or 6-animal study should follow the criteria given in 3.3.5.3.3.*

3.3.2.3 *Classification based on Defined Approaches[2] (Tier 2 in Figure 3.3.1)*

Defined Approaches consist of a rule-based combination of data obtained from a predefined set of different information sources (e.g. *in vitro* methods, *ex vivo* methods, physico-chemical properties, non-test methods). It is recognised that most single *in vitro/ex vivo* methods are not able to replace *in vivo* methods fully for most regulatory endpoints. Thus, Defined Approaches can be useful strategies of combining data for classifying substances and mixtures. Results obtained with a Defined Approach that is validated according to international procedures, such as an OECD Defined Approach Guideline or an equivalent approach, is conclusive for classification for serious eye damage/eye irritation if the criteria of the Defined Approach are fulfilled (see 3.3.5.3.4)[3]. Data from a Defined Approach can only be used for classification when the tested substance is within the applicability domain of the Defined Approach used. Additional limitations described in the published literature should also be taken into consideration.

3.3.2.4 *Classification based on in vitro/ex vivo data (Tier 2 in Figure 3.3.1)*

3.3.2.4.1 The classification criteria for the currently available *in vitro/ex vivo* test methods adopted by the OECD in Test Guidelines 437, 438, 460, 491, 492, 494 and 496 are described in Table 3.3.6 (see 3.3.5.3.5.1). When considered individually, these *in vitro/ex vivo* OECD Test Guidelines address serious eye damage and/or no classification for eye hazard, but do not address eye irritation. Therefore, data from a single *in vitro/ex vivo* OECD Test Guideline can only be used to conclude on either classification in Category 1 or no classification, according to the criteria defined in Table 3.3.6, and cannot be used to conclude on classification in Category 2. When the result of a single *in vitro/ex vivo* method is "no stand-alone prediction can be made" (e.g. see Table 3.3.6), a classification in Category 1, Category 2 or for no classification cannot be excluded on the basis of that single result and further data are necessary for classification (see 3.3.5.3.4.3 and 3.3.5.3.4.4). ~~If no adequate Defined Approach is available (see 3.3.2.3) nor other *in vitro/ex vivo* data to allow a stand-alone prediction (see 3.3.2.4.2), the classification should be based, where possible, on a within-tier weight of evidence assessment (see Figure 3.3.2 and paragraph 3.3.5.3.4.4).~~

3.3.2.4.2 Other validated *in vitro/ex vivo* test methods accepted by some competent authorities are described in 3.3.5.3.5.2. Some of these *in vitro/ex vivo* test methods may be useful to classify in Category 2. A competent authority

[2] *According to the OECD, and as defined in Guidance Document No. 255 on the reporting of Defined Approaches to be used within Integrated Approaches to Testing and Assessment, a Defined Approach to testing and assessment consists of a fixed data interpretation procedure (DIP) applied to data generated with a defined set of information sources to derive a result that can either be used on its own, or together with other information sources within an overall weight of evidence ~~approach~~assessment, to satisfy a specific regulatory need.*

[3] *Some Defined Approaches have been proposed for serious eye damage/eye irritation (Alépée et al., 2019a,b) but no classification criteria have yet been agreed internationally.*

may decide which classification criteria, if any, should be applied for these test methods to conclude on classification, including ~~or~~ that a substance is not classified for effects on the eye.

3.3.2.4.3 *In vitro/ex vivo* data can only be used for classification when the tested substance is within the applicability domain of the test method(s) used. Additional limitations described in the published literature should also be taken into consideration.

3.3.2.4.4 *Serious eye damage (Category 1)/irreversible effects on the eye*

3.3.2.4.4.1 Where tests have been undertaken in accordance with OECD Test Guidelines 437, 438, 460, 491 and/or 496 a substance is classified for serious eye damage in Category 1 based on the criteria in Table 3.3.6 (see 3.3.5.3.5.1).

3.3.2.4.4.2 Although the currently available OECD *in vitro/ex vivo* Test Guidelines and equivalent methods have not been developed to identify substances inducing discolouration of the eye, some comparable effects may be observed in these tests. Therefore, where, after washing, discolouration of the cornea or of the tested cells compared to the control is observed in OECD Test Guidelines 437, 438, 492 or 494, or in other equivalent methods, suggesting a permanent effect, a competent authority may require classification of a substance for serious eye damage in Category 1.

3.3.2.4.5 *Eye irritation (Category 2)/Reversible effects on the eye*

3.3.2.4.5.1 A positive result in an *in vitro/ex vivo* test method that is validated according to international procedures for identification of substances inducing eye irritation can be used to classify for eye irritation in Category 2/2A[4].

3.3.2.4.5.2 Where competent authorities adopt Categories 2A and 2B, it is important to note that the currently validated *in vitro/ex vivo* test methods for effects on the eye do not allow discrimination between these two categories. In this situation, if the criteria for classification in Category 2 have been considered fulfilled, and no other relevant information is available, classification in Category 2/2A should be applied.

3.3.2.4.6 *No classification for effects on the eye*

OECD Test Guidelines 437, 438, 491, 492 and 496 (see Table 3.3.6) can be used to conclude that a substance is not classified for effects on the eye.

3.3.2.5 *Classification based on conclusive human data; standard animal data; or in vitro/ex vivo data for skin corrosion (Tier 3 in Figure 3.3.1)*

Substances classified as corrosive to skin (Skin Category 1) based on conclusive human data, standard animal data or *in vitro/ex vivo* data for skin corrosion according to the criteria in Chapter 3.2 are also deemed as inducing serious eye damage (Eye Category 1). Skin irritation (Skin Category 2), mild skin irritation (Skin Category 3) and no classification for skin irritation, as well as human patch data (as described in Chapter 3.2), cannot be used alone to conclude on eye irritation or no classification for effects on the eye, but may be considered in an overall weight of evidence assessment.

3.3.2.6 *Classification based on other existing skin or eye animal data (Tier 4 in Figure 3.3.1)*

Other existing skin or eye data in animals may be used for classification, but there may be limitations regarding the conclusions that can be drawn (see 3.3.5.3.6).

3.3.2.7 *Classification based on ~~chemical properties~~extreme pH (pH ≤ 2 or ≥ 11.5) and acid/alkaline reserve (Tier 5 in Figure 3.3.1)*

Eye effects may be indicated by pH extremes such as ≤ 2 or ≥ 11.5 especially when associated with significant acid/alkaline reserve. Generally, such substances are expected to produce significant effects on the eyes. A substance is considered to cause serious eye damage (Category 1) in this Tier if it has an extreme pH (pH ≤ 2 or ≥ 11.5)

[4] *Although no classification criteria have yet been agreed internationally for some validated and/or accepted in vitro/ex vivo test methods proposed for identifying substances inducing eye irritation, these test methods may still be accepted by some competent authorities (see 3.3.2.4.2). If a Defined Approach (see 3.3.2.3) is not available or is not adequate for classification, data from these methods may be considered in a weight of evidence assessment within this tier.*

with significant acid/alkaline reserve or no data for acid/alkaline reserve. However, if consideration of acid/alkaline reserve suggests the substance may not cause serious eye damage despite the extreme pH value, the result is considered inconclusive within this Tier (see Figure 3.3.1). A pH > 2 and < 11.5 is considered inconclusive and cannot be used for classification purposes. Acid/alkaline reserve and pH can be determined by different methods including those described in OECD Test Guideline 122 and Young et al. (1988), acknowledging that there are some differences between these methods (see 3.3.5.3.7). A competent authority may decide which criteria for significant acid/alkaline reserve should be applied.

3.3.2.8 *Classification based on non-test methods for serious eye damage/eye irritation or for skin corrosion (Tier 6 in Figure 3.3.1)*

3.3.2.8.1 Classification, including the conclusion not classified for effects on the eye, can be based on non-test methods for serious eye damage/eye irritation, with due consideration of reliability and applicability, on a case-by-case basis. Such methods include computer models predicting qualitative structure-activity relationships (structural alerts, SAR) or quantitative structure-activity relationships (QSARs), computer expert systems, and read-across using analogue and category approaches.

3.3.2.8.2 Read-across using analogue or category approaches requires sufficiently reliable test data on similar substance(s) and justification of the similarity of the tested substance(s) with the substance(s) to be classified. Where adequate justification of the read-across approach is provided, it has in general higher weight than (Q)SARs.

3.3.2.8.3 Classification based on (Q)SARs requires sufficient data and validation of the model. The validity of the computer models and the prediction should be assessed using internationally recognised principles for the validation of (Q)SARs. With respect to reliability, lack of alerts in a SAR or expert system is not sufficient evidence for no classification.

3.3.2.8.4 Conclusive non-test data for skin corrosion may be used for classification for effects on the eye. Thus, substances classified as corrosive to skin (Skin Category 1) according to the criteria in Chapter 3.2 are also deemed ~~to also be classified~~ as inducing serious eye damage (Eye Category 1). Skin irritation (Skin Category 2), mild skin irritation (Skin Category 3) and no classification for skin irritation according to Chapter 3.2 cannot be used alone to conclude eye irritation or no classification for effects on the eye, but may be considered in an overall weight of evidence ~~approach~~assessment.

3.3.2.9 *Classification based on an overall weight of evidence assessment (Tier 7 in Figure 3.3.1)*

3.3.2.9.1 An overall weight of evidence assessment is indicated where none of the previous tiers resulted in a definitive conclusion on classification for serious eye damage or eye irritation or on no classification. In some cases, where the classification decision was postponed until the overall weight of evidence, but no further data are available, a classification may still be possible.

3.3.2.9.2 If a substance with an extreme pH (pH ≤ 2 or ≥ 11.5) and non-significant acid/alkaline reserve is identified and considered inconclusive in Tier 5 (see 3.3.2.7), but no other information is available, the substance should be classified as Eye Category 1 in this Tier. If inconclusive information is available from other Tiers and the overall weight of evidence assessment determination remains inconclusive, a substance with an extreme pH (pH ≤ 2 or ≥ 11.5) and non-significant acid/alkaline reserve should still be classified as Eye Category 1 in this Tier. In both situations, mixtures with an extreme pH (pH ≤ 2 or ≥ 11.5) and a non-significant acid/alkaline reserve should be assessed using the bridging principles described in 3.3.3.2 (see 3.3.3.1.3).

3.3.2.9.3 If a skin corrosion Category 1 classification is communicated via an SDS, by a registrant or as part of a regulatory classification list, but the underlying data that led to this classification are not known, the skin corrosion Category 1 classification may be considered within this Tier. Where no other data relevant for serious eye damage/eye irritation are available, classification for Eye Category 1 may be indicated.

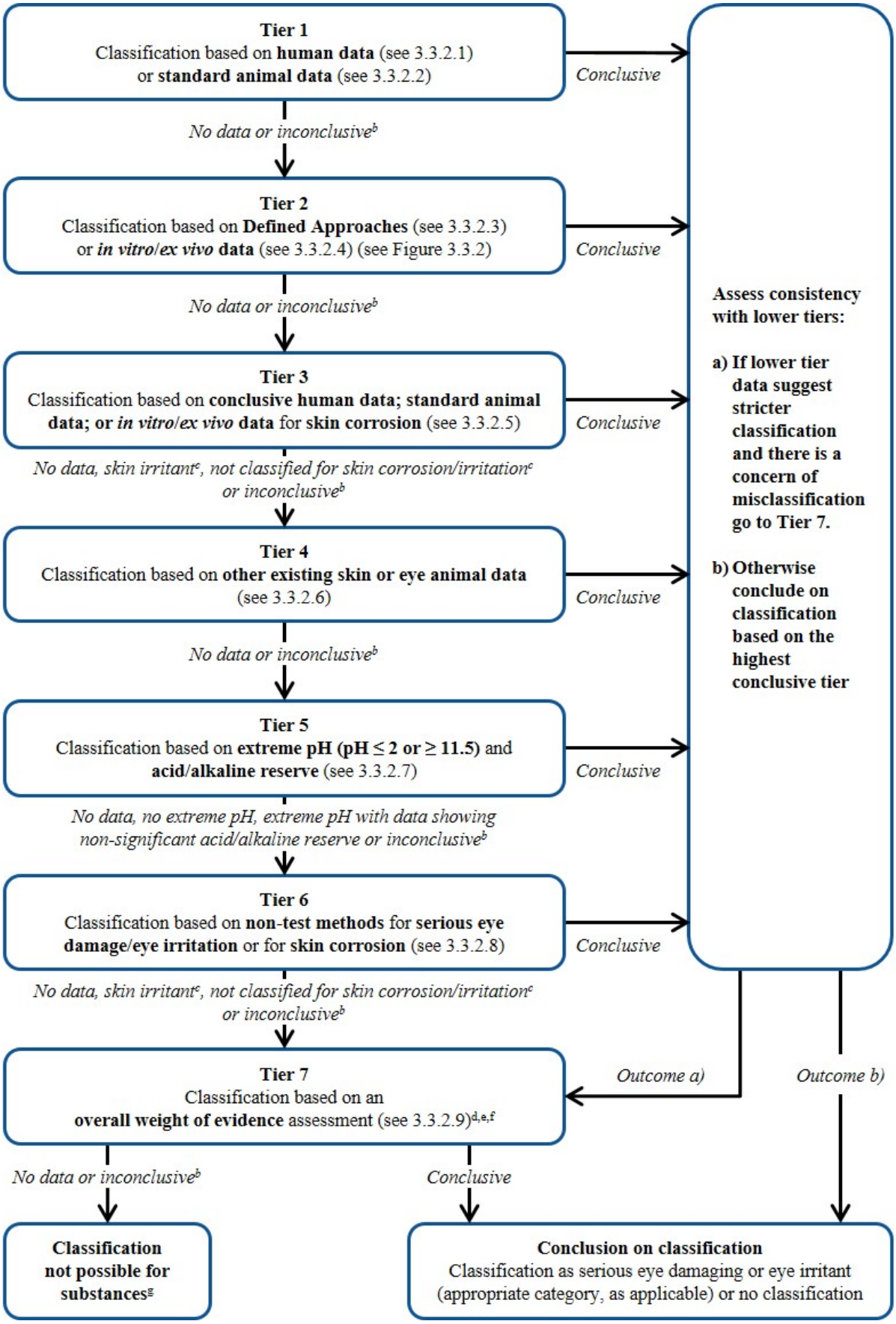
3.3.2.10 *Classification in a tiered approach (Figure 3.3.1)*

3.3.2.10.1 A tiered approach to the evaluation of initial information should be considered where applicable (Figure 3.3.1), recognising that not all elements may be relevant. However, all available and relevant information of sufficient quality needs to be examined for consistency with respect to the resulting classification.

3.3.2.10.2 In the tiered approach (Figure 3.3.1), existing human and animal data for eye effects form the highest tier, followed by Defined Approaches and *in vitro/ex vivo* data for eye effects, ~~and then~~ existing human/standard animal/*in vitro*/*ex vivo* data for skin corrosion, ~~followed by~~ other existing animal test data for eye, and then~~reafter~~ other sources of information. Where information from data within the same tier is inconsistent and/or conflicting, the conclusion from that tier is determined by a weight of evidence ~~approach~~assessment.

3.3.2.10.3 Where information from several tiers is inconsistent and/or conflicting with respect to the resulting classification, information of sufficient quality from a higher tier is generally given a higher weight than information from a lower tier. However, when information from a lower tier would result in a stricter classification than information from a higher tier and there is concern for misclassification, then classification is determined by an overall weight of evidence ~~approac~~hassessment. For example, having consulted the guidance in 3.3.5.3 as appropriate, classifiers concerned with a negative result for serious eye damage in an *in vitro/ex vivo* study when there is a positive result for serious eye damage in other existing eye data in animals would utilise an overall weight of evidence ~~approach~~assessment. The same would apply in the case where there is human data indicating irritation but positive results from an *in vitro/ex vivo* test for serious eye damage.

Figure 3.3.1: Application of the tiered approach for serious eye damage/eye irritation[a]



[a] *Before applying the approach, the explanatory text in 3.3.2.10 as well as the guidance in 3.3.5.3 should be consulted. Only adequate and reliable data of sufficient quality should be included in applying the tiered approach.*

[b] *Information may be inconclusive for various reasons, e.g.:*

- *The available data may be of insufficient quality, or otherwise insufficient/inadequate for the purpose of classification, e.g. due to quality issues related to experimental design and/or reporting.*
- *The available data may be insufficient to conclude on the classification, e.g. they might be indicative for absence of serious eye damage, but inadequate to demonstrate eye irritation.*
- *Where competent authorities make use of the eye irritation Categories 2A and 2B, the available data may not be capable of distinguishing between Category 2A and Category 2B.*

[c] *It is recognised that not all skin irritants are eye irritants and that not all substances that are non-irritant to skin are non-irritant to the eye~~. Therefore, skin irritation (Category 2), mild skin irritation (Category 3) and no classification for skin irritation, as well as human patch data (as described in Chapter 3.2), cannot be used alone to conclude eye irritation or no classification for effects on the eye, but may be considered in an overall weight of evidence assessment.~~ Expert judgment should be exercised prior to making such determinations (see 3.3.2.5 and 3.3.2.8.4).*

[d] *Where no other information is available, substances with an extreme pH (pH ≤ 2 or ≥ 11.5) and non-significant acid/alkaline reserve should be classified as Eye Category 1 in the overall weight of evidence assessment (i.e. in Tier 7). If inconclusive information is available from other Tiers and the overall weight of evidence assessment determination remains inconclusive, a substance with an extreme pH (pH ≤ 2 or ≥ 11.5) and non-significant acid/alkaline reserve should still be classified as Eye Category 1 in Tier 7 (see 3.3.2.9.2).*

[e] *Where no other information on the mixture itself is available, mixtures with an extreme pH (pH ≤ 2 or ≥ 11.5) and non-significant acid/alkaline reserve should be assessed using the bridging principles described in 3.3.3.2. If inconclusive information is available from other Tiers on the mixture itself and the overall weight of evidence assessment determination remains inconclusive, mixtures with an extreme pH (pH ≤ 2 or ≥ 11.5) and non-significant acid/alkaline reserve should also be assessed using the bridging principles described in 3.3.3.2 (see 3.3.3.1.3).*

[f] *If a skin corrosion Category 1 classification is communicated via an SDS, by a registrant or as part of a regulatory classification list, but the underlying data that led to this classification are not known, the skin corrosion Category 1 classification may be considered in the overall weight of evidence assessment (i.e. in Tier 7) (see 3.3.2.9.3). Where no other data relevant for serious eye damage/eye irritation are available, classification for Eye Category 1 may be indicated.*

[g] *For mixtures, decision logic 3.3.2 for classification based on similar tested mixtures and/or ingredients should be applied.*

3.3.3 Classification criteria for mixtures

3.3.3.1 *Classification of mixtures when data are available for the complete mixture*

3.3.3.1.1 In general, the mixture should be classified using the criteria for substances, and taking into account the tiered approach to evaluate data for this hazard class (as illustrated in Figure 3.3.1) and 3.3.3.1.2 and 3.3.3.1.3 below. If classification is not possible using the tiered approach, then the approach described in 3.3.3.2 (bridging principles), or, if that is not applicable, 3.3.3.3 (calculation method) should be followed.

3.3.3.1.2 Defined Approaches and/or *in vitro/ex vivo* test methods validated according to international procedures may not have been validated using mixtures; although these approaches/methods are considered broadly applicable to mixtures, they can only be used for classification of mixtures when all ingredients of the mixture fall within the applicability domain of the Defined Approach or test method(s) used. Specific limitations regarding applicability domains are described in the respective Defined Approaches and test methods, and should be taken into consideration as well as any further information on such limitations from the published literature. Where there is reason to assume or evidence indicating that the applicability domain of a particular Defined Approach or test method is limited, data interpretation should be exercised with caution, or the results should be considered not applicable.

3.3.3.1.3 A mixture is considered to cause serious eye damage (~~Eye~~ Category 1) in Tier 5 if it has an extreme pH (pH ≤ 2 or ≥ 11.5) with significant acid/alkaline reserve or no data for acid/alkaline reserve. However, if consideration of acid/alkaline reserve suggests the mixture may not cause serious eye damage despite the extreme pH value, the result is considered inconclusive within Tier 5 (see Figure 3.3.1). Where no other other information on the mixture itself is available, mixtures with an extreme pH (pH ≤ 2 or ≥ 11.5) and non-significant acid/alkaline reserve should be assessed using the bridging principles described in 3.3.3.2. If inconclusive information is available from other

Tiers on the mixture itself and the overall weight of evidence assessment determination remains inconclusive, mixtures with an extreme pH (pH ≤ 2 or ≥ 11.5) and non-significant acid/alkaline reserve should also be assessed using the bridging principles described in 3.3.3.2. In both situations, when the bridging principles are not applicable, the mixture should be classified as Eye Category 1. A pH > 2 and < 11.5 is considered inconclusive and cannot be used for classification purposes. Acid/alkaline reserve and pH can be determined by different methods including those described in OECD Test Guideline 122 and Young et al. (1988), acknowledging that there are some differences between these methods (see 3.3.5.3.7). A competent authority may decide which criteria for significant acid/alkaline reserve should be applied.

3.3.3.2 ***Classification of mixtures when data are not available for the complete mixture: bridging principles***

3.3.3.2.1 Where the mixture itself has ~~not been tested to determine its skin corrosivity or~~no conclusive information to decide on its potential to cause serious eye damage or eye irritation, but there are sufficient data on both the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data will be used in accordance with the following agreed bridging principles. This ensures that the classification process uses the available data to the greatest extent possible in characterising the hazards of the mixture without the necessity for additional testing in animals.

3.3.3.2.2 *Dilution*

If a tested mixture is diluted with a diluent which has an equivalent or lower classification for serious eye damage/eye irritation than the least seriously eye damaging/eye irritant original ingredient and which is not expected to affect the serious eye damage /eye irritancy of other ingredients, then the new diluted mixture may be classified as equivalent to the original tested mixture. Alternatively, the method explained in 3.3.3.3 could be applied.

3.3.3.2.3 *Batching*

The serious eye damage/eye irritation potential of a tested production batch of a mixture can be assumed to be substantially equivalent to that of another untested production batch of the same commercial product when produced by or under the control of the same manufacturer, unless there is reason to believe there is significant variation such that the serious eye damage/eye irritation potential of the untested batch has changed. If the latter occurs, a new classification is necessary.

3.3.3.2.4 *Concentration of mixtures of the highest serious eye damage/eye irritation category*

If a tested mixture classified for serious eye damage (Category 1) is concentrated, the more concentrated untested mixture should be classified for serious eye damage (Category 1) without additional testing. If a tested mixture classified for eye irritation (Category 2 or 2A) is concentrated and does not contain serious eye damage ingredients, the more concentrated untested mixture should be classified in the same category (Category 2 or 2A) without additional testing.

3.3.3.2.5 *Interpolation within one hazard category*

For three mixtures (A, B and C) with identical ingredients, where mixtures A and B have been tested and are in the same serious eye damage/eye irritation hazard category, and where untested mixture C has the same toxicologically active ingredients as mixtures A and B but has concentrations of toxicologically active ingredients intermediate to the concentrations in mixtures A and B, then mixture C is assumed to be in the same serious eye damage/eye irritation category as A and B.

3.3.3.2.6 *Substantially similar mixtures*

Given the following:

(a) Two mixtures: (i) A + B
(ii) C + B;

(b) The concentration of ingredient B is essentially the same in both mixtures;

(c) The concentration of ingredient A in mixture (i) equals that of ingredient C in mixture (ii);

(d) Data on serious eye damage/eye irritation for A and C are available and substantially equivalent, i.e. they are in the same hazard category and are not expected to affect the serious eye damage/eye irritation potential of B.

If mixture (i) or (ii) is already classified by testing, the other mixture can be assigned in the same hazard category.

3.3.3.2.7 *Aerosols*

An aerosol form of a mixture may be classified in the same hazard category as the tested non-aerosolised form of mixture provided that the added propellant does not affect the serious eye damage/eye irritation properties of the mixture upon spraying[5].

3.3.3.3 ***Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture: calculation method***

3.3.3.3.1 In order to make use of all available data for purposes of classifying the serious eye damage/eye irritation properties of the mixtures, the following assumption has been made and is applied where appropriate in the tiered approach to mixtures (see 1.3.2.3 and 1.3.3):

The "relevant ingredients" of a mixture are those which are present in concentrations ≥ 1% (w/w for solids, liquids, dusts, mists and vapours and v/v for gases), unless there is a presumption (e.g. in the case of corrosive ingredients) that an ingredient present at a concentration < 1% can still be relevant for classifying the mixture for serious eye damage/eye irritation.

3.3.3.3.2 In general, the approach to classification of mixtures as seriously damaging to the eye or eye irritant when data are available on the ingredients, but not on the mixture as a whole, is based on the theory of additivity, such that each corrosive or serious eye damaging/eye irritant ingredient contributes to the overall serious eye damage/eye irritation properties of the mixture in proportion to its potency and concentration. A weighting factor of 10 is used for corrosive and serious eye damaging ingredients when they are present at a concentration below the concentration limit for classification with Category 1, but are at a concentration that will contribute to the classification of the mixture as serious eye damaging/eye irritant. The mixture is classified as seriously damaging to the eye or eye irritant when the sum of the concentrations of such ingredients exceeds a threshold cut-off value/concentration limit.

3.3.3.3.3 Table 3.3.3 provides the cut-off value/concentration limits to be used to determine if the mixture should be classified as seriously damaging to the eye or an eye irritant.

3.3.3.3.4 Particular care must be taken when classifying mixtures containing certain types of ~~chemicals~~substances such as acids and bases, inorganic salts, aldehydes, phenols, and surfactants. The approach explained in 3.3.3.3.1 and 3.3.3.3.2 might not work given that many such substances are seriously damaging to the eye/eye irritating at concentrations < 1%. For mixtures containing strong acids or bases, the pH should be used as classification criterion (see 3.3.3.1.~~3~~2) since pH will be a better indicator of serious eye damage ~~(subject to consideration of acid/alkaline reserve)~~ than the concentration limits in Table 3.3.3. ~~Where no other information than pH value and acid/alkaline reserve is available, mixtures with a pH ≤ 2 or a pH ≥ 11.5 and non-significant acid/alkaline reserve should be classified as Category 1 (see Decision logic 3.3.2 in paragraph 3.3.5.2).~~A mixture containing corrosive or serious eye damaging/eye irritating ingredients that cannot be classified based on the additivity approach applied in Table 3.3.3 due to chemical characteristics that make this approach unworkable, should be classified as Eye Category 1 if it contains ≥ 1% of a corrosive or serious eye damaging ingredient and as Eye Category 2 when it contains ≥ 3% of an eye irritant ingredient. Classification of mixtures with ingredients for which the approach in Table 3.3.3 does not apply is summarised in Table 3.3.4.

3.3.3.3.5 On occasion, reliable data may show that the irreversible/reversible eye effects of an ingredient will not be evident when present at a level above the generic cut-off values/concentration limits mentioned in Tables 3.3.3

[5] *Bridging principles apply for the intrinsic hazard classification of aerosols, however, the need to evaluate the potential for "mechanical" eye damage from the physical force of the spray is recognised.*

and 3.3.4. In these cases the mixture could be classified according to those data (see also 1.3.3.2 *"Use of cut-off values/Concentration limits"*). On occasion, when it is expected that the skin corrosion/irritation or the irreversible/reversible eye effects of an ingredient will not be evident when present at a level above the generic concentration/cut-off levels mentioned in Tables 3.3.3 and 3.3.4, testing of the mixture may be considered. Where new testing data become available~~In those cases~~, the tiered weight of evidence approach should be applied as referred to in section 3.3.3, Figure 3.3.1 and explained in detail in this chapter.

3.3.3.3.6 If there are data showing that (an) ingredient(s) may be corrosive to the skin or seriously damaging to the eye/eye irritating at a concentration of < 1% (corrosive to the skin or seriously damaging to the eye) or < 3% (eye irritant), the mixture should be classified accordingly (see also 1.3.3.2 *"Use of cut-off values/concentration limits"*).

Table 3.3.3: Concentration of ingredients of a mixture classified as skin Category 1 and/or eye Category 1 or 2 that would trigger classification of the mixture as hazardous to the eye (Category 1 or 2)

Sum of ingredients classified as	Concentration triggering classification of a mixture as	
	Serious eye damage	Eye irritation
	Category 1	Category 2/2A
Skin Category 1 + Eye Category 1[a]	≥ 3%	≥ 1% but < 3%
Eye Category 2		≥ 10% [b]
10 × (skin Category 1 + eye Category 1)[a] + eye Category 2		≥ 10%

[a] *If an ingredient is classified as both skin Category 1 and eye Category 1 its concentration is considered only once in the calculation.*

[b] *A mixture may be classified as eye Category 2B when all relevant ingredients are classified as eye Category 2B.*

Table 3.3.4: Concentration of ingredients of a mixture when the additivity approach does not apply, that would trigger classification of the mixture as hazardous to the eye

Ingredient	Concentration	Mixture classified as: Eye
Acid with pH ≤ 2	≥ 1%	Category 1
Base with pH ≥ 11.5	≥ 1%	Category 1
Other corrosive (eye Category 1) ingredient	≥ 1%	Category 1
Other eye irritant (eye Category 2) ingredient	≥ 3%	Category 2

3.3.4 Hazard communication

General and specific considerations concerning labelling requirements are provided in *Hazard communication: Labelling* (Chapter 1.4). Annex 1 contains summary tables about classification and labelling. Annex 3 contains examples of precautionary statements and pictograms which can be used where allowed by the competent authority.

Table 3.3.5: Label elements for serious eye damage/eye irritation [a]

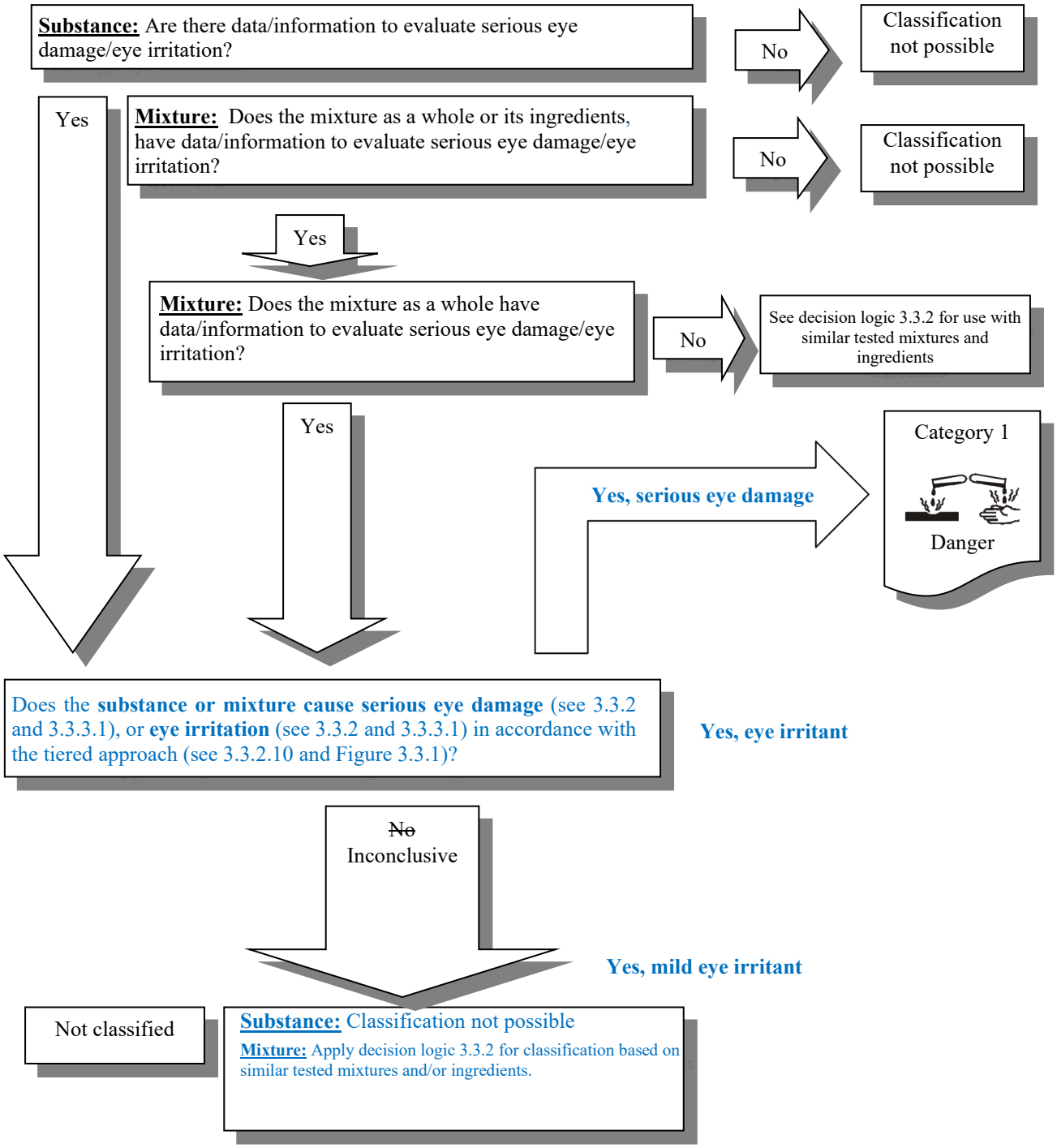
	Category 1	Category 2A	Category 2B
Symbol	Corrosion	Exclamation mark	*No symbol*
Signal word	Danger	Warning	Warning
Hazard statement	Causes serious eye damage	Causes serious eye irritation	Causes eye irritation

[a] *Where a chemical is classified as skin Category 1, labelling for serious eye damage/eye irritation may be omitted as this information is already included in the hazard statement for skin Category 1 (Causes severe skin burns and eye damage) (see Chapter 1.4, para. 1.4.10.5.3.3).*

3.3.5 Decision logics and guidance

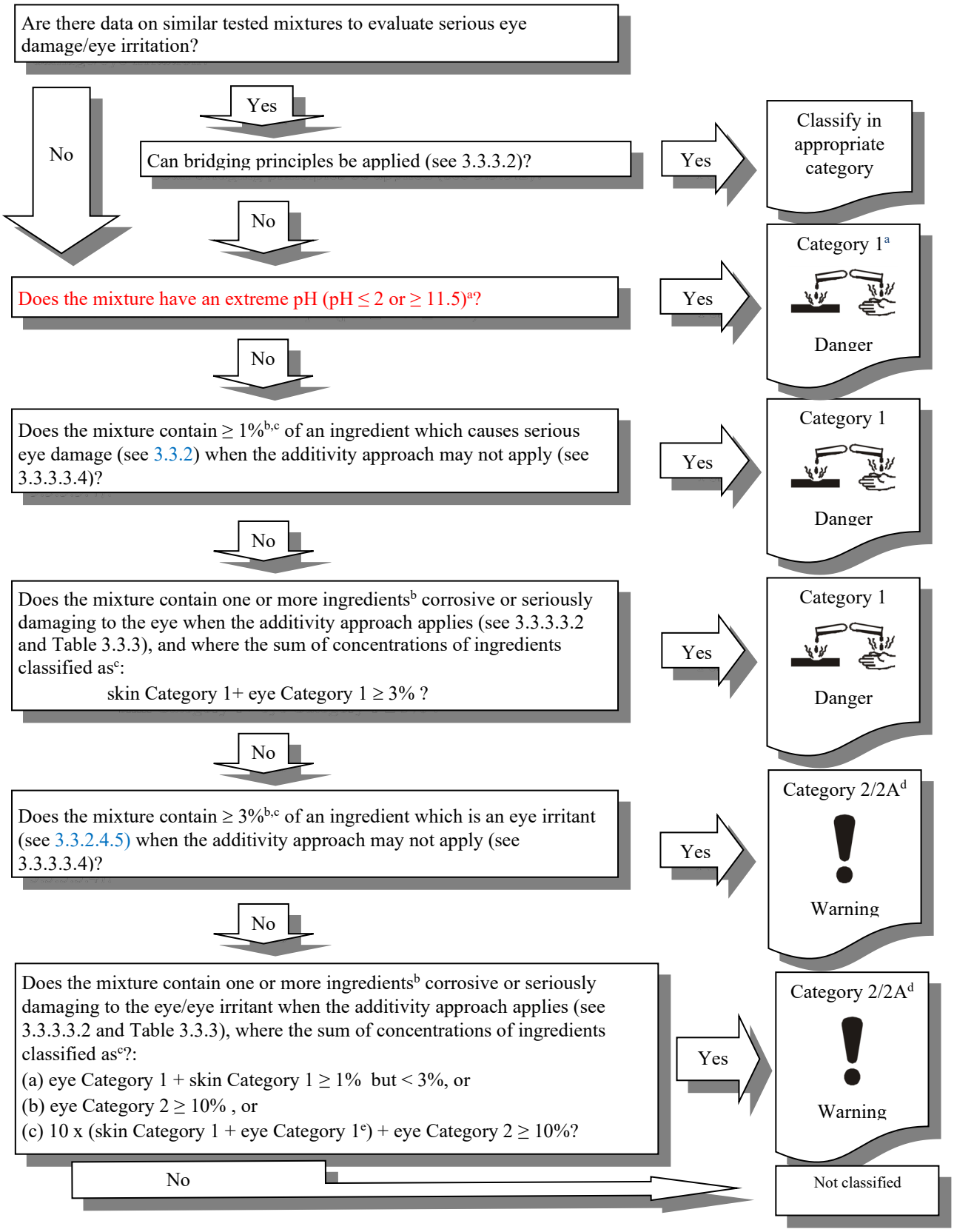
The decision logics which follow are not part of the harmonised classification system but are provided here as additional guidance. It is strongly recommended that the person responsible for classification study the criteria before and during use of the decision logics.

3.3.5.1 *Decision logic 3.3.1 for serious eye damage/eye irritation*



3.3.5.2 ***Decision logic 3.3.2 for serious eye damage/eye irritation***

Classification of mixtures on the basis of information/data on similar tested mixtures and ingredients



[a] *Category 1 applies at this stage of the tiered approach to mixtures independently of the acid/alkaline reserve of the mixture (see 3.3.3.1.3).*

[b] *Where relevant < 1%, see 3.3.3.3.1.*

[c] *For specific concentration limits, see 3.3.3.3.5 and 3.3.3.3.6. See also Chapter 1.3, para. 1.3.3.2 "Use of cut-off values/concentration limits".*

[d] *A mixture may be classified as eye Category 2B in case all relevant ingredients are classified as eye Category 2B.*

[e] *If an ingredient is classified as both skin Category 1 and eye Category 1 its concentration is considered only once in the calculation.*

3.3.5.3 *Background guidance*

3.3.5.3.1 *Relevant guidance documents*

Helpful information on the strengths and weaknesses of the different test and non-test methods, as well as useful guidance on how to apply a weight of evidence ~~approach~~assessment, is provided in OECD Guidance Document 263, an Integrated Approach on Testing and Assessment (IATA) for serious eye damage and eye irritation.

3.3.5.3.2 *Guidance on the use of human data for classification as Serious Eye Damage/ Eye Irritation*

The availability of human data for serious eye damage/eye irritation is limited and the data available may contain some uncertainty. However, where such data exist, they should be considered based on their quality. Human data may be obtained from epidemiological studies, human experience (e.g. consumer experience), poison control centres, national and international home accident surveillance programs, case studies, or worker experience and accidents. Human case studies may have limited predictive value as often the presence of a substance or mixture in the eye will result in pain and quickly washing of the eyes. Therefore, the effects observed may underestimate the intrinsic property of the substance or the mixture to affect the eye without washing.

3.3.5.3.3 *Classification based on standard animal tests with more than 3 animals*

3.3.5.3.3.1 Classification criteria for the skin and eye hazard classes are detailed in the GHS in terms of a 3-animal test. It has been identified that some older test methods may have used up to 6 animals. However, the GHS criteria do not specify how to classify based on existing data from tests with more than 3 animals. Guidance on how to classify based on existing data from studies with 4 or more animals is given in the following paragraphs.

3.3.5.3.3.2 Classification criteria based on a 3-animal test are detailed in 3.3.2.~~1~~2. Evaluation of a 4, 5 or 6 animal study should follow the criteria in the following paragraphs, depending on the number of animals tested. Scoring should be done at 24, 48 and 72 hours after instillation of the test material.

3.3.5.3.3.3 In the case of a study with 6 animals the following principles apply:

(a) The substance or mixture is classified as Eye Category 1 if:

(i) at least in one animal effects on the cornea, iris or conjunctiva are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(ii) at least 4 out of 6 animals show a mean score per animal of ≥ 3 for corneal opacity and/or > 1.5 for iritis.

(b) The substance or mixture is classified as Eye Category 2/2A if at least 4 out of 6 animals show a mean score per animal of:

(i) ≥ 1 for corneal opacity; and/or

(ii) ≥ 1 for iritis; and/or

(iii) ≥ 2 for conjunctival redness; and/or

(iv) ≥ 2 for conjunctival oedema (chemosis),

and which fully reverses within an observation period of normally 21 days.

(c) The substance or mixture is classified as irritant to eyes (Category 2B) if the effects listed in sub-paragraph (b) above are fully reversible within 7 days of observation.

3.3.5.3.3.4 In the case of a study with 5 animals the following principles apply:

(a) The substance or mixture is classified as Eye Category 1 if:

(i) at least in one animal effects on the cornea, iris or conjunctiva are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(ii) at least 3 out of 5 animals show a mean score per animal of ≥ 3 for corneal opacity and/or > 1.5 for iritis.

(b) The substance or mixture is classified as Eye Category 2/2A if at least 3 out of 5 animals show a mean score per animal of:

(i) ≥ 1 for corneal opacity; and/or

(ii) ≥ 1 for iritis; and/or

(iii) ≥ 2 for conjunctival redness; and/or

(iv) ≥ 2 for conjunctival oedema (chemosis),

and which fully reverses within an observation period of normally 21 days.

(c) The substance or mixture is classified as irritant to eyes (Category 2B) if the effects listed in sub-paragraph (b) above are fully reversible within 7 days of observation.

3.3.5.3.3.5 In the case of a study with 4 animals the following principles apply:

(a) The substance or mixture is classified as Eye Category 1 if:

(i) at least in one animal effects on the cornea, iris or conjunctiva are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

(ii) at least 3 out of 4 animals show a mean score per animal of ≥ 3 for corneal opacity and/or > 1.5 for iritis.

(b) Classification as Eye Category 2/2A if at least 3 out of 4 animals show a mean score per animal of:

(i) ≥ 1 for corneal opacity; and/or

(ii) ≥ 1 for iritis; and/or

(iii) ≥ 2 for conjunctival redness; and/or

(iv) ≥ 2 for conjunctival oedema (chemosis),

and which fully reverses within an observation period of normally 21 days.

(c) The substance or mixture is classified as irritant to eyes (Category 2B) if the effects listed in sub-paragraph (b) above are fully reversible within 7 days of observation.

3.3.5.3.4 *Guidance on the use of Defined Approaches and/or in vitro/ex vivo data for classification within Tier 2 of Figure 3.3.1*

3.3.5.3.4.1 Defined Approaches (DAs) consist of a predefined set of different information sources (e.g. *in vitro* methods, *ex vivo* methods, physico-chemical properties, non-test methods) which, combined together through a fixed Data Interpretation Procedure (DIP) to convert input data into a prediction (or result), can provide a conclusion on the classification of a substance or mixture. A fixed DIP is defined as any fixed algorithm for interpreting data from one or

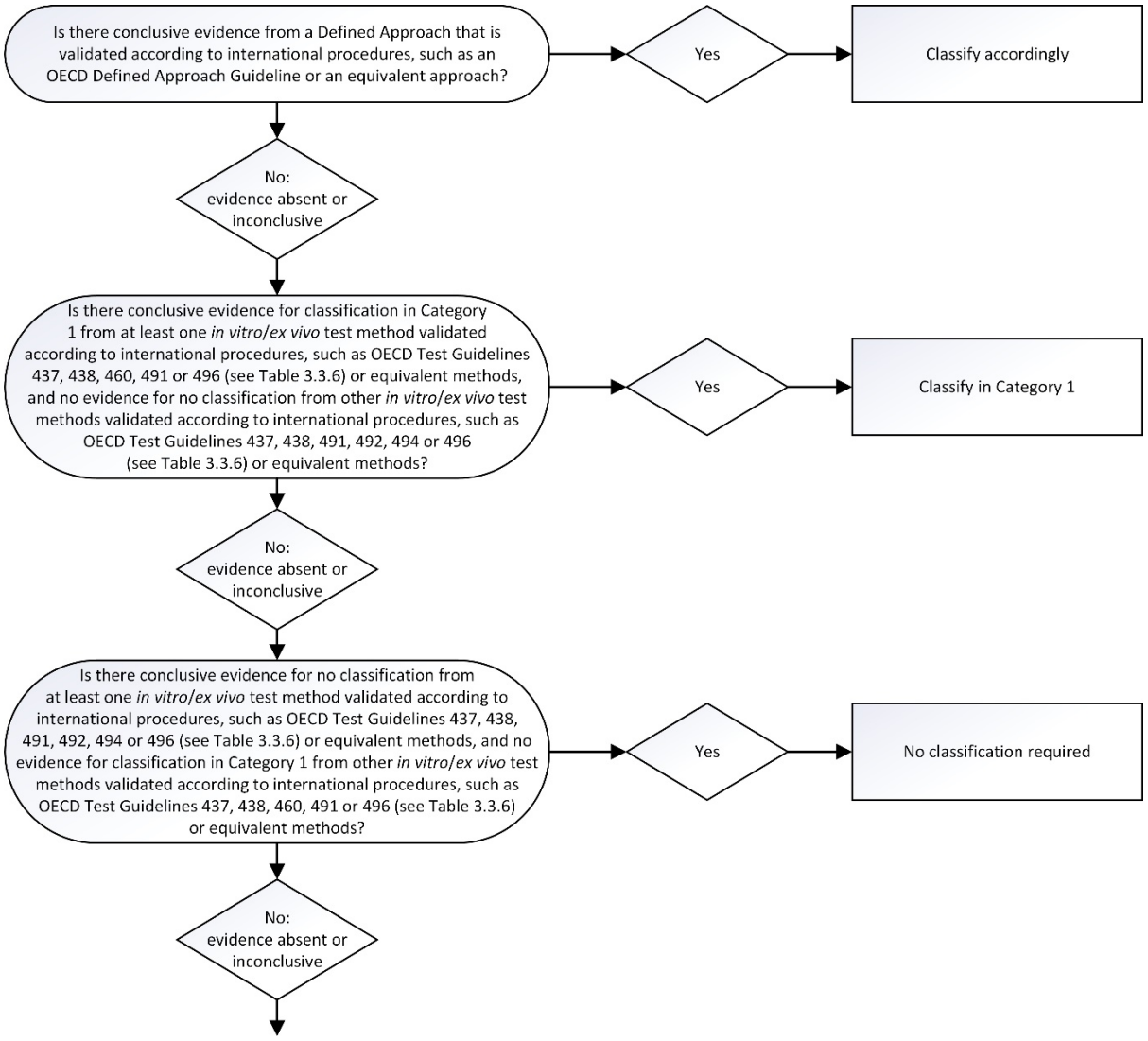
typically several information sources and is rule-based in the sense that it is based, for example on a formula or an algorithm (e.g. decision criteria, rule or set of rules) that do not involve expert judgment. The output of a DIP generally is a prediction of a biological effect of interest or regulatory endpoint. Since in a DA the information sources are prescribed and the set of rules on how to integrate and interpret them is predetermined, the same conclusion will always be reached by different assessors on the same set of data as there is no room for subject interpretation. In contrast, in a weight of evidence ~~approach~~assessment, expert judgment is applied on an ad hoc basis to the available information, which may lead to different conclusions because there are no fixed rules for interpreting the data.

3.3.5.3.4.2 A stepwise approach to the evaluation of information derived from Tier 2 of Figure 3.3.1, i.e. Defined Approaches and/or *in vitro*/*ex vivo* test methods, should be considered where applicable (Figure 3.3.2), recognising that not all elements may be relevant. However, all available and relevant information of sufficient quality needs to be examined for consistency with respect to the resulting classification. ~~Conclusive animal or human data should not be used as part of a Defined Approach in Tier 2 (see Figure 3.3.1), but should be used as described in the criteria for Tier 1.~~ The outcome of a Defined Approach containing conclusive animal and/or human data may also eventually be considered during the overall weight of evidence in Tier 7 (see Figure 3.3.1).

3.3.5.3.4.3 Current *in vitro*/*ex vivo* test methods are not able to distinguish between certain *in vivo* effects, such as corneal opacity, iritis, conjunctiva redness or conjunctiva chemosis, but they have shown to correctly predict substances inducing serious eye damage/eye irritation independently of the types of ocular effects observed *in vivo*. However, it should be considered that substances inducing serious eye damage are identified by many of these test methods with a high specificity but a limited sensitivity when used to distinguish Category 1 from Category 2/not classified. This means that it is reasonably certain that a substance identified as Category 1 by OECD Test Guideline 437, 438, 460, 491 or 496 (see Table 3.3.6) is indeed inducing irreversible eye effects, whereas some substances inducing serious eye damage will be under-predicted by these *in vitro*/*ex vivo* test methods when used in isolation. Furthermore, many of the current *in vitro*/*ex vivo* test methods can identify substances not requiring classification with high sensitivity and limited specificity when used to distinguish not classified from classified substances. This means that it is reasonably certain that a substance identified as not requiring classification by OECD Test Guideline 437, 438, 491, 492, 494 or 496 (see Table 3.3.6) is indeed not inducing eye effects warranting classification, whereas some substances not requiring classification will be over-predicted by these *in vitro*/*ex vivo* test methods when used in isolation. As a consequence, a single *in vitro*/*ex vivo* OECD Test Guideline method is currently sufficient to conclude on either Category 1 or no classification according to the criteria defined in Table 3.3.6, but not to conclude Category 2. When the result of an *in vitro*/*ex vivo* method is "no stand-alone prediction can be made" (e.g. see Table 3.3.6), a decision on classification in Category 1 or Category 2 or for no classification cannot be made on the basis of that single result and further data are necessary for classification. Some *in vitro*/*ex vivo* test methods validated according to international procedures but not adopted as OECD Test Guidelines may be accepted by some competent authorities to classify in Category 2 (see 3.3.5.3.5.2). Moreover, combinations of *in vitro*/*ex vivo* methods in tiered approaches or their integration in Defined Approaches (see 3.3.2.3) may reduce the number of false predictions and show adequate performance for classification purposes.

3.3.5.3.4.4 In the absence of an adequate Defined Approach (see 3.3.2.3) or other *in vitro*/*ex vivo* data allowing a stand-alone prediction (see 3.3.2.4.2), classification solely from *in vitro*/*ex vivo* test results should be based, where possible, on a within-tier weight of evidence assessment of data from more than one method. For example, for the *in vitro*/*ex vivo* OECD Test Guideline methods listed in Table 3.3.6 and other equivalent methods not able to classify in Category 2, a within-tier weight of evidence assessment of data from more than one method would be needed if none of the available data results in Category 1 or no classification, or if Category 1 and no classification conclusions are obtained for the same substance or mixture with different methods. A within-tier weight of evidence assessment of data from more than one method may however, not be conclusive for classification for effects on the eye. In this case, data from lower tiers may be required to reach a conclusion (see Figure 3.3.1).

Figure 3.3.2: Classification based on Defined Approaches and/or *in vitro*/*ex vivo* data within Tier 2 of Figure 3.3.1[a]



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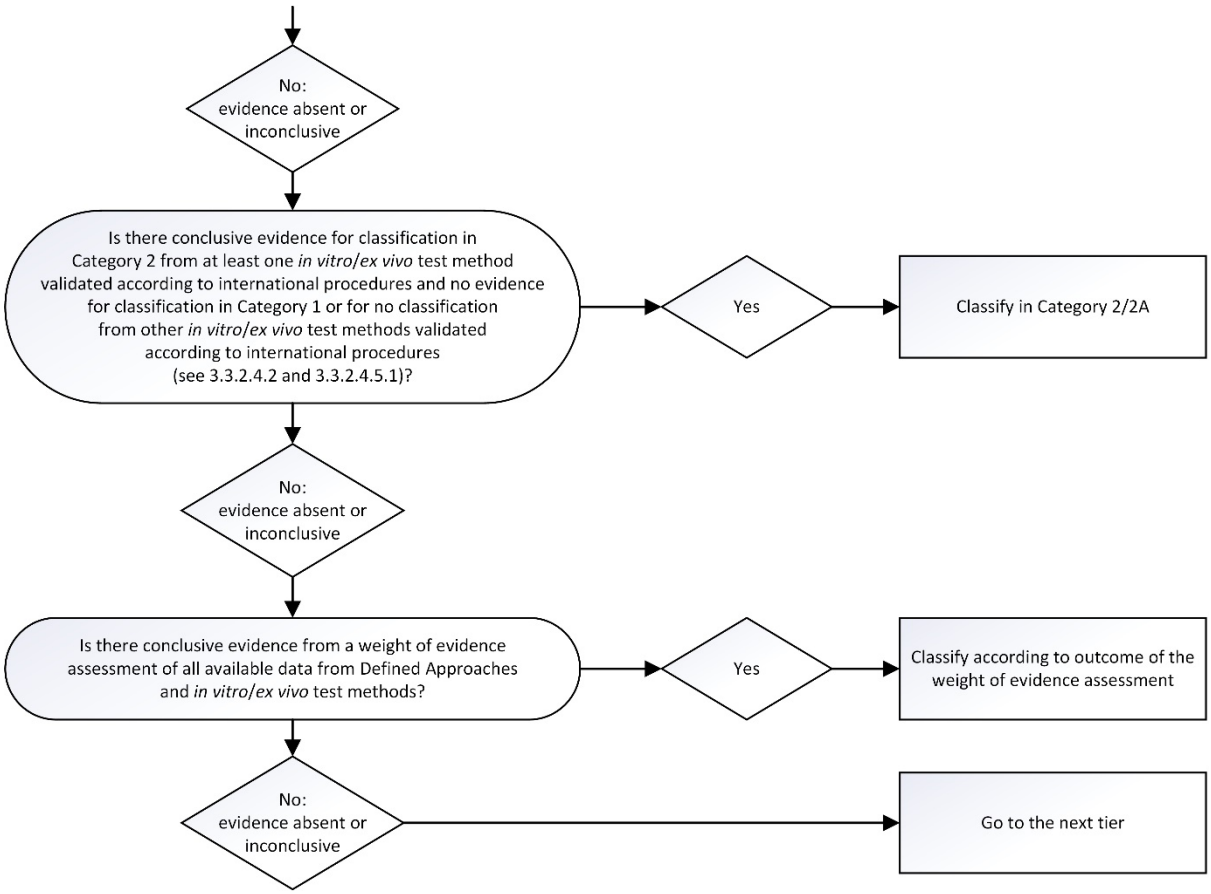

[a] *Evidence is considered conclusive if the data fulfil the criteria of the Defined Approach or the method and there is no contradicting in vitro/ex vivo information.*

3.3.5.3.5 *Classification criteria based on in vitro/ex vivo data*

3.3.5.3.5.1 Where *in vitro/ex vivo* tests have been undertaken in accordance with OECD Test Guidelines 437, 438, 460, 491, 492, 494 and/or 496, the criteria for classification in Category 1 for serious eye damage/irreversible effects on the eye and for no classification are set out in Table 3.3.6

Table 3.3.6: Serious eye damage/Irreversible effects on the eye and for no classification [a] *for in vitro/ex vivo* methods

Category	OECD Test Guideline 437 Bovine Corneal Opacity and Permeability test method	OECD Test Guideline 438 Isolated Chicken Eye test method	OECD Test Guideline 460 Fluorescein Leakage test method	OECD Test Guideline 491 Short Time Exposure test method	OECD Test Guideline 492 Reconstructed human Cornea-like Epithelium (RhCE)-based test methods: Methods 1, 2, 3 and 4 as numbered in Annex II of OECD Test Guideline 492	OECD Test Guideline 494 Vitrigel-Eye Irritancy Test Method	OECD Test Guideline 496 *In vitro* Macromolecular Test Method (test method 1)
	Organotypic *ex vivo* assay using isolated corneas from the eyes of freshly slaughtered cattle. Test chemicals are applied to the epithelial surface of the cornea. Damage by the test chemical is assessed by quantitative measurements of: - Corneal opacity changes measured using a light transmission opacitometer (opacitometer 1) or a laserlight-based opacitometer (LLBO, opacitometer 2) - Permeability (sodium fluorescein dye). Both measurements are used to calculate an *In Vitro* Irritancy Score (IVIS) when using opocitometer 1 or a LLBO Irritancy Score (LIS) when using opacitometer 2. **Criteria based on IVIS or LIS.**	Organotypic *ex vivo* assay based on the short-term maintenance of chicken eyes *in vitro*. Test chemicals are applied to the epithelial surface of the cornea. Damage by the test chemical is assessed by (i) a quantitative measurement of increased corneal thickness (swelling), (ii) a qualitative assessment of corneal opacity, (iii) a qualitative assessment of damage to epithelium based on application of fluorescein to the eye, and (iv) a qualitative evaluation of macroscopic morphological damage to the surface. Histopathology can be used to increase the sensitivity of the method for identifying Category 1 non-extreme pH (2 < pH < 11.5) detergents and surfactants. [b] **Criteria based on the scores of corneal swelling, opacity and fluorescein retention, which are used to assign ICE classes (I, II, III or IV) to each endpoint, and on macroscopic and histopathology assessment [b]**	Cytotoxicity and cell-function based *in vitro* assay that is performed on a confluent monolayer of Madin-Darby Canine Kidney (MDCK) CB997 tubular epithelial cells cultured on permeable inserts. The toxic effects of a test chemical are measured after a short exposure time (1 minute) by an increase in permeability of sodium fluorescein through the epithelial monolayer of MDCK cells. The amount of fluorescein leakage that occurs is proportional to the chemical-induced damage to the tight junctions, desmosomal junctions and cell membranes, and is used to estimate the ocular toxicity potential of a test chemical. **Criteria based on mean percent fluorescein leakage following a defined exposure period**	Cytotoxicity-based *in vitro* assay that is performed on a confluent monolayer of Statens Seruminstitut Rabbit Cornea (SIRC) cells. Each test chemical is tested at both 5 % and 0.05 % concentrations. Following five-minute exposure, cell viability is assessed by the enzymatic conversion in viable cells of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from cells. **Criteria based on mean percent cell viability following a defined exposure period**	Three-dimensional RhCE tissues are reconstructed from either primary human cells or human immortalised corneal epithelial cells, which have been cultured for several days to form a stratified, highly differentiated squamous epithelium, consisting of at least 3 viable layers of cells and a non-keratinised surface, showing a cornea-like structure morphologically similar to that found in the human cornea. Following exposure and post-treatment incubation (where applicable), tissue viability is assessed by the enzymatic conversion in viable cells of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from the tissues. **Criteria based on mean percent tissue viability following defined exposure and post-exposure (where applicable) periods**	*In vitro* assay using human corneal epithelium models fabricated in a collagen vitrigel membrane (CVM) chamber. The eye irritation potential of the test chemical is predicted by analysing time-dependent changes in transepithelial electrical resistance values using the value of three indexes. Resistance values are measured at intervals of 10 seconds for a period of three minutes after exposure to the test chemical preparation. **Criteria based on the 3 measured indexes: time lag, intensity and plateau level of electrical resistance.**	*In vitro* assay consisting of a macromolecular plant-based matrix obtained from jack bean *Canavalis enisformis*. This matrix serves as the target for the test chemical and is composed of a mixture of proteins, glycoproteins, carbohydrates, lipids and low molecular weight components, which form a highly ordered and transparent gel structure upon rehydration. Test chemicals causing ocular damage lead to the disruption and disaggregation of the highly organised macromolecular reagent matrix, and produce turbidity of the macromolecular reagent. Such phenomena is quantified, by measuring changes in light scattering. **Criteria based on a Maximum Qualified Score (MQS) derived from the Optical Density readings at different concentrations, calculated via a software.**

1	Opacitometer 1 IVIS > 55	Opacitometer 2 LIS > 30 and lux/7 ≤ 145 and OD490 > 2.5, OR LIS > 30 and lux/7 > 145	At least 2 ICE class IV, OR Corneal opacity = 3 at 30 min (in at least 2 eyes), OR Corneal opacity = 4 at any time point (in at least 2 eyes), OR Severe loosening of the epithelium (in at least 1 eye), OR Certain histopathological effects[b]	Chemical concentration causing 20 % of Fluorescein Leakage (FL_{20}) ≤ 100 mg/mL	Viability ≤ 70 % at 5 % and 0.05 %	No stand-alone prediction can be made				No stand-alone prediction can be made	MQS > 30.0
2/2A/2B	No stand-alone prediction can be made.	No stand-alone prediction can be made	No stand-alone prediction can be made	No stand-alone prediction can be made	No stand-alone prediction can be made	No stand-alone prediction can be made				No stand-alone prediction can be made	No stand-alone prediction can be made
Not Classified	Opacitometer 1 IVIS ≤ 3	Opacitometer 2 LIS ≤ 30	ICE class I for all 3 endpoints, OR ICE class I for 2 endpoints and ICE class II for the other endpoint, OR ICE class II for 2 endpoints and ICE class I for the other endpoint	No stand-alone prediction can be made	Viability > 70 % at 5 % and 0.05 %	Test method 1 Liquids and Solids: Viability > 60 %	Test method 2 Liquids: Viability > 60 %; Solids: Viability > 50 %	Test method 3 Liquids and Solids: Viability > 40 %	Test method 4 Liquids: Viability > 35 %; Solids: Viability > 60 %	Time lag > 180 seconds and Intensity < 0.05 %/seconds and Plateau level ≤ 5.0 %	MQS ≤ 12.5

[a] *Grading criteria are understood as described in OECD Test Guidelines 437, 438, 460, 491, 492, 494 and 496.*

[b] *For criteria, please consult OECD Test Guideline 438*

3.3.5.3.5.2 A non-exhaustive list of other validated *in vitro*/*ex vivo* test methods accepted by some competent authorities but not adopted as OECD Test Guidelines are listed below. A competent authority may decide which classification criteria, if any, should be applied for these test methods:

- Time to Toxicity (ET_{50}) tests using the Reconstructed human Cornea-like Epithelia (RhCE) described in OECD Test Guideline 492 (Kandarova et al., 2018; Alépée et al., 2020);
- Porcine Ocular Cornea Opacity/Reversibility Assay (PorCORA): an *ex vivo* assay that uses excised porcine corneal tissues kept in culture for up to 21 days and monitors tissue recovery to model both reversible and non-reversible eye effects. The tissues are stained with fluorescent dye and effects on the corneal epithelia are visualised by the retention of fluorescent dye (Piehl et al., 2010; Piehl et al., 2011);
- *Ex Vivo* Eye Irritation Test (EVEIT): an *ex vivo* assay that uses excised rabbit corneal tissues kept in culture for several days and monitors tissue recovery to model both reversible and non-reversible eye effects. Full-thickness tissue recovery is monitored non-invasively using optical coherence tomography (OCT) (Frentz et al., 2008; Spöler et al., 2007; Spöler et al., 2015);
- *In vitro* Macromolecular Test Method (test method 2), similar to test method 1 described in OECD Test Guideline 496 (Choksi et al., 2020);
- Metabolic activity assay: *In vitro* assay consisting of measuring changes to metabolic rate in test-material treated L929 cell monolayer (Harbell et al., 1999; EURL ECVAM, 2004a; Hartung et al., 2010; Nash et al., 2014);
- Hen's Egg Test on the Chorio-Allantoic Membrane (HET-CAM): an organotypic assay that uses the vascularised membrane of fertile chicken eggs to assess a test material's potential to cause vascular changes (Spielmann et al., 1993; Balls et al., 1995; Spielmann et al., 1996; Brantom et al., 1997; ICCVAM, 2007; ICCVAM, 2010);
- Chorio-Allantoic Membrane Vascular Assay (CAMVA): an organotypic assay that uses the vascularised membrane of fertile chicken eggs to assess a test material's potential to cause vascular changes (Bagley et al., 1994; Brantom et al., 1997; Bagley et al., 1999; Donahue et al., 2011);
- Neutral Red Release (NRR) assay: *In vitro* assay that quantitatively measures a substance's ability to induce damage to cell membranes in a monolayer of normal human epidermal keratinocytes (NHEK) (Reader et al. 1989; Reader et al., 1990; Zuang, 2001; EURL ECVAM, 2004b; Settivari et al., 2016); and
- Isolated Rabbit Eye (IRE) test, similar to OECD Test Guideline 438 but using isolated rabbit eyes instead of isolated chicken eyes (Burton et al., 1981; Whittle et al. 1992; Balls et al., 1995; Brantom et al., 1997; ICCVAM, 2007; ICCVAM, 2010).

3.3.5.3.6 *Guidance on the use of other existing skin or eye data in animals for classification as serious eye damage or eye irritation*

3.3.5.3.6.1 The availability of other animal data for serious eye damage/eye irritation may be limited as tests with the eye as route of exposure are not normally performed. An exception could be historical data from the Low Volume Eye Test (LVET) that might be used in a weight of evidence ~~approach~~assessment. The LVET is a modification of the standard OECD TG 405 test method.

3.3.5.3.6.2 Existing data from the LVET test could be considered for the purpose of classification and labelling, but must be carefully evaluated. The differences between the LVET and OECD 405 may result in a classification in a lower category (or no classification) based on LVET data, than if the classification were based on data derived from the standard *in vivo* test (OECD TG 405). Thus, positive data from the LVET test could be a trigger for considering classification in Category 1 on its own, but data from this test are not conclusive for a Category 2 classification or no classification (ECHA, 2017). Such data may however, be used in an overall weight of evidence assessment. It is noted that the applicability domain of the LVET is limited to household detergent and cleaning products and their main ingredients (surfactants) (see scientific opinion of the EURL ECVAM Scientific Advisory Committee (ESAC): https://ec.europa.eu/jrc/sites/jrcsh/files/esac31_lvet_20090922.pdf).

3.3.5.3.6.3 Effects on the eyes may or may not be observed in acute or repeated dose inhalation studies with full body exposure. However, normally no scoring according to the Draize criteria is performed and the follow up period may be shorter than 21 days. Also, the effects on the eyes will likely depend upon the concentration of the substance/mixture and the exposure duration. As there are no criteria for minimal concentration and duration, the absence of effects on the eyes or eye irritation may not be conclusive for the absence of serious eye damage.

~~Substances/mixtures inducing irreversible effects on the eye are, however, deemed as inducing serious eye damage (Category 1).~~The presence of irreversible effects on the eye should be considered within a weight of evidence assessment.

3.3.5.3.6.4 Substances/mixtures classified as corrosive to skin (Skin Category 1) based on other existing skin data ~~that lead to classification as skin corrosion Category 1~~ according to the criteria in Chapter 3.2 are also deemed as inducing serious eye damage (Eye Category 1~~), should also lead to classification of a substance/mixture for serious eye damage (Category 1), unless other available data conclusively show that it should not be classified as such.~~ Other existing skin data leading to classification in skin Category 2, 3 or no classification, are considered inconclusive and can only be used in the overall weight of evidence assessment~~. Importantly, additional testing in animals for assessment of serious eye damage/eye irritation should not be conducted for the purpose of contradicting a Category 1 classification derived from skin data.~~

3.3.5.3.7 *Guidance on the use of pH and acid/alkaline reserve for classification as serious eye damage*

3.3.5.3.7.1 ~~Eye effects may be indicated by pH extremes such as ≤ 2 and ≥ 11.5, especially when associated with significant acid/alkaline reserve.~~ Methods to determine the pH value such as OECD TG 122 and the method described by Young et al. (1988) differ in the concentration of the substance or mixture for which the pH is determined and include values of 1%, 10% and 100%. These methods also differ in the way the acid/alkaline reserve is determined, namely up to a pH of 7 for both acids and bases (OECD TG 122) or up to a pH of 4 for acids and a pH of 10 for bases (Young et al., 1988). Also there are differences between OECD TG 122 and Young et al. (1988) in the units used to express the acid/alkaline reserve.~~Also the values for acid/alkaline reserve differ as OECD TG 122 expresses this as g sulphuric acid/100 g for bases and as g sodium hydroxide/100 g for acids, whereas Young et al. (1988) uses the same approach for acids but expresses the value for bases as the amount of sodium hydroxide per 100 g equivalent to the amount of sulphuric acid per 100 g~~.

3.3.5.3.7.2 Criteria to identify substances and mixtures requiring classification in Category 1 based on pH and acid/alkaline reserve have been developed for effects on the skin (Young et al., 1988) and the same criteria are applied for effects on the eye. These criteria were developed using a combination of pH and acid/alkaline reserve values that were determined in a specific way (Young et al., 1988). Therefore, these criteria may not be directly applicable when other test concentrations or methods are used to measure pH and acid/alkaline reserve. Furthermore, the calibration and validation of these criteria was based on a limited dataset for effects on the skin. Thus, the predictive value of the combination of pH and acid/alkaline reserve for classification in Category 1 for effects on the eye is limited, especially for substances and mixtures with an extreme pH but a non-significant acid/alkaline reserve. The criteria developed by Young et al. (1988) for classification in Category 1 may be used as a starting point for determining whether a substance or a mixture has a significant acid/alkaline reserve or a non-significant acid/alkaline reserve. A competent authority may decide which criteria for significant acid/alkaline reserve should be applied.~~3.3.5.3.7.3 Additional testing in animals for assessment of serious eye damage/eye irritation should not be conducted for the purpose of contradicting a Category 1 classification derived from pH data, even in cases where the acid/alkaline reserve of the substance or mixture is non-significant.~~

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