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

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**Work on the Globally Harmonized System of Classification and Labelling of Chemicals: 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 at the forty-first session in December 2021.

## Background

2. At the thirty-ninth session, the Sub-Committee agreed to keep the work on the use of non-animal testing methods for classification of health and environmental hazard classes on its programme of work for the 2021-2022 biennium[1] and updates on the progress of the work were provided to the Sub-Committee at the fortieth and forty-first sessions.[2]

3. The informal working group presently has approximately 60 members, reflecting the importance of, and interest in, this work. Its membership includes experts with specialised knowledge of test methods and their application to classification, and experts on national legislation that implements GHS. Discussions are often lively and detailed, but overall are propelled by a strong desire to make progress on the informal working group's mandate and ensure that non-animal test methods are consistently incorporated in the GHS in a way that reflects their growing importance and scientific relevance, whilst recognising their limitations.

## Status report

4. Since the last update to the Sub-Committee in December 2021, the informal working group has continued to work hard on the revision of Chapter 3.4 for skin sensitization for the inclusion of non-animal testing methods via correspondence, virtual meetings (13 January 2022; 3 March 2022; 12 April 2022; and 9 June 2022) and at a face-to-face informal group meeting that will be held during the forty-second session in July 2022. 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

[1] See ST/SG/AC.10/C.4/78

[2] See informal documents INF.18 (40th session) and INF.11 (41st session)

Chapter 3.4 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.

5. The working draft (Version 5; 17 May 2022) that was considered by the working group during their virtual meeting on 9 June 2022 is provided in the Annex of this document. Comments received from members during the June 2022 meeting have not yet been incorporated into this draft. New text relative to the 9th 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 3.4 should be processed further to achieve adoption by the Sub-Committee.

## On-going work

6. The informal working group will continue to work hard on resolving the outstanding issues and the revision of Chapter 3.4 during its next meeting on 6 July 2022 followed, if necessary, by further virtual meetings. There is tentative hope that it will be possible to finalise the revision of Chapter 3.4 in time for adoption by the Sub-Committee in the current biennium.

7. The Sub-Committee is invited to note the progress of the revision of Chapter 3.4 (as provided in the Annex of this document) and the issues outlined in this informal document.

# Annex

## Working draft of Chapter 3.4 (Version 5; 17 May 2022)

Black text is from the current Chapter 3.4 in the 9th Revision of the GHS.
Blue text is new in this draft chapter, as agreed by the working group.
Red text requires further discussion.
Deleted text from previous versions of the draft chapter 3.4 are shown in red with strike through.
Deleted original text is shown in black with strike-through.

**"CHAPTER 3.4**
**RESPIRATORY OR SKIN SENSITIZATION**

### 3.4.1 Definitions and general considerations

3.4.1.1 *Respiratory sensitization* refers to hypersensitivity of the airways occurring after inhalation of a substance or a mixture.

*Skin sensitization* refers to an allergic response occurring after skin contact with a substance or a mixture.

3.4.1.2 For the purpose of this chapter, sensitization includes two phases: the first phase is induction of specialized immunological memory in an individual by exposure to an allergen. The second phase is elicitation, i.e. production of a cell-mediated or antibody-mediated allergic response by exposure of a sensitized individual to an allergen.

3.4.1.3 For respiratory sensitization, the pattern of induction followed by elicitation phases is shared in common with skin sensitization. For skin sensitization, an induction phase is required in which the immune system learns to react; clinical symptoms can then arise when subsequent exposure is sufficient to elicit a visible skin reaction (elicitation phase). As a consequence, predictive tests usually follow this pattern in which there is an induction phase, the response to which is measured by a standardized elicitation phase, typically involving a patch test. The local lymph node assay is the exception, directly measuring the induction response. Evidence of skin sensitization in humans normally is assessed by a diagnostic patch test.

3.4.1.4 Usually, for both skin and respiratory sensitization, lower levels are necessary for elicitation than are required for induction. Provisions for alerting sensitized individuals to the presence of a particular sensitizer in a mixture can be found in 3.4.4.2.

3.4.1.5 The hazard class "respiratory or skin sensitization" is differentiated into:

(a) Respiratory sensitization; and

(b) Skin sensitization

### 3.4.2 Classification criteria for substances

#### 3.4.2.1 *Respiratory sensitizers*

For clarity these paragraphs are removed but remain unchanged.

#### 3.4.2.2 *Skin sensitizers*

3.4.2.2.1 *Hazard categories*

3.4.2.2.1.1 Skin sensitizers shall be classified in Category 1 where sub-categorization is not required by a competent authority or where data are not sufficient for sub-categorization.

3.4.2.2.1.2 Where data are sufficient and where required by a competent authority, a refined evaluation according to 3.4.2.2.1.5 allows the allocation of skin sensitizers into sub-category 1A, strong sensitizers, or sub-category 1B for other skin sensitizers.

3.4.2.2.1.3 For classification of skin sensitisers, all available and relevant information is collected and its quality in terms of adequacy and reliability is assessed. Classification should be based on data/results generated using methods and/or defined approaches that are validated according to international procedures. These include both OECD Guidelines and equivalent methods/defined approaches (see 1.3.2.4.3). Sections 3.4.2.2.2 to 3.4.2.2.6 provide classification criteria for the different types of information that may be available.

3.4.2.2.1.4 A tiered approach (see 3.4.2.2.7) organizes the available information on skin sensitisation 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 is made on the basis of weight-of-evidence within that tier. In some cases where information from different tiers gives inconsistent and/or conflicting results (see 3.4.2.2.7.7) or where data are insufficient to conclude on the classification, an overall weight of evidence assessment is used (see 3.4.2.2.7.6).

3.4.2.2.1.5 Guidance on the interpretation of criteria and references to relevant guidance documents are provided in 3.4.5.3.

3.4.2.2.2 ***Classification based on human data-(Tier 1 in Figure 3.4.1)***

3.4.2.2.2.1 A substance is classified as a skin sensitizer in category 1 if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons.

3.4.2.2.2.2 Substances showing a high frequency of occurrence in humans, can be presumed to have the potential to produce significant sensitization and are classified in category 1A. Severity of reaction may also be considered. Human evidence for sub-category 1A can include:

(a) positive responses at ≤ 500 µg/cm$^2$ (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;

(c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

3.4.2.2.2.3 Substances showing a low to moderate frequency of occurrence in humans can be presumed to have the potential to produce sensitization and are classified in category 1B. Severity of reaction may also be considered. Human evidence for sub-category 1B can include:

(a) positive responses at > 500 µg/cm$^2$ (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure;

(c) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

3.4.2.2.3 ***Classification based on standard animal data (Tier 1 in Figure 3.4.1)***

3.4.2.2.3.1 A substance is classified as a skin sensitizer if there are positive results from an appropriate animal test. For Category 1, when an adjuvant type test method for skin sensitization is used, a response of at least 30 % of the animals is considered as positive. For a non-adjuvant Guinea pig test method a response of at least 15 % of the animals is considered positive. For Category 1, a stimulation index of three or more is considered a positive response in the local lymph node assay. Test methods for skin sensitization are described in the OECD Guideline 406 (the Guinea Pig Maximisation test and the Buehler guinea pig test) and Guideline 429 (Local Lymph Node Assay). Other methods may be used provided that they are well-validated and scientific justification is given. The Mouse Ear Swelling Test (MEST), appears to be a reliable screening test to detect moderate to strong sensitizers, and can be used as a first stage in the assessment of skin sensitization potential.

3.4.2.2.3.2 Substances showing a high potency in animals, can be presumed to have the potential to produce significant sensitization in humans and are classified in category 1A. Severity of reactions may also be considered. Animal test results for sub-category 1A can include data with values indicated in Table 3.4.3 below:

**Table 3.4.3: Animal test results for sub-category 1A**

| Assay | Criteria |
|---|---|
| Local lymph node assay | EC3 value ≤ 2 % |
| Guinea pig maximisation test | ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or<br>≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose |
| Buehler assay | ≥15 % responding at ≤ 0.2 % topical induction dose or<br>≥ 60 % responding at > 0.2 % to ≤ 20 % topical induction dose |

3.4.2.2.3.3 Substances showing a low to moderate potency in animals can be presumed to have the potential to produce sensitization in humans and are classified in category 1B. Severity of reaction may also be considered. Animal test results for sub-category 1B can include data with values indicated in Table 3.4.4 below:

**Table 3.4.4: Animal test results for sub-category 1B**

| Assay | Criteria |
|---|---|
| Local lymph node assay | EC3 value > 2 % |
| Guinea pig maximisation test | ≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or<br>≥ 30 % responding at > 1 % intradermal induction dose |
| Buehler assay | ≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or<br>≥ 15 % responding at > 20 % topical induction dose |

3.4.2.2.4 ***Classification based on defined approaches (Tier 1 or Tier 2 in Figure 3.4.1)***

3.4.2.2.4.1 Defined approaches consist of a rule-based combination of data obtained from a predefined set of different information sources (e.g. *in chemico* methods, *in vitro* methods, physico-chemical properties, non-test methods). It is recognized that most single non animal 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 validated according to international procedures, such as OECD Guideline 497 or an equivalent approach, are conclusive for classification for skin sensitisation if the criteria of the defined approach are fulfilled (see Table 3.4.6)[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.

3.4.2.2.4.2 Where the results from defined approaches are assigned a level of confidence as for example in OECD Guideline 497, a low confidence outcome of a defined approach cannot be used on its own to classify but may be considered in combination with other data in Tier 2.

3.4.2.2.5 ***Classification based on in chemico/in vitro data (Tier 1 or Tier 2 in Figure 3.4.1)***

3.4.2.2.5.1 The currently available *in chemico/in vitro* methods address specific biological mechanisms leading to the acquisition of skin sensitization as defined in the OECD Adverse Outcome Pathway for Skin Sensitisation. Individual test methods that are validated according to international procedures and are accepted as stand-alone methods, can be used to conclude on the classification in Tier 1. A competent authority may decide which methods and classification criteria, if any, can be used to conclude on the classification in Tier 1. The guidance in 3.4.5.3.5 provides information on the currently available methods in OECD TG 442C.

3.4.2.2.5.2 Other non-stand-alone *in chemico/in vitro* methods that are validated according to international procedures such as OECD Test Guidelines 442C, 442D and 442E, are accepted as supportive evidence and should within Tier 1 only be used in combination with other types of data. ~~preferably~~ in defined approaches ~~or alternatively in a weight of evidence assessment in subsequent tiers~~. When already considered within a defined approach in Tier 1, non-stand-alone *in chemico/in vitro* methods should not be considered as an additional line of evidence in subsequent Tiers (see 3.4.2.2.7.4). ~~However,~~ Where non-stand alone *in chemico/in vitro* data is available and it is not used in define approaches within Tier1

[3] *Additional defined approaches have been proposed for skin sensitization (OECD 2016b) but no classification criteria have yet been agreed internationally.*

they should be used in combination with other data in a weight-of evidence in Tier 2 or in an overall weigh-of-evidence in Tier 3. In case a positive result from a non-stand-alone *in chemico/in vitro* method validated according to international procedures is the only evidence available, this can be used to classify into category 1.

3.4.2.2.5.3 Other validated *in chemico/in vitro* test methods accepted by some competent authorities are described in 3.4.5.3.6.1[4]. A competent authority may decide which classification criteria, if any, should be applied for these test methods to conclude on classification.

3.4.2.2.5.~~3~~.4 *In chemico/in vitro* 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.

3.4.2.2.6 ***Classification based on non-test methods (Tier 2 in Figure 3.4.1)***

3.4.2.2.6.1 Classification, including the conclusion not classified, can be based on non-test methods, with due consideration of reliability and applicability, on a case-by-case basis. Specific non-test methods may also be used in a defined approach. When already considered within a defined approach, these specific non-test methods should not be considered as an additional line of evidence (see 3.4.2.2.7.4). Non-test 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.

3.4.2.2.6.2 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. For conclusions on no classification from read-across the adequacy and robustness of the scientific reasoning and of the supporting evidence should be well substantiated and normally requires multiple negative substances with good structural and physical (related to toxicokinetics) similarity to the substance being classified ~~and the~~ as well as a clear absence of positive substances with good structural and physical similarity to the substance being classified.

3.4.2.2.6.3 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. For conclusions on no classification from (Q)SARs the adequacy and robustness of the scientific reasoning and of the supporting evidence should be well substantiated and normally requires multiple negative substances with good structural and physical (related to toxicokinetics) similarity to the substance being classified, ~~show~~ as well as a clear ~~and the~~ absence of positive substances with good structural and physical similarity to the substance being classified.

3.4.2.2.7 ***Classification in a tiered approach (Figure 3.4.1)***

3.4.2.2.7.1 All available and relevant information of sufficient quality should be considered according to the tiered approach (Figure 3.4.1).

3.4.2.2.7.2 For classification of a substance, evidence in Tier 1 may include any or all of the following lines of evidence. Where information from data within Tier 1 is inconsistent and/or conflicting, the conclusion is determined using a weight of evidence approach:

(a) ~~Positive~~ Data from ~~well run~~ clinical/experimental studies (e.g., predictive patch testing, HRIPT, HMT), ~~(with large sample size),~~ normally obtained in more than one dermatology clinic ~~(e.g.)~~ (see Chapter 1.3. paragraph 1.3.2.4.7, see criteria in 3.4.2.2.2.1(a) and 3.4.2.2.2.2.(a) and guidance 3.4.5.3.2);

(b) Epidemiological studies (e.g., case control studies, prospective studies including data obtained by diagnostic patch testing) showing allergic contact dermatitis caused by the substance (see Chapter 1.3. paragraph 1.3.2.4.7, criteria in 3.4.2.2.2.1(b) and (c), 3.4.2.2.2.2(b) and (c), and guidance 3.4.5.3.2); ~~Situations in which a high proportion of those exposed exhibit characteristic symptoms are to be looked at with special concern, even if the number of cases is small;~~

[4] *Additional in chemico/in vitro methods have been proposed for skin sensitisation (see 3.4.5.3.5.1) but no classification criteria have yet been agreed internationally.*

(c) Data from appropriate animal studies (see criteria in 3.4.2.2.3, and guidance 3.4.5.3.3.);

~~(d) Positive data from experimental studies in humans (see Chapter 1.3, paragraph 1.3.2.4.7);~~

(e~~d~~) Well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic ~~(generally small sample size)~~, case studies in which a high proportion of those exposed exhibit characteristic symptoms are to be looked at with special concern, even if the number of cases is small (see paragraph 3.4.5.3.2), data obtained from clinical or diagnostic studies, case histories (severity of reaction may also be considered);

(g~~e~~) ~~Positive~~ Data from defined approaches validated according to international procedures (see 3.4.2.2.4, guidance3.4.5.3.4, and Table 3.4.7);

(~~h~~f) ~~Positive~~ Data from stand-alone in *chemico/in vitro* methods validated according to international procedures (see 3.4.2.2.5, guidance 3.4.5.3.5, and Table 3.4.8).

3.4.2.2.7.3 In case a definitive conclusion on classification, including sub-categorization where required by a competent authority, cannot be derived from Tier 1, additional lines of evidence shall be considered in a weight-of-evidence in Tier 2. These may include:

a) Data from non-stand alone in *chemico/in vitro* methods (see 3.4.2.2.5 and 3.4.5.3.5)

b) Data from non-test methods (see 3.2.2.2.6)

c) Low confidence/inconclusive results from defined approaches (see 3.4.2.2.4.2)

3.4.2.2.7.4 Evidence from non-stand alone *in chemico/in vitro* methods and from non-test methods should not be considered at this stage if this data is already used in a defined approach under 3.4.2.2.7.2 with a conclusive result.

3.4.2.2.7.5 Individual non-stand alone ~~*in vitro/*~~*in chemico/in vitro* methods validated according to international procedures, non-test methods (including read-across) and low confidence/inconclusive data from defined approaches can be applied in a weight-of-evidence assessment together with inconclusive data from ~~the~~ Tier 1 and should be used in this second Tier because they can usually not be used as stand-alone (with the exception of good quality read across). However, a competent authority may decide that a positive result with one of these non-~~test~~stand alone in chemico/in vitro methods, ~~in the absence of contradicting results from other methods~~, may be used on its own to classify in category 1.

3.4.2.2.7.6 In case a definitive conclusion on classification ~~or no classification~~ including sub-categorization where required by a competent authority, ~~classification into sub-categories 1A or 1B~~ cannot be derived from the previous tiers, an overall weight-of-evidence assessment using expert judgment should be used.~~is indicated~~ ~~If none of the above-mentioned conditions are met, the substance need not be classified as a skin sensitizer. However,~~ that may include a combination of two or more indicators of skin sensitization as listed below. ~~may alter the decision. This shall be considered on a case-by-case basis.~~

(a) Isolated episodes of allergic contact dermatitis;

(b) Epidemiological studies of limited power, e.g. where chance, bias or confounders have not been ruled out fully with reasonable confidence;

(c) Data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in 3.4.2.2.3, but which are sufficiently close to the limit to be considered significant;

(d) ~~Positive~~ data from non-standard methods;

(e) ~~Positive results from close structural analogues.~~

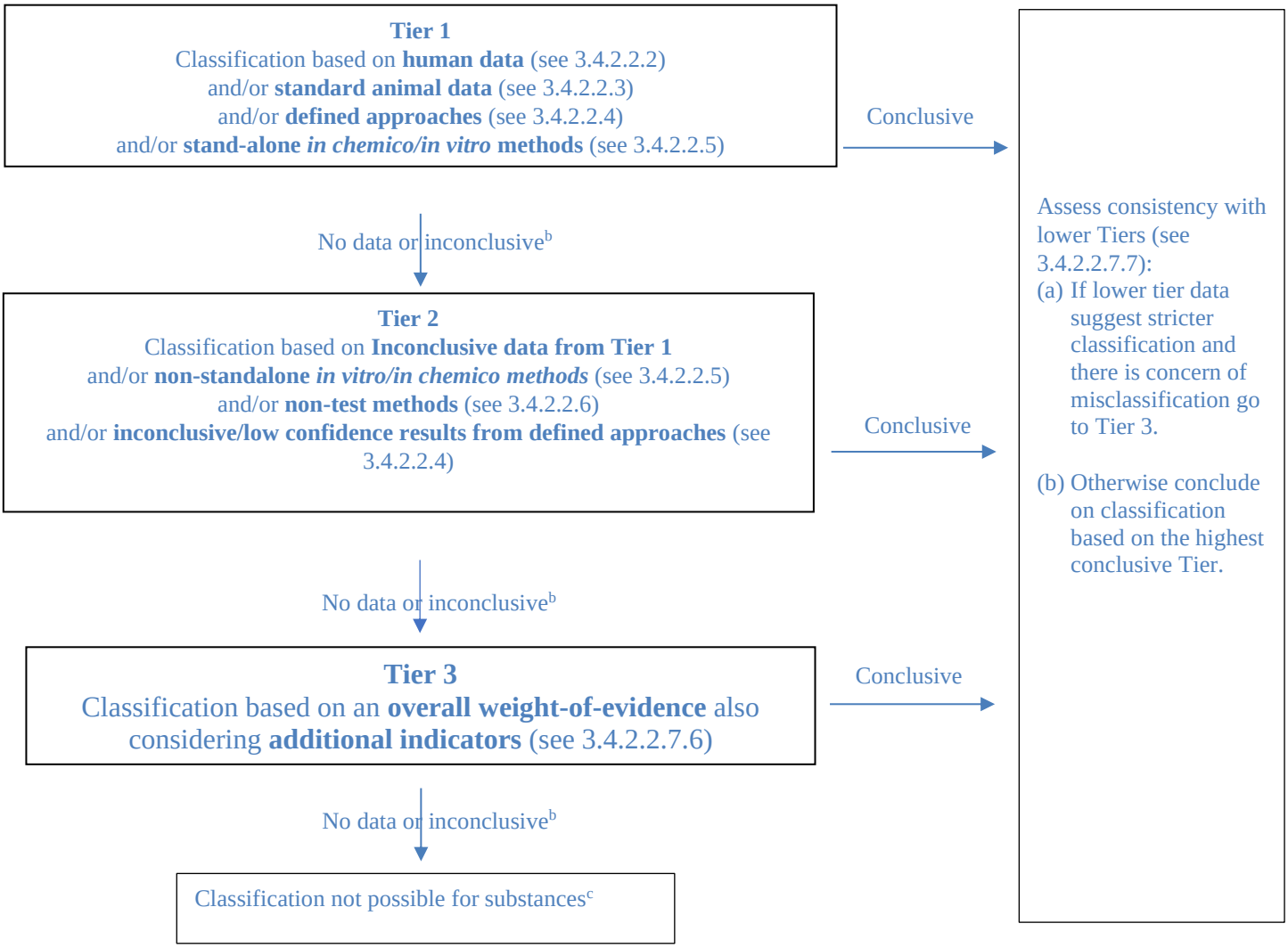
3.4.2.2.7.7 Where information from the various tiers is inconsistent and/or conflicting with respect to the resulting classification, information of sufficient quality from a higher tier is generally given a higher weight than information from a lower tier. However, when information from a lower tier 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 assessment (i.e. in Tier 3). For example, having consulted the guidance in 3.4.5.3 as appropriate, classifiers concerned with a negative result for skin sensitisation in a Buehler study when there is a clear positive result in humans for very similar substances (from read across) would utilise an overall weight of evidence approach.

3.4.2.2.7.~~7~~8 Immunological contact urticaria

Substances meeting the criteria for classification as respiratory sensitizers may in addition cause immunological contact urticaria. Consideration should be given to classifying these substances also as skin sensitizers. Substances which cause immunological contact urticaria without meeting the criteria for respiratory sensitizers should also be considered for classification as skin sensitizers.

There is no recognized animal model available to identify substances which cause immunological contact urticaria. Therefore, classification will normally be based on human evidence which will be similar to that for skin sensitization.

**Figure 3.4.1: Application of the tiered approach for skin sensitization[a]**



[a] *Before applying the approach, the explanatory text in 3.4.2.2.7 as well as the guidance in 3.4.5.3 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;*

- *Where competent authorities make use of the skin sensitsisation sub-categories 1A and 1B, the available data may not be capable of distinguishing between sub-category 1A and sub-category 1B.*

[c] *For mixtures, the flow chart in Figure 3.4.2 should be followed.[depending on discussion for mixtures]*

### 3.4.3 Classification criteria for mixtures

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

When reliable and good quality evidence from human experience or appropriate studies in experimental animals, as described in the criteria for substances, is available for the mixture, then the mixture can be classified by weight of evidence evaluation of these data. Care should be exercised in evaluating data on mixtures that the dose used does not render the results inconclusive. (For special labelling required by some competent authorities, see the note to Table 3.4.5 of this chapter and 3.4.4.2.)

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

3.4.3.2.1 Where the mixture itself has not been tested to determine its sensitizing properties, but there are sufficient data on both the individual ingredients and similar tested mixtures to adequately characterize 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 characterizing the hazards of the mixture without the necessity for additional testing in animals.

3.4.3.2.2 *Dilution*

If a tested mixture is diluted with a diluent which is not a sensitizer and which is not expected to affect the sensitization of other ingredients, then the new diluted mixture may be classified as equivalent to the original tested mixture.

3.4.3.2.3 *Batching*

The sensitizing properties 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 sensitization potential of the untested batch has changed. If the latter occurs, a new classification is necessary.

3.4.3.2.4 *Concentration of mixtures of the highest sensitizing category/sub-category*

If a tested mixture is classified in Category 1 or sub-category 1A, and the concentration of the ingredients of the tested mixture that are in Category 1 and sub-category 1A is increased, the resulting untested mixture should be classified in Category 1 or sub-category 1A without additional testing.

3.4.3.2.5 *Interpolation within one category/sub-category*

For three mixtures (A, B and C) with identical ingredients, where mixtures A and B have been tested and are in the same category/sub-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 category/sub-category as A and B.

3.4.3.2.6 *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) Ingredient B is a sensitizer and ingredients A and C are not sensitizers;

(e) A and C are not expected to affect the sensitizing properties of B.

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

3.4.3.2.7 *Aerosols*

An aerosol form of the mixture may be classified in the same hazard category as the tested non-aerosolized form of the mixture provided that the added propellant does not affect the sensitizing properties of the mixture upon spraying.

**3.4.3.3 *Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture***

The mixture should be classified as a respiratory or skin sensitizer when at least one ingredient has been classified as a respiratory or skin sensitizer and is present at or above the appropriate cut-off value/concentration limit for the specific endpoint as shown in Table 3.4.5 for solid/liquid and gas respectively.

**Table 3.4.5: Cut-off values/concentration limits of ingredients of a mixture classified as either respiratory sensitizers or skin sensitizers that would trigger classification of the mixture**

| **Ingredient classified as:** | **Cut-off values/concentration limits triggering classification of a mixture as:** | | |
|---|---|---|---|
| | **respiratory sensitizer Category 1** | | **skin sensitizer Category 1** |
| | **Solid/Liquid** | **Gas** | **All physical states** |
| **Respiratory sensitizer Category 1** | ≥ 0.1 % (see note) | ≥ 0.1 % (see note) | -- |
| | ≥ 1.0 % | ≥ 0.2 % | |
| **Respiratory sensitizer sub-category 1A** | ≥ 0.1 % | ≥ 0.1 % | |
| **Respiratory sensitizer sub-category 1B** | ≥ 1.0 % | ≥ 0.2 % | |
| **Skin sensitizer Category 1** | -- | -- | ≥ 0.1 % (see note) |
| | -- | -- | ≥ 1.0 % |
| **Skin sensitizer sub-category 1A** | -- | -- | ≥ 0.1 % |
| **Skin sensitizer sub-category 1B** | -- | -- | ≥ 1.0 % |

***NOTE:*** *Some competent authorities may require SDS and/or supplemental labelling only, as described in 3.4.4.2 for mixtures containing a sensitizing ingredient at concentrations between 0.1 and 1.0 % (or between 0.1 and 0.2 % for a gaseous respiratory sensitizer). While the current cut-off values reflect existing systems, all recognize that special cases may require information to be conveyed below that level.*

**3.4.4 Hazard communication**

3.4.4.1 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 3.4.6 below presents specific label elements for substances and mixtures that are classified as respiratory and skin sensitizers based on the criteria in this chapter.

**Table 3.4.6: Label elements for respiratory or skin sensitization**

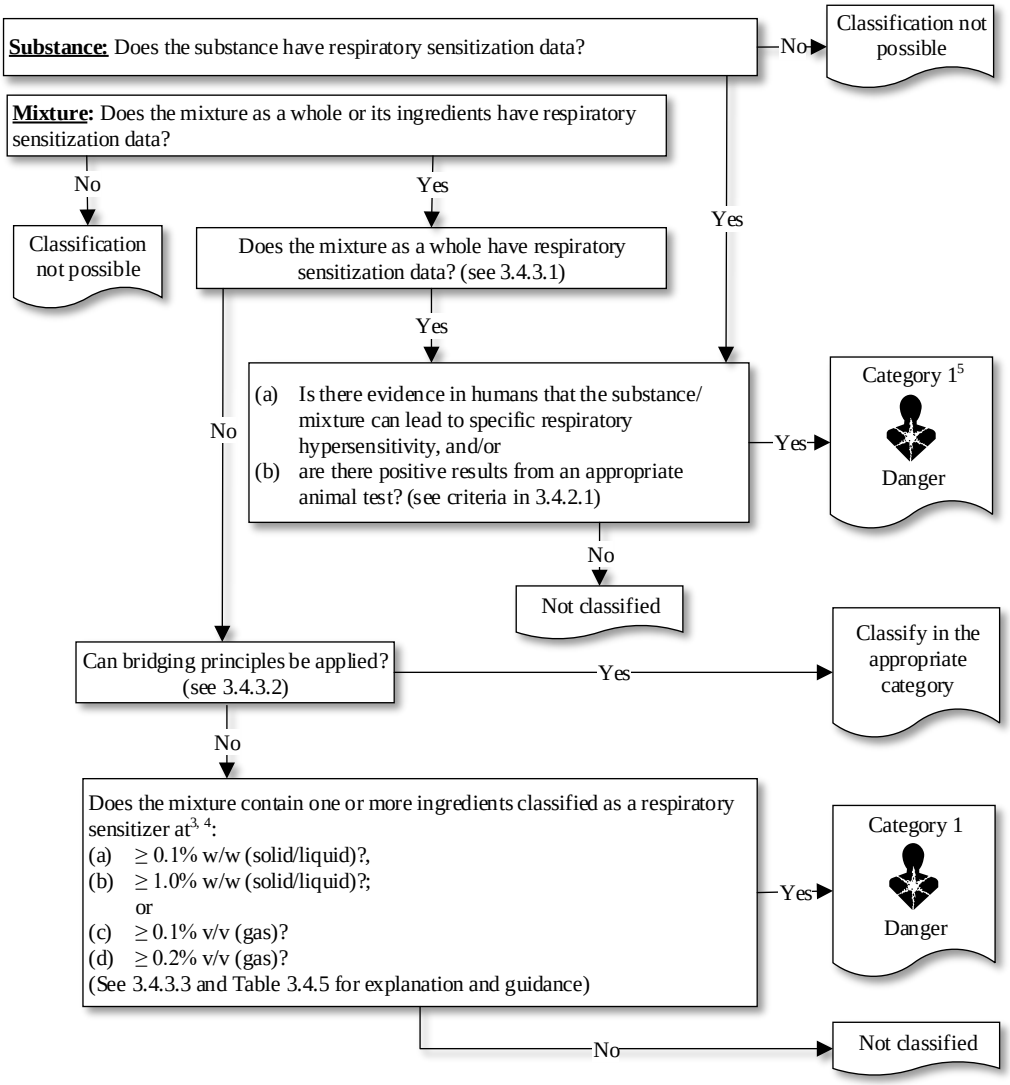
| | **Respiratory sensitization**<br>**Category 1 and sub-categories 1A and 1B** | **Skin sensitization**<br>**Category 1 and sub-categories 1A and 1B** |
|---|---|---|
| **Symbol** | Health hazard | Exclamation mark |
| **Signal word** | Danger | Warning |
| **Hazard statement** | May cause allergy or asthma symptoms or breathing difficulties if inhaled | May cause an allergic skin reaction |

3.4.4.2 Some chemicals that are classified as sensitizers may elicit a response, when present in a mixture in quantities below the cut-offs established in Table 3.4.5, in individuals who are already sensitized to the chemicals. To protect these individuals, certain authorities may choose to require the name of the ingredients as a supplemental label element whether or not the mixture as a whole is classified as sensitizer.

### 3.4.5 Decision logic and guidance

The decision logics which follow are not part of the harmonized 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.

### 3.4.5.1 *Decision logic 3.4.1 for respiratory sensitization*

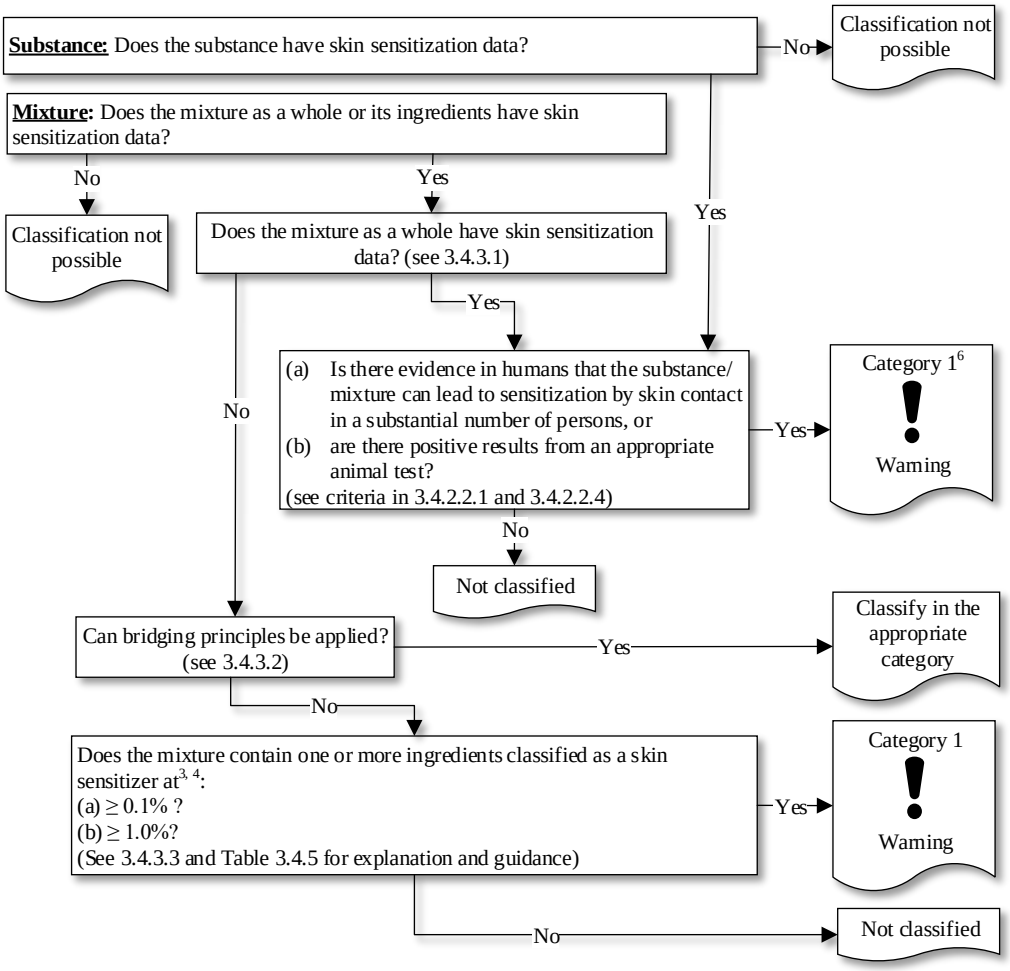



[5] *For specific concentration limits, see "The use of cut-off values/concentration limits" in Chapter 1.3, paragraph 1.3.3.2.*

[6] *See 3.4.4.2.*

[7] *See 3.4.2.1.1 for details on use of Category 1 sub-categories.*

### 3.4.5.2 *Decision logic 3.4.2 for skin sensitization*




### 3.4.5.3 Background guidance

[3] *For specific concentration limits, see "The use of cut-off values/concentration limits" in Chapter 1.3, paragraph 1.3.3.2.*

[4] *See 3.4.4.2.*

[8] *See 3.4.2.2.1 for details on use of Category 1 sub-categories.*

3.4.5.3.1 *Relevant guidance documents*

Mechanistic information on the process of skin sensitisation is available in the OECD document on the Adverse Outcome Pathway for skin sensitisation. This information can be helpful in understanding the value of the individual *in chemico* and *in vitro* methods compared to the *in vivo* methods.

### 3.4.5.3.2 *Guidance on the use of human data*

The classification of a substance can be based on human evidence generated from a variety of sources. These sources include human predictive patch testing, epidemiological studies, case studies, case reports or histories, diagnostic patch testing and medical surveillance reports, and poison control center information. This data may have been generated for consumers, workers, or the general population. When considering human evidence, consideration should be given to the size, exposure level, and exposure frequency of the exposed population (EU CLP, 2017).

Positive data from predictive patch testing (HRIPT or HMT) conducted through human experimental and clinical studies, showing allergic contact dermatitis caused by the test substance can be used to classify substances for skin sensitization These studies are generally conducted in controlled clinical settings using statistically significant population sizes. Criteria for evaluating this data are provided in paragraph 3.4.2.2.2.1 and 3.4.2.2.2.

Positive data from well-run epidemiological studies (according to WHO COIMS guidelines, 2009) can be used for classifying substances for skin sensitization. Some examples of epidemiological studies may include case control studies, cohort studies, cross-sectional studies, or longitudinal studies. These studies should have large sample sizes with well-documented exposures to a substance.

A specific type of epidemiological study (such as randomized control studies or trials) may include information from diagnostic patch testing. Diagnostic patch testing is considered by some competent authorities to be the gold standard in diagnosing contact allergy in dermatitis patients (Johansen et al, 2015). Importantly, due consideration needs to be given to the appropriate selection of vehicle, substance and patch test concentrations for the purpose of not causing false negatives, false positives, irritant reactions or inducing contact allergy (skin sensitisation). Positive data from data from experimental/clinical/diagnostic studies in man and/or well-documented episodes of allergic contact dermatitis may be used to classify substances for skin sensitization, when it can be assumed with sufficient likelihood that the tested substance was indeed the most likely cause for induction of sensitisation. Therefore, at least a general likelihood that the respective patient(s) had been previously exposed to the substance should be established. On the other hand, negative results from such tests are not sufficient to prove that the test substance should not be classified as a skin sensitiser. ~~Normally~~

Human data ~~are~~ not generated in controlled experiments with volunteers for the purpose of hazard classification (e.g. case studies, case reports and case histories, and poison control center information) ~~but rather as part of risk assessment to confirm lack of effects seen in animal tests. Consequently~~, ~~positive human data are usually derived from case control or other, less defined studies~~ can be used~~. Evaluation of human data must therefore be carried out~~ with caution. Consideration should be given to the frequency of cases, the inherent properties of the substances, as well as factors such as the exposure situation, bioavailability, individual predisposition and preventive measures taken.

Special consideration should be given to negative human data as full dose-response information is generally not available. For example, a negative result in an HRIPT or HMT at a low concentration may not allow for the conclusion that the substance does not have skin sensitising properties as such effect at a higher concentration may not be excluded. In addition, negative human data should not necessarily be used to negate positive results from animal studies and/or defined approaches, but can be used as part of a weight of evidence. For both animal and human data, consideration should be given to the impact of the vehicle. ~~In case multiple line of evidence are available under Tier 1 and when good quality human data are available, and it can be assumed that the effects observed are not underestimated by the above mentioned factors, these should be given higher weight as this gives information directly relevant to the species of interest.~~

For example, negative results from substances tested in a predictive patch test at DSA (dose per skin area) < 500 $\mu g/cm^2$ imply that a classification for skin sensitisation might not be needed at all, however, classification as category 1A or 1B cannot be ruled, because the concentration tested was not high enough to exclude these possibilities. The same holds for test results for which it is unknown whether the test concentration corresponded to a DSA < 500 $\mu g/cm^2$ Negative results from substances tested at DSA ≥ 500 $\mu g/cm^2$ suggest that classification might not be needed. but, while classification as category 1A can be ruled out, classification as category 1B cannot, because a higher test concentration might have resulted

in a positive test result. However, a negative test result at a concentration of 100% would reliably indicate the absence of a need for classification (based on this test).

Can we say something about the risk of classifying substances as 1B as the criteria are fulfilled whereas 1A cannot be excluded?

*3.4.5.3.3 Guidance on the use of animal data*

When classification into sub-categories is required by a competent authority and data fulfil the criteria for Category 1B but Category 1A cannot be excluded, Category 1 should be applied instead of Category 1B. This is particularly important if only data are available from certain tests like the Buehler, the GPMT and the rLLNA showing a high response after exposure to a high concentration but where lower concentrations, which could show the presence of effects at lower doses which would fulfil the criteria for Category 1A, have not been tested (in line with some test protocols where only one maximised dose should be used). For example, if in a GPMT a response in 100% of the animals is observed after intradermal exposure to 2% the criteria for category 1B are fulfilled. However, a response above 60% after intradermal exposure to 1%, which would fulfil the criteria for Category 1A, cannot be excluded. Therefore, classification into Category 1 should be applied.

A positive result in a guinea pig test is defined as a score above zero according to the applicable grading scale such as the Magnusson and Kligman grading scale for OECD TG 406 at one or more of the two observations. A score of 0.5, which is sometimes reported, is therefore also considered a positive result.

When in guinea pig tests positive results are also observed in the control animals, a correction should be made to the number of responding animal in the test group based on the number responding in the control group corrected for group size using subtraction to determine whether the criteria are fulfilled. For example when in a GPMT a response is observed in 9 out of 20 test animals and 2 out of 10 control animals (meaning 4 out of 20 after correction for group size), the response rate is (9-4)/(20-4) = 5/16 = 0.31 or 31%. This percentage of response would warrant classification. An alternative approach would be to base the correction on the percentage of responders which in this example would result in 45% - 20% = 25%. This percentage of response would not warrant classification.

3.4.5.3.4 *Data from defined approaches*

Defined approaches validated according to international procedures and described in OECD Guideline 497 have been characterized for the level of confidence that can be assigned to the predictions based on the applicability domain of the individual information sources used and the data interpretation procedure (DIP) applied (see Table 3.4.6).

Other defined approaches under consideration but not yet validated according to international procedures and described in OECD Guidance Document 256 according to internationally agreed criteria for their reporting (OECD Guidance Document 255) may be accepted by some competent authorities.

3.4.5.3.5 Data from non-stand-alone in *chemico/in vitro* methods

Individual *in chemico/in vitro* methods such as those reported in OECD Test Guidelines 442C, 442D and 442E, due to the limited mechanistic coverage, cannot be used on their own to conclude on Category 1 or no classification according to the criteria defined in table 3.4.5 and further data are necessary for classification in Tier 2. In addition, although some of these methods provides quantitative information, these cannot be used for the purposes of subcategorization into sub-category 1A and subcategory 1B since the criteria have not been validated according to international procedure. Nevertheless, such quantitative information may be accepted by a competent authority when used in weight-of-evidence under Tier 2 for the purpose of subcategorization.

3.4.5.3.6 Non-standard data

Validated but not yet adopted *in chemico/in vitro* methods such as those reported under 3.4.5.3.6.1 as well as *in vivo* test methods which do not comply with internationally agreed guidelines for the identification of skin sensitisers or assessment of skin sensitising potency may provide supportive evidence when used in an overall weight-of-evidence assessment (i.e. Tier 3).

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

- ~~The Genomic Allergen Rapid Detection (GARD) is a transcriptomics based *in vitro* assay addressing the third key event of the skin sensitisation Adverse Outcome Pathway (activation of dendritic cells) (Johansson et al., 2011; Johansson et al., 2014; Johansson et al., 2017; Corsini et al., 2021)~~

- The GARDpotency is a transcriptomics-based *in vitro* assay addressing the third key event of the skin sensitisation Adverse Outcome Pathway (activation of dendritic cells) similar to the GARDskin but uses a different gene signature that provides sub-categorization of skin sensitisers (Gradin et al., 2020; Zeller et al., 2017; Corsini et al. 2021)

- The SENS-IS assay is a genomic approach applied to a Reconstructed Human Epidermis (RHE) (Cottrez et al., 2015; Cottrez et al., 2016).

- The Epidermal Sensitization Assay (EpisensA) is based on the measurement of the upregulation of four genes in a reconstructed human epidermis (RhE) to discriminate between sensitisers and non-sensitisers (Saito et al., 2017).

# Table 3.4.7: Criteria for defined approaches

| Category | OECD GL 497 on Defined Approaches for Skin Sensitisation<br>"2 out of 3" (2o3) defined approach | OECD GL 497 on Defined Approaches for Skin Sensitisation<br>Integrated testing strategy (ITSv1) defined approach and<br>Integrated testing strategy (ITSv2 defined approach) |
|---|---|---|
| | **2o3** defined approach to skin sensitization hazard identification based on *in chemico* (KE1 -DPRA) and *in vitro* (KE2-KeratinoSens™/KE3-h-CLAT)<br><br>Assays are run for two KEs, and if these assays provide consistent results, then the chemical is predicted accordingly as sensitizer or non-sensitizer. If the first two assays provide discordant results, the assay for the remaining KE is run. The overall result is based on the two concordant findings taking into account the confidence on the obtained predictions as described in the GL | **ITSv1** based on *in chemico* (KE1-DPRA) and *in vitro* (KE3-h-CLAT) data, and *in silico* (Derek Nexus) predictions.<br>**ITSv2** based on *in chemico* (KE1 -DPRA) and *in vitro* (KE3 -h-CLAT) data, and in silico (OECD QSAR Toolbox) predictions.<br><br>Quantitative results of h-CLAT and DPRA are converted into a score from 0 to 3. For the *in silico* prediction (Derek or OECD QSAR TB), a positive outcome is assigned a score of 1; a negative outcome is assigned a score of 0. When these scores have been assessed, a total battery score ranging from 0 to 7, calculated by summing the individual scores, is used to predict the sensitizing potential (hazard identification; UN GHS Cat. 1 vs. UN GHS NC) and potency (UN GHS Cat. 1A, Cat. 1B and NC). |
| **1** | 2 out of 3 or 3 out of 3 positive predictions | Total battery score ≥ 2 |
| **1A** | Not Applicable | Total battery score 6-7 |
| **1B** | Not Applicable | Total battery score 2-5 |
| **Not classified** | 2 out of 3 or 3 out of 3 negative predictions | Total battery score < 2 |

# Table 3.4.8: Criteria for individual *in chemico/in vitro* methods

| Category | OECD TG 442C<br>Key event-based Test Guideline for *in chemico* skin sensitisation assays addressing the AOP Key Event on covalent binding to proteins | | | OECD TG 442D<br>Key event-based Test Guideline for *in vitro* skin sensitisation assays addressing the AOP Key Event on keratinocyte activation<br>ARE-Nrf2 luciferase methods | | OECD TG 442E<br>In vitro skin sensitisation assays addressing the AOP Key Event on activation of dendritic cells | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Method described in Appendix I**<br>**The Direct Peptide Reactivity Assay (DPRA)[a]** | **Method described in Appendix II**<br>**The Amino acid Derivative Reactivity Assay (ADRA)[a]** | **Method described in Appendix III**<br>**The kinetic Direct Peptide Reactivity Assay (kDPRA)[b]** | **Method described in Appendix 1A**<br>**KeratinoSens™[a]** | **Method described in Appendix 1B**<br>**Lusens[a]** | **Method described in Annex I**<br>**human Cell Line Activation Assay (h-CLAT)[a]** | **Method described in Annex II**<br>**U937 Cell Line Activation Test[a]** | **Method described Annex III**<br>**IL-8 Luc assay[a]** | **Method described Annex IV**<br>**GARD skin ™** |
| | Methods: in chemico methods addressing the process of haptenation by quantifying the reactivity of test chemicals towards model synthetic peptides containing either lysine or cysteine (DPRA and kDPRA) or towards model synthetic amino acid derivatives containing either cysteine (NAC) or lysine (NAL) (ADRA).<br>The criteria are based on the mean of cysteine and lysine peptides percent depletion (DPRA), kinetic rates of cysteine depletion (kDPRA) and mean NAC and NAL percent depletion value (ADRA). Predictions models based on the cysteine or NAC percent depletion value alone in case the unreacted lysine peptide or NAL cannot be reliably measured can be applied for the DPRA and ADRA. | | | Methods: ~~Two similar~~ cell-based methods addressing the process of keratinocytes activation, by assessing with the help of luciferase, the Nrf2-mediated activation of antioxidant response element (ARE)-dependent genes following exposure of the cells to the test chemical.<br>Cell viability is quantitatively measured in parallel by enzymatic conversion of the dye MTT.<br>The criteria are based on the induction of the luciferase gene above a given threshold, quantified at subtoxic concentrations. Criteria should be met in 2 of 2 or in 2 of 3 repetitions. | | Methods: ~~three~~ cell-based methods ~~are~~ addressing the process of monocytes/dendritic cell activation by either quantifying the change in the expression of cell surface marker(s) (e.g. CD54, CD86) or the change in IL-8 expression or the transcriptional patterns of an endpoint-specific genomic biomarker signature following exposure of the cells to the test chemical.<br><br>Criteria should be met in 2 of 2 or in at least 2 of 3 repetitions for test methods described in Annexes I, II and III or in three valid biological replicates for test method described in Annex IV. | | | |
| **1** | The mean cysteine/lysine % depletion > 6.38%<br>Or<br>the mean cysteine % depletion > 13.89 % | The mean NAC and NAL % depletion ≥ 4.9%<br>Or<br>NAC% depletion ≥ 5.6% | Not applicable | The following 4 conditions are all met in 2 of 2 or in the same 2 of 3 repetitions:<br>1. Imax equal or higher than (≥) 1.5 fold and statistically significantly different to the solvent control<br>2. The cellular viability is higher than (>) 70% at the lowest concentration with induction of luciferase | The following conditions are all met in 2 of 2 or in the same 2 of 3 repetitions:<br>1. A luciferase induction above or equal to (≥) 1.5 fold as compared to the solvent control is observed in at least 2 consecutive non-cytotoxic tested | At least one of the following conditions is met in 2 of 2 or in at least 2 of 3 independent runs:<br>The Relative Fluorescence Intensity of CD86 is equal to or greater than 150% at any tested concentration | The following condition is met in 2 of 2 or in at least 2 of 3 independent runs:<br>The stimulation index of CD86 is equal or higher (≥) than 150% and/or interference is observed | The Ind-IL8LA is equal or higher than (≥) 1.4 and the lower limit of the 95% confidence interval of Ind-IL8LA is equal or higher than (≥) 1.0 in at least 2 out of a maximum | The mean Decision Value (DV) is ≥0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | activity equal or above 1.5 fold<br>3. The $EC_{1.5}$ value is less than (<) 1000 µM (or < 200 µg/mL for test chemicals with no defined MW)<br>4. There is an apparent overall dose-dependent increase in luciferase induction | concentrations (i.e. cellular viability is equal or higher than (≥) 70%)<br>2. At least three tested concentrations should be non-cytotoxic (cellular viability equal or higher than (≥) 70%). | (with cell viability ≥ 50%)<br>or<br>the Relative Fluorescence Intensity of CD54 is equal to or greater than 200% at any tested concentration (with cell viability ≥ 50%). | | of 4 independent runs | |
| **1A** | Not applicable | | $\log k_{max} \geq -2.0$ | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable |
| **1B** | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable |
| **Not classified** | The mean cysteine/lysine % depletion ≤ 6.38%<br>or<br>the mean cysteine % depletion ≤ 13.89 % | The mean NAC and NAL % depletion < 4.9%<br>Or<br>NAC% depletion < 5.6% | Not applicable | At least one of the conditions for Category 1 is not met | At least one of the conditions for Category 1 is not met | None of the conditions for Caterory 1 is met | The stimulation index of CD86 is < 150% at all non-cytotoxic concentrations (cell viability ≥ 70%) and if no interference is observed | The Ind-IL8LA is less than (<) 1.4 and/or the lower limit of the 95% confidence interval of Ind-IL8LA is less than (<) 1.0 in at least 3 out of a maximum of 4 independent runs | The mean Decision Value (DV) is <0 |

[a] Data can be used as stand-alone to conclude on classification in sub-category 1A

[b] Data cannot be used as stand-alone to conclude on classification in Category 1 or on no classification in tier 1 but could be used to conclude on classification in category 1 in Tier 2.

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