Secretariat

Distr.: General
15 September 2022

Original: English

___

**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**

Forty-third session
Geneva, 7-9 December 2022
Item 3 (c) of the provisional agenda
**Work on the Globally Harmonized System of Classification and
Labelling of Chemicals: use of non-animal testing methods
for classification of health hazards**

Revision of Chapter 3.4 to fully incorporate non-animal testing methods for skin sensitization

Transmitted by the experts from the United Kingdom and the Netherlands on behalf of the informal working group on the use of non-animal testing methods for classification of health and environmental hazards*

Introduction

1.   This document summarises the work of the informal working group on "Use of non-animal testing methods for classification of health hazards" (NATM IWG) on Chapter 3.4 (Respiratory or Skin Sensitization) for skin sensitization in accordance with the programme of work for the 2021-2022 biennium[1]. This document, together with informal document INF.3, presents for the agreement of the Sub-Committee a revision of this chapter to better reflect the increased capability, availability and utility for classification of *in chemico/in vitro* test methods, defined approaches and of non-test methods such as computer models and read-across. This proposal is limited to changes to the criteria for the classification of substances. The discussion on the guidance and the criteria for mixtures will continue. The group anticipates that updated proposals on the guidance will be provided in a supplementary informal document that the group plans to submit for the consideration of the Sub-Committee at the forty-third session, with proposals for mixtures anticipated to be submitted early in the next biennium. In addition, to align chapters 1.2, 3.2 and 3.3 with Chapter 3.4, a number of conforming changes are also proposed to those chapters (see ST/SG/AC.10/C.4/2022/15).

___

*   A/75/6 (Sect.20), para. 20.51.
[1]   See ST/SG/AC.10/C.4/78

Background

2.   The terms of reference the Sub-Committee gave to the informal working group (see informal document INF.26 from the thirty-ninth session) set out five main activities:

(a)   To identify and evaluate[2] the available *in vitro* and *in chemico* test methods, validated at the international level, and the existing guidance on *in silico* methods (including grouping approaches, quantitative structure activity relationship (QSARs) and read-across), taking into account their limitations, uncertainties and expected future developments, that could be useful for GHS hazard classification for health hazard and environmental hazard classes, using a step-wise approach and starting with a hazard class to be determined by group;

(b)   For each relevant hazard class and category, to assess:

(i)   Where substances and mixtures may be classified using non-animal methods, utilizing all relevant scientific information and whether new or amended GHS classification criteria are needed to facilitate the use of such methods for hazard classification; and

(ii)   Whether an integrated or tiered evaluation approach taking into account all relevant scientific information and combination of methods for hazard classification should be developed.

(c)   To prepare draft amendments and additions to the GHS to facilitate hazard classification using non-animal methods where appropriate, taking into account relevant limitations and uncertainties. The amendments and additions should include as appropriate: classification criteria, notes, decision logics, tiered evaluation and guidance; and should take into account the needs of all sectors; and so far as possible, should provide a consistent approach across the different hazard classes.

(d)   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 for implementation or present them in a working paper directly to the Sub-Committee;

(e)   To report progress to the Sub-Committee as appropriate. The latest status update will be provided as an informal document for the forty-third session.

3.   The informal working group has around sixty members, reflecting the importance of, and interest in, its work. The group's discussions are very detailed and are propelled by a strong desire to make progress on the group's mandate to ensure that non-animal testing methods are consistently incorporated in the GHS in a way that reflects their growing importance and scientific relevance, whilst recognising their limitations.

4.   In May 2021, the group commenced their work on updating Chapter 3.4 for skin sensitization for the inclusion of non-animal testing methods. The informal working group agreed that the update of Chapter 3.4 would be in line as far as possible with Chapter 3.2 (skin corrosion/irritation) and Chapter 3.3 (serious eye damage/eye irritation) that were revised to include non-animal testing methods.

5.   The group is very active, both via correspondence and through virtual meetings, to resolve the issues. For example, during the 2021-2022 biennium the group held, or have been scheduled before the end of the forty second session, fifteen webinars and two face-to-face meetings (July and December 2022), the first two focused on completing Chapter 3.3, with the remaining meetings focussed on completing the group's work on revising Chapter 3.4 for skin sensitization this biennium. In addition, a small subgroup led by colleagues from the United States of America, with the assistance from the United Kingdom, Netherlands,

---

[2]   It is not foreseen to have a complete evaluation of all existing guidance or to cover all new developments. The work by the informal working group should focus on relevant information in relation to the possible amendments or additions to GHS classification.

Canada, the European Chemicals Agency (ECHA), the European Commission's Joint Research Centre (JRC) and Germany, have focussed on particular technical issues which has greatly assisted the group in reaching a resolution to those issues and hence the progression the revision of the chapter. After each meeting, the Netherlands and the United Kingdom, as joint leads, together with the JRC, have revised the draft text of 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 members of the group.

6.   Paragraphs 7 to 26 below provide the Sub-Committee with an indication of the nature of the work that has been undertaken, the key issues that have been identified, and the solutions that have been adopted. The proposed changes made to Chapter 3.4 are provided in the annex to this document. For clarity the full text of the revised Chapter 3.4 is set out in informal document INF.3 with indication of where the text has changed relative to the ninth revised edition of GHS.

Key issues and outcomes

Tiered approach

7.   In line with chapters 3.2 and 3.3, the tiered approach in Chapter 3.4 for skin sensitization is applied with some necessary amendments. Similar to chapters 3.2 and 3.3, a weight of evidence assessment can also be applied where the available information gives inconsistent and/or conflicting results within a tier, and an overall weight of evidence assessment where there are inconsistent and/or conflicting results between tiers. The order of the tiers in Chapter 3.3 is as follows:

Tier 1 - Classification based on human data, standard animal data, defined approaches or stand-alone *in chemico/in vitro* methods;

Tier 2 - Classification based on inconclusive data from Tier 1, non-standalone *in vitro/in chemico* methods, non-test methods or inconclusive/low confidence results from defined approaches;

Tier 3 – Classification based on overall weight-of-evidence, including additional indicators.

8.   In addition, and to assist readers of Chapter 3.4 in the GHS for skin sensitization classification, amendments to section headings under "Classification criteria for substances" are also proposed to include a reference, in brackets, linking the section to the applicable tier in Figure 3.4.1.

In chemico/in vitro and non-test methods

9.   In line with chapters 3.2 and 3.3, a new sub-section has been added on how to classify for skin sensitization based on non-test methods (3.4.2.2.6). The non-test methods include computer models predicting structure-activity relationships, computer expert systems and read-across using analogue and category approaches.

10.   Unlike for the hazard classes provided in Chapters 3.2 and 3.3 where ex vivo test methods are available, there are no such validated methods currently available for classification for skin sensitization. In terms of non-animal test methods only *in vitro* (cell based systems) and *in chemico* (test tube methods) are currently available for skin sensitization and have been included as *in chemico*/*in vitro* data (3.4.2.2.5).

11.   The criteria for classification for skin sensitization and for no classification based on OECD individual *in chemico*/*in vitro* test methods are set out in Table 3.4.7 of the background guidance.

Defined approaches

12.   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.

13.   The working group agreed that since several defined approaches have already been developed/validated for classification for skin sensitization that are already accepted at the national level and validated according to international procedures (such as OECD Guideline 497 or an equivalent approach), the use of defined approach guidelines could be included within the tiered approach in Chapter 3.4 of the GHS for skin sensitization.

14.   The outcomes of the group discussions on the inclusion of defined approaches are new sub-sections in the main text and background guidance to Chapter 3.4 as follows:

>   (a)   Classification based on defined approaches (3.4.2.2.4);
>
>   (b)   Classification in a tiered approach (3.4.2.2.7);
>
>   (c)   Inclusion of defined approaches within Tier 1 on the application of the tiered approach for skin sensitization (Figure 3.4.1);
>
>   (d)   Inclusion of inconclusive/low confidence results from defined approaches within Tier 2 on the application of the tiered approach for skin sensitization (Figure 3.4.1);
>
>   (e)   Guidance on the use of defined approaches for classification for skin sensitization (3.4.5.3.4 and Table 3.4.6);

15.   As the concept of defined approaches is being proposed for skin sensitization classification in Chapter 3.4 in addition to being included in the adopted amendments for serious eye damage/eye irritation in Chapter 3.3 (see ST/SG/AC.10/C.4/2021/4 and ST/SG/AC.10/C.4/80) at the fortieth session, the group considered that it would also be appropriate to include explanatory text on defined approaches within Chapter 1.2 as provided in ST/SG/AC.10/C.4/2022/15.

Application of a weight-of-evidence evaluation for classification for skin sensitisation based on *in chemico/in vitro* methods

16.   The proposed GHS criteria require a weight-of-evidence assessment in case there are conflicting or inconsistent data.

17.   To develop guidance, an exercise was performed for which experts were requested to provide a classification for each combination of outcomes (category 1A, 1B, 1, no classification) for each type of data (human data, animal data, defined approaches and stand-alone in vitro data) and the rules by which these classifications were derived.

18.   Based on an analysis of the results, some suggestions for guidance on the application of the weight-of-evidence assessment were discussed but since no agreement had been reached at the time of submission of this document, the discussions on this issue will continue and an update will be provided to the Sub-Committee at the forty-third session.

Classification in category 1 or 1B when category 1A cannot be excluded

19.   In the exercise for the development of guidance on the weight-of-evidence assessment it was found out that there are differences between competent authorities in the interpretation of the criteria when the available data fulfil the numerical criteria for classification in sub-category 1B but where the data do not allow exclusion that category 1A is applicable.

20.   Some competent authorities require classification in category 1B whereas others require classification in category 1. This has no consequences for the labelling of substances. However, it introduces disharmonization of the classification of mixtures depending on the applied cut-off value/concentration limit for ingredients classified as category 1.

21.   Changing the criteria is not within the scope of the NATM IWG but the use of guidance to explain the difference in interpretation of the criteria was discussed. Based on this guidance, competent authorities could decide on their interpretation and provide legal clarity via guidance to their legislation. Although at the September webinar no agreement was reached, the group propose to include a short paragraph to provide guidance on the weight of evidence assessment (see 3.4.5.3.7) and will undertake further discussions with the aim of providing an updated proposal on this issue for consideration at the forty-third session.

Classification of mixtures

22.   The applicability of the non-animal methods for the classification of mixtures based on data for the whole mixture was discussed as for the OECD methods, the validation for mixtures was limited and there was unclarity in the applicability of the defined approaches to mixtures.

23.   Although at the September meeting no agreement was reached, the group agreed to continue the discussions with the aim of providing formal proposals on the amendments to the classification of mixtures for skin sensitization sections of Chapter 3.4 for the consideration of the Sub-Committee early in the 2023-2024 biennium.

Test method neutrality

24.   For health and environmental hazards, paragraph 1.3.2.4.3 of the GHS sets out the principle that "tests that determine hazardous properties, which are conducted according to internationally recognized scientific principles, can be used for purposes of a hazard determination.

25.   The GHS criteria are test method neutral, allowing different approaches as long as they are scientifically sound and validated according to international procedures and criteria already referred to in existing systems for the hazard of concern and produce mutually acceptable data." In revising Chapter 3.4 the informal working group has tried hard to maintain this principle, whilst continuing to provide practical information to GHS users.

Presentation of classification criteria and background guidance

26.   As well as adding new sub-sections on how to classify for skin sensitization based on defined approaches (3.4.2.2.4), *in chemico/in vitro* data (3.4.2.2.5) and on non-test methods (3.4.2.2.6), the opportunity has been taken to pull together in one sub-section (3.4.2.2.2) existing text on classification using human data and one sub-section (3.4.2.2.3) for existing text on classification using animal data, with a cross reference to the related background guidance to Chapter 3.4 where five new sub-sections are introduced:

  (a)   Guidance on the use of human data (3.4.5.3.2) and animal data (3.4.5.3.3) for the classification skin sensitizers; and

  (c)   Guidance on the use of defined approaches (3.4.5.3.4); non-stand-alone *in chemico/in vitro* methods (3.4.5.3.5); and non-standard data (3.4.5.3.6) for the classification skin sensitizers.

Conforming changes to chapters 3.2, 3.3 and 1.2

27.   The proposed changes to Chapter 3.4 necessitate a number of conforming changes to be made to chapters 3.2 and 3.3 to ensure consistency of approach between the three chapters.

28.   Specifically, in relation to the proposed changes in Chapter 3.4, new text is also proposed to be included in chapters 3.2 and 3.3 on classification based on non-test methods

(new 3.2.2.6.4 and 3.3.2.8.5, with amendment to 3.3.2.8.1) and in Chapter 3.3 on classification based on defined approaches (new 3.3.2.3,2 and 3.3.2.3.3; and amendment to 3.3.2.8.1) and *in vitro/ex vivo* data (new 3.3.2.4.2) as proposed in ST/SG/AC.10/C.4/2022/15.

29.   In addition, amendments will also be required to Chapter 1.2, as proposed in ST/SG/AC.10/C.4/2022/15 to include a new definition on defined approaches as this concept will now be included in two chapters of the GHS, upon adoption of Chapter 3.4.

Action and next steps

30.   The Sub-Committee is invited to agree the revised Chapter 3.4 as set out in the annex to this document and as provided in full in informal document INF.3.

31.   Looking ahead, the informal working group recognizes the longer-term nature of this work to ensure that non-animal testing methods are consistently incorporated in the GHS in a way that reflects their growing importance and scientific relevance, whilst recognising their limitations. Activities planned include a further update to Chapter 3.4 with a focus on non-animal methods for respiratory sensitisation in line with the informal working group's mandate and proposed workplan for the 2023-2024 biennium.

Annex

Proposed amendments to Chapter 3.4 for skin sensitisation

3.4.2.2.1.2   Amend the reference to "3.4.2.2.1.3" to read: "3.4.2.2.2 – 3.4.2.2.6".

3.4.2.2.1.3   Replace with the following:

"3.4.2.2.1.3   For classification of skin sensitizers, all available and relevant information is collected and its quality in terms of adequacy and reliability is assessed. Classification should be based on mutually acceptable 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."

3.4.2.2.1.3   Delete Table 3.4.2.

3.4.2.2.1.4 and 3.4.2.2.1.5   Insert the following new two paragraphs:

"3.4.2.2.1.4   A tiered approach (see 3.4.2.2.7) organizes the available information on skin sensitization 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 3.4.2.2.7.7) or where data individually are insufficient to conclude on the classification, an overall weight of evidence assessment is used (see 3.4.2.2.7.6 and 1.3.2.4.9).

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   Replace the heading with the following:

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

3.4.2.2.2.1   Insert the following new paragraph:

"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 (former 3.4.2.2.2.1)   Insert two new sentences at the beginning of 3.4.2.2.2.2 to read:

"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."

3.4.2.2.2.3 (former 3.4.2.2.2.2)   Insert two new sentences at the beginning of 3.4.2.2.2.3 to read:

"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."

3.4.2.2.3   Replace the heading with the following:

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

3.4.2.2.3.1   Insert a new first sentence at the beginning of 3.4.2.2.3.1 to read:

"A substance is classified as a skin sensitizer if there are positive results from an appropriate animal test."

3.4.2.2.3.2   Insert two new sentences at the beginning 3.4.2.2.3.2 to read:

"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."

3.4.2.2.3.2   In 3.4.2.2.3.2 and table header, amend the references to table 3.4.3 to read: "Table 3.4.2".

3.4.2.2.3.3   Insert two new sentences at the beginning of 3.4.2.2.3.3 to read:

"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."

3.4.2.2.3.3   In 3.4.2.2.3.3 and table header, amend the references to table 3.4.4 to read: "Table 3.4.3".

3.4.2.2.4   Replace 3.4.2.2.4 heading and paragraphs 3.4.2.2.4.1; 3.4.2.2.4.2; and 3.4.2.2.4.3 with the following (including the related new footnotes 3 and 4):

"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 sensitization 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.

3.4.2.2.4.3   Some evidence can be used individually and in defined approaches. Evidence used within defined approaches should then not also be used individually within a weight of evidence assessment.

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 described, for example, in the OECD Adverse Outcome Pathway for Skin Sensitisation (see OECD, 2014). 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 whether to use the method

described in Appendix III to OECD Test Guideline 442C as a stand-alone method to discriminate between category 1A and those not categorized as category 1A (see 3.4.5.3.5).

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 (Annex I and II), 442D and 442E, are accepted as supportive evidence and should within Tier 1 only be used in combination with other types of data in defined approaches. The use of these methods in Tier 2 is described in 3.4.2.2.7.5. When already considered within a defined approach, non-stand-alone *in chemico/in vitro* methods should not be considered as an additional line of evidence (see 3.4.2.2.7.4).

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.

3.4.2.2.5.4   *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 to be classified. Where adequate justification of the read-across approach is provided, it has in general higher weight than (Q)SARs.

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 recognized 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.

3.4.2.2.6.4   For conclusions on no classification from read-across and (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, as well as a clear 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   A tiered approach to the evaluation of information should be considered, where applicable (Figure 3.4.1), recognizing that not all tiers as well as information within a tier may be relevant. However, all available and relevant information of sufficient quality needs to be examined for consistency with respect to the resulting classification.

3.4.2.2.7.2   Tier 1 - Classification based on human data, standard animal data, defined approaches or stand-alone *in chemico/in vitro* methods

For classification of a substance, evidence in Tier 1 may include data from 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 in a weight of evidence assessment:

(a)   Experimental studies in humans (e.g., predictive patch testing, human repeated insult patch test (HRIPT), human maximization test (HMT) (see paragraph 1.3.2.4.7, criteria in 3.4.2.2.2.2 (a) and 3.4.2.2.2.3 (a) and guidance 3.4.5.3.2);

(b)   Epidemiological studies (e.g., case control studies, prospective studies) assessing allergic contact dermatitis (see paragraph 1.3.2.4.7, criteria in 3.4.2.2.2.2 (b and c) and 3.4.2.2.2.3 (b and c) and guidance 3.4.5.3.2);

(c)   Well-documented cases of allergic contact dermatitis (see criteria in 3.4.2.2.2.2 (b) and 3.4.2.2.2.3 (b) and guidance 3.4.5.3.2);

(d)   Appropriate animal studies (see criteria in 3.4.2.2.3, and guidance 3.4.5.3.3);

(e)   Defined approaches validated according to international procedures (see 3.4.2.2.4, guidance 3.4.5.3.4, and Table 3.4.6);

(f)   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 3.4.7).

3.4.2.2.7.3   Tier 2 - Classification based on inconclusive data from Tier 1, non-stand alone *in chemico/in vitro* methods, non-test methods or low confidence/inconclusive results from defined approaches

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.

3.4.2.2.7.5   Individual non-stand alone *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–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-stand alone *in chemico/in vitro* methods, may be used on its own to classify in category 1 (see Table 3.4.7).

3.4.2.2.7.6   Tier 3 - Classification based on overall weight-of-evidence, including additional indicators

In case a definitive conclusion on classification including sub-categorization where required by a competent authority, cannot be derived from the previous tiers, an overall weight-of-evidence assessment using expert judgment should be used that may include a combination of two or more indicators of skin sensitization as listed below.

(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)   Data from non-standard methods;

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 sensitization 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."

3.4.2.2.4.4   Current paragraph 3.4.2.2.4.4 'Immunological contact urticaria' becomes new section 3.4.2.2.8 and amend the two currently unnumbered sub-paragraphs under this heading to read as follows:

"3.4.2.2.8.1   Substances meeting the criteria for…."

"3.4.2.2.8.2   There is no recognized animal model available…"

Insert the following new footnotes 3 and 4 at the bottom of the page in relation to paragraphs 3.4.2.2.4.1 (for footnote 3) and 3.4.2.2.5.3 (for footnote 4):

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

"[4] *Additional in chemico/in vitro methods have been proposed for skin sensitization (see 3.4.5.3.6.1) but no classification criteria have yet been agreed internationally.*"

Figure 3.4.1   Insert new Figure 3.4.1 "*Application of the tiered approach for skin sensitization*" after the new paragraph 3.4.2.2.8 (former 3.4.2.2.4.4), as follows:
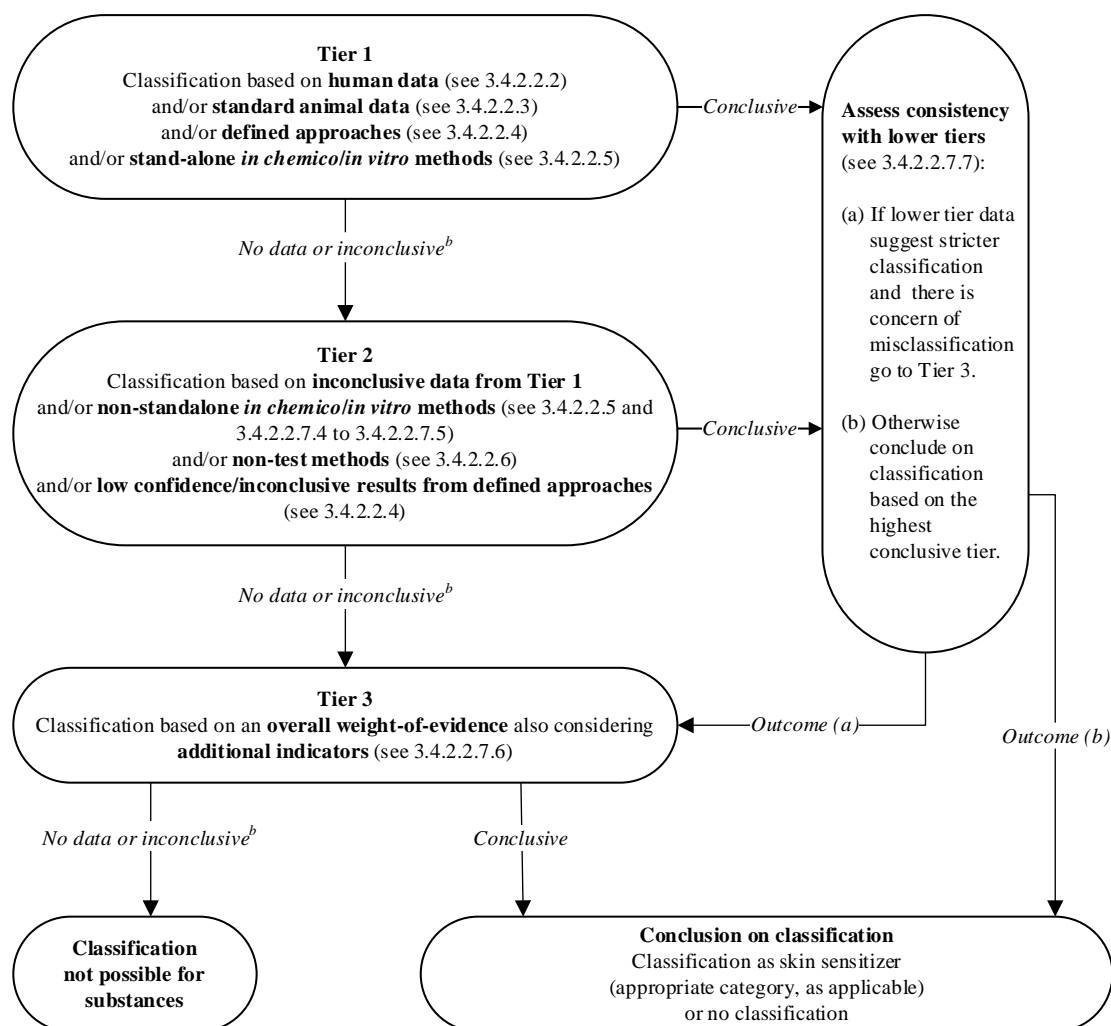
Figure 3.4.1: Application of the tiered approach for skin sensitization[a]

Figure 3.4.1   Insert the following notes to the new Figure 3.4.1 as follows:

"[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 sensitization sub-categories 1A and 1B, the available data may not be capable of distinguishing between sub-category 1A and sub-category 1B."*

3.4.3.1   Within the brackets of 3.4.3.1, amend the reference to table 3.4.5 to read: "Table 3.4.4".

3.4.3.3   In 3.4.3.3 and the table heading, amend the references to table 3.4.5 to read: "Table 3.4.4".

3.4.4.1   In 3.4.4.1 and the table heading, amend the references to table 3.4.6 to read: "Table 3.4.5".

3.4.4.2   In 3.4.4.2, amend the reference to table 3.4.5 to read: "Table 3.4.4".

3.4.5   Replace the heading with: "**3.4.5   Decision logic and guidance**"

3.4.5.1   In decision logic 3.4.1, for respiratory sensitization':

- In the second right hand text box from the top, replace "Category 1[5]" with "Category 1[7]".

- In the bottom left hand text box starting with "Does the mixture contain…":
  - Amend the footnote references '3, 4' to read: "5, 6".
  - Amend the reference to table 3.4.5 to read: "Table 3.4.4".

- Renumber the current footnotes beneath decision logic 3.4.1 as follows:
  - Amend footnote number '3' to read: "5".
  - Amend footnote number '4' to read: "6".
  - Amend footnote number '5' to read: "7".

3.4.5.2   In 'Decision logic 3.4.2, for skin sensitization':

- In the central text box starting with "(a) Is there evidence…":
  - Amend the beginning of sub-paragraph (a) to read as follows: "(a) Is there evidence in humans that the substance (see 3.4.2.2.1 and 3.4.2.2.7.8)/mixture (see 3.4.3) can lead to….".
  - Delete "(see criteria in 3.4.2.2.1 and 3.4.2.2.4)"

- In the second right hand text box from the top, replace "Category 1[6]" with "Category 1[8]"

- In the bottom left hand text box starting with "Does the mixture contain…":
  - Amend the footnote references '3, 4' to read: "5, 6".
  - Amend the reference to table 3.4.5 to read: "Table 3.4.4".

- Renumber the current footnotes beneath 'Decision logic 3.4.2 as follows:
  - Amend the footnote number '3' to read: "5".
  - Amend the footnote number '4' to read: "6".
  - Amend the footnote number '6' to read: "8".

3.4.5.3   Insert the following new text:

"3.4.5.3   *Background guidance*

3.4.5.3.1   *Relevant guidance documents*

        Mechanistic information on the process of skin sensitization is available in the OECD document on the Adverse Outcome Pathway for skin sensitization (OECD, 2014). 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*

3.4.5.3.2.1   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 centre 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. Guidance for evaluating human evidence and the criteria in 3.4.2.2.2 are provided by some competent authorities (e.g., ECHA Guidance on the Application of the CLP Criteria, 2017).

3.4.5.3.2.2   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 and the larger the population size, the more reliable the study outcome is. Criteria for evaluating this data are provided in paragraph 3.4.2.2.2.1 and 3.4.2.2.2.

3.4.5.3.2.3   Positive data from well-run epidemiological studies (in accordance with 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.

3.4.5.3.2.4   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 sensitization). Positive 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, it should be established that there is at least a general likelihood that the respective patient(s) had been previously exposed to the substance. 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.

3.4.5.3.2.5   Human data 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 centre information) can be used 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, cross-reactivity and preventive measures taken.

3.4.5.3.2.6   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 (e.g. Wright et al, 2001 and Kligman, 1966).

3.4.5.3.2.7   For example, negative results from substances tested in a predictive patch test at DSA (dose per skin area) < 500 μg/cm^2 imply that a classification for skin sensitization 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 μg/cm^2. Negative results from substances tested at DSA ≥ 500 μg/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 indicate that no classification is needed (based on this test). However, negative results at low concentrations may be informative for mixtures containing the substance.

3.4.5.3.3   *Guidance on the use of standard animal data*

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 Test Guideline 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.

3.4.5.3.4   *Guidance on the use of 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 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   *Guidance on the use of 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 3.4.7 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. This is also in line with the statement in these Test Guidelines that "Depending on the regulatory framework, positive results generated with these methods may be used on their own to classify a chemical into UN GHS Category 1." Therefore, GHS also allows a competent authority to decide that a positive result with one of these non-stand alone in *chemico/in vitro* methods, may be used on its own to classify in category 1 and whether 442C (appendix III) kinetic Direct Peptide Reactivity Assay (kDPRA) can be used to differentiate between category 1A versus no category 1A.

3.4.5.3.6   *Guidance on the use of* non-standard data

3.4.5.3.6.1   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 sensitizers or assessment of skin sensitising potency may provide supportive evidence when used in an overall weight-of-evidence assessment (i.e. Tier 3).

3.4.5.3.6.2   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:

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

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

(c)   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).

3.4.5.3.7   *Guidance on the weight of evidence assessment*

In some situations where several results from test or non-test methods are available and in disagreement with each other with respect to the classification outcome, the tiered approach to classification for skin sensitisation requires a weight-of-evidence assessment."

Table 3.4.6   Insert new Table 3.4.6 '*Criteria for defined approaches*' after paragraph 3.4.5.3.7, as follows:

Table 3.4.6: Criteria for defined approaches

Category	OECD Guideline 497 on Defined Approaches for Skin sensitization "2 out of 3" (2o3) defined approach	OECD Guideline 497 on Defined Approaches for Skin sensitization Integrated testing strategy (ITSv1) defined approach and Integrated testing strategy (ITSv2 defined approach)
	2o3 defined approach to skin sensitization hazard identification based on *in chemico* (key event 1 - Direct Peptide Reactivity Assay (KE1-DPRA)) and *in vitro* (key event 2-OECD 442D Appendix IA, key event 3 - human Cell Line Activation Test (KE3-h-CLAT))	**ITSv1** based on *in chemico* (KE1-DPRA) and *in vitro* (KE3-h-CLAT) data, and *in silico* (Derek Nexus) predictions. **ITSv2** based on *in chemico* (KE1 -DPRA) and *in vitro* (KE3 -h-CLAT) data, and in silico (OECD QSAR Toolbox) predictions.
	Assays are run for two key events, 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 key event 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 Guideline	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 ToolBox), 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; GHS Cat. 1 vs. no classification) and potency (GHS Cat. 1A, Cat. 1B and no classification).
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 3.4.7   Insert new Table 3.4.7 '*Criteria for individual in chemico/in vitro methods*' after Table 3.4.6, as follows:

Table 3.4.7: Criteria for individual *in chemico/in vitro* methods

Category	OECD Test Guideline 442C Key event-based Test Guideline for *in chemico* skin sensitization assays addressing the adverse outcome pathway (AOP) Key Event on covalent binding to proteins			OECD Test Guideline 442D Key event-based Test Guideline for *in vitro* skin sensitization assays addressing the AOP Key Event on keratinocyte activation antioxidant response element-nuclear factor-erythroid 2-related factor 2 (ARE-Nrf2) luciferase methods		OECD Test Guideline 442E In vitro skin sensitization assays addressing the AOP Key Event on activation of dendritic cells			
	Method described in Appendix I **The Direct Peptide Reactivity Assay (DPRA)**[a]	**Method described in Appendix II** **The Amino acid Derivative Reactivity Assay (ADRA)**[a]	**Method described in Appendix III** **The kinetic Direct Peptide Reactivity Assay (kDPRA)**[b]	**Method described in Appendix 1A**[a]	**Method described in Appendix 1B** **Lusens**[a]	**Method described in Annex I** **human Cell Line Activation Assay (h-CLAT)**[a]	**Method described in Annex II** **U937 Cell Line Activation Test**[a]	**Method described in Annex III** **Interleukin-8 luciferase (IL-8 Luc) assay**[a]	**Method described in Annex IV**[a]
	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 N-(2-(1-naphthyl) acetyl)-L-cysteine (NAC) or α-N-(2-(1-naphthyl) acetyl)-L-lysine (NAL) (ADRA). 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: 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. Cell viability is quantitatively measured in parallel by enzymatic conversion of the dye 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). 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: cell-based methods addressing the process of monocytes/dendritic cell activation by either quantifying the change in the expression of cell surface marker(s) (e.g. cluster of differentiation 54 (CD54), cluster of differentiation 86 (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. 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% Or the mean cysteine % depletion > 13.89 %	The mean NAC and NAL % depletion ≥ 4.9% Or 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: 1. Imax equal or higher than (≥) 1.5 fold and statistically significantly different to the solvent control 2. The cellular viability is higher than (>) 70% at the lowest concentration with induction of luciferase activity equal or above 1.5 fold 3. The $EC_{1.5}$ value is less than (<) 1000 µM (or < 200 µg/mL for test chemicals with no defined molecular weight) 4. There is an apparent overall dose-dependent increase in luciferase induction	The following conditions are all met in 2 of 2 or in the same 2 of 3 repetitions: 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 concentrations (i.e. cellular viability is equal or higher than (≥) 70%) 2. At least three tested concentrations should be non-cytotoxic (cellular viability equal or higher than (≥) 70%).	At least one of the following conditions is met in 2 of 2 or in at least 2 of 3 independent runs: The Relative Fluorescence Intensity of CD86 is equal to or greater than 150% at any tested concentration (with cell viability ≥ 50%) or the Relative Fluorescence Intensity of CD54 is equal to or greater than 200% at any tested concentration (with cell viability ≥ 50%).	The following condition is met in 2 of 2 or in at least 2 of 3 independent runs: The stimulation index of CD86 is equal or higher (≥) than 150% and/or interference is observed	The induction of normalised interleukin-8 luciferase activity (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 of 4 independent runs	The mean Decision Value (DV) is ≥0
1A	Not applicable		log kmax ≥ -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% or the mean cysteine % depletion ≤ 13.89 %	The mean NAC and NAL % depletion < 4.9% Or 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 Category 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

Table 3.4.7   Insert the following notes beneath Table 3.4.7 as follows:

"[a]   *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 depending on the decision of the competent authority for their regulatory framework.*
[b]   *A competent authority may decide that data can be used as stand-alone to conclude on classification in sub-category 1A.*"

Insert the following references:

"_____

* *References:*

Corsini, E., Clewell, R., Cotgreave, I., Eskes, C., Kopp-Schneider, A., Westmoreland, C., Alves, P.M., Navas, J.M. and Piersma, A., ESAC Opinion on the Scientific Validity of the GARDskin and GARDpotency Test Methods, Asturiol Bofill, D., Casati, S. and Viegas Barroso, J.F. editor(s), Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-40345-6, doi:10.2760/626728, JRC125963.

Cottrez F, Boitel E, Auriault C, Aeby P, Groux H.Genes specifically modulated in sensitized skins allow the detection of sensitizers in a reconstructed human skin model. Development of the SENS-IS assay. Toxicol In Vitro. 2015 Jun;29(4):787-802. doi: 10.1016/j.tiv.2015.02.012.

Cottrez F, Boitel E, Ourlin JC, Peiffer JL, Fabre I, Henaoui IS, Mari B, Vallauri A, Paquet A, Barbry P, Auriault C, Aeby P, Groux H. SENS-IS, a 3D reconstituted epidermis based model for quantifying chemical sensitization potency: Reproducibility and predictivity results from an inter-laboratory study. Toxicol In Vitro 2016 Apr;32:248-60. doi: 10.1016/j.tiv.2016.01.007.

ECHA Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017

Gradin R., Johansson A., Forreryd A., Aaltonen E., Jerre A., Larne O., Mattson U., Johansson H. (2020) The GARDpotency assay for potency-associated subclassification of chemical skin sensitizers – Rationale, method development, and ring trial results of predictive performance and reproducibility. Toxicol. Sci. 176(2):423-432. Doi: 10.1093/toxsci/kfaa068

Johansson H., Lindstedt M., Albrekt A.S., Borrebaeck C.A. (2011) A genomic biomarker signature can predict skin sensitizers using a cell-based in vitro alternative to animal tests. BMC Genomics 12:399. Doi: 10.1186/1471-2164-12-399.

Johansson H., Rydnert F., Kühnl J., Schepky A., Borrebaeck C., Lindstedt M. (2014) Genomic allergen rapid detection in-house validation – A proof of concept. Toxicol. Sci. 139(2):362- 370. Doi: 10.1093/toxsci/kfu046.

Johansson H., Gradin R., Forreryd A., Agemark M., Zeller K., Johansson A., Larne O., van Vliet E., Borrebaeck C., Lindstedt M. (2017) Evaluation of the GARD assay in a blind Cosmetics Europe study. ALTEX 34(4):515-523. Doi: 10.14573/altex.1701121

OECD 2016a. Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used Within Integrated Approaches to Testing and Assessment (IATA) for Skin sensitization. Series on Testing & Assessment No. 256. http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm

Kligman A.M. (1966): The identification of contact allergens by human assay: II. Factors influencing the induction and measurement of allergic contact dermatitis. Journal of Investigative Dermatology 47 (5), 375-392. DOI: 10.1038/jid.1966.159

OECD (2014). The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris. Available at [https://doi.org/10.1787/9789264221444-en]

OECD 2016b. Annex I: Case Studies to the Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used Within Integrated Approaches to Testing and Assessment (IATA) for Skin sensitization. Series on Testing & Assessment No. 256. http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm

OECD (2012). Series on Testing and Assessment No. 168. The Adverse Outcome Pathway for Skin sensitization Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence. Organisation for Economic

Cooperation and Development, Paris. Available at [https://www.oecd.org/chemicalsafety/testing/series-testingassessment-publications-number.htm]

Saito K, Takenouchi O, Nukada Y, Miyazawa M, Sakaguchi H. An in vitro skin sensitization assay termed EpiSensA for broad sets of chemicals including lipophilic chemicals and pre/pro-haptens. Toxicol In Vitro. 2017 Apr;40:11-25. doi: 10.1016/j.tiv.2016.12.005. Epub 2016 Dec 10.PMID: 27965148

Wright ZM, Basketter PA, Blaikie L, Cooper KJ, Warbrick EV, Dearman RJ, Kimber I. Vehicle effects on skin sensitizing potency of four chemicals: assessment using the local lymph node assay. Int J Cosmet Sci. 2001 Apr;23(2):75-83. doi: 10.1046/j.1467-2494.2001.00066.x. PMID: 18498452

Zeller K.S., Forreryd A., Lindberg T., Gradin R., Chawade A., Lindstedt M. (2017) The GARD platform for potency assessment of skin sensitizing chemicals. ALTEX 34(4):539-559. Doi: 10.14573/altex.1701101.".

_____