**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**   **10 November 2022**

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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**

Amendments to the proposal in ST/SG/AC.10/2022/14

**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 informal document proposes revised paragraphs and sections following the outcome of the discussions of the informal working group on the proposed revision of Chapter 3.4 (document ST/SG/AC.10/C.4/2022/14 and INF.3) to incorporate non-animal testing methods for skin sensitization.

2.   The amendments listed under paragraph 13 of this document address additional changes to the text proposed in ST/SG/AC.10/C.4/2022/14 for the following paragraphs, sections, figure, decision logic, table and reference list: 3.4.2.2.1.4; 3.4.2.2.4 (3.4.2.2.4.1 to 3.4.2.2.4.3); 3.4.2.2.5 (3.4.2.2.5.1 to 3.4.2.2.5.3); 3.4.2.2.6.1; 3.4.2.2.7 (3.4.2.2.7.3 to 3.4.2.2.7.7); Figure 3.4.1; Decision logic 3.4.2; 3.4.5.3.1; 3.4.5.3.2 (3.4.5.3.2.1 to 3.4.5.3.2.4; 3.4.5.3.2.6 to 3.4.5.3.2.7); 3.4.5.3.3; 3.4.5.3.5; 3.4.5.3.6.1; 3.4.5.3.7; Table 3.4.7; and the reference list. The revised text of Chapter 3.4, including the amendments listed in ST/SG/AC.10/C.4/2022/14, as modified by those proposed within this document, are provided in informal document INF.3/Rev.1.

Background

3.   As planned, the informal working group held meetings on 1 September, 6 October and 2 November 2022 after the formal proposals to revise Chapter 3.4 (documents ST/SG/AC.10/C.4/2022/14 and INF.3) and the consequential amendments to chapters 1.2, 3.2 and 3.3 (document ST/SG/AC.10/C.4/2022/15) had been submitted for discussion at the forty-third session. A key purpose of these meetings was to undertake a final review of these documents and to continue the discussions on skin sensitization mixtures sections of Chapter 3.4 in preparation for formal proposals on these issues early in the next biennium.

4.   However, a number of minor editorial changes to the paragraphs and sections listed in paragraph 2 of this document, were agreed to improve the clarity and readability of the proposed changes to Chapter 3.4.

5.   The revised text for the proposed new paragraph 3.4.2.2.4.3 provides improved clarity regarding when evidence that has already been used in a defined approach cannot then also be used as additional evidence in a weight of evidence assessment for skin sensitization classification for a particular substance. In addition, the revised text means that the related

last sentence in 3.4.2.2.5.2 and the second sentence in 3.4.2.2.6.1 (as initially proposed in ST/SG/AC.10/C.4/2022/14) can now both be deleted as they are no longer required.

6.　　Furthermore, the revised new text for the proposed paragraph 3.4.2.2.4.3 means that the related proposed consequential changes to Chapter 3.3 as provided in paragraphs 4, 5 and 6 in ST/SG/AC.10/C.4/2022/15 are required to be amended. These are provided in INF.9.

7.　　The revised text for the last two sentences of the proposed new paragraph 3.4.5.3.2.7 in also provides improved clarity from the originally proposed text in ST/SG/AC.10/C.4/2022/14.

8.　　The revised Figure 3.4.1 to remove reference to 'low confidence/inconclusive results from defined approaches' in the Tier 2 text box on the grounds that consideration of such results would be included within the assessment undertaken under Tier 1. In addition, this new change requires further amendments to the proposed paragraphs 3.4.2.2.7.3 and 3.4.2.2.7.5 in ST/SG/AC.10/C.4/2022/14 and INF.3 to remove reference to such results.

9.　　The revised text for the proposed new paragraphs 3.4.5.3.3 (guidance for the use of standard animal data) and 3.4.5.3.7 (guidance for the use of weight of evidence assessments) both provide improved clarity and details from the originally proposed text in ST/SG/AC.10/C.4/2022/14.

10.　　In addition, a number of typographical errors were identified within the text previously proposed in ST/SG/AC.10/C.4/2022/14 and INF.3. Hence, for consistency with other chapters in the GHS:

- Where hyphens have been incorrectly used in the term 'weight of evidence', these should be removed. This error has occurred in the following paragraphs: 3.4.2.2.1.4; 3.4.2.2.7.3; 3.4.2.2.7.5; 3.4.2.2.7.6 (error occurs twice); Figure 3.4.1 (in Tier 3); 3.4.5.3.5; 3.4.5.3.6.1; and 3.4.5.3.7.

- Where hyphens have not been used in the term 'stand-alone', these should be inserted. This error has occurred in the following paragraphs: 3.4.2.2.7.3 (error occurs twice); 3.4.2.2.7.4; 3.4.2.2.7.5 (error occurs twice); Figure 3.4.1 (in Tier 2) and 3.4.5.3.5.

11.　　A number of other minor typographical errors were also identified where corrections are necessary to improve the clarity, readability of the text and consistency with terms used elsewhere in the GHS and include:

- Inserting the word 'assessment' following the term 'weight of evidence' in paragraphs 3.4.2.2.7.3; 3.4.2.2.7.6; 3.4.2.2.7.7; 3.4.5.3.2.6; and 3.4.5.3.5.

- Correcting the reference to WHO guidelines in 3.4.5.3.2.3.

- Correcting various other minor errors or additional words in paragraphs 3.4.5.3.2.1; 3.4.5.3.2.2; 3.4.5.3.2.7; 3.4.5.3.5; and 3.4.5.3.6.1.

12.　　These amendments are listed under paragraph 13. When applicable, new or replacement text is shown in **bold black** and **underlined**, and deleted text (from that proposed in ST/SG/AC.10/C.4/2022/14 and INF.3) is shown in underlined black ~~strikethrough~~. Text shown in plain black is the text previously proposed in ST/SG/AC.10/C.4/2022/14, and consolidated in INF.3/Rev.1, that remains unchanged.

Proposed amendments to ST/SG/AC.10/2022/14

13.　　Amend the proposals in ST/SG/AC.10/2022/14 for the paragraphs below as follows:

3.4.2.2.1.4　　Replace "weight-of-evidence" with "**weight of evidence**".

3.4.2.2.4.1　　In the brackets of footnote "3" referenced at the end of the fourth sentence, replace "OECD 2016b" with "**OECD (2017)**"

3.4.2.2.4.2   After the words '…outcome of a defined approach' insert the words: "**in Tier 1 is inconclusive and thus**".

3.4.2.2.4.3   Amend as follows:

["3.4.2.2.4.3   ~~Some evidence can be used individually and in defined approaches.~~ **Individual** ~~e~~**E**vidence **considered** ~~used~~ within **a** defined approach~~es~~ should ~~then~~ not ~~also~~ be **considered as an additional line of evidence** ~~used individually~~ within a weight of evidence assessment."]

3.4.2.2.5.1   Replace "see OECD, 2014" with "**see OECD (2014)**".

3.4.2.2.5.2   Replace "non-stand alone" with "**non stand-alone**"; and delete the last sentence "When already… (see 3.4.2.2.7.4)".

3.4.2.2.5.3   Replace the reference "3.4.5.3.6.1" with "**3.4.5.3.6.2**" (including in the related new footnote 4).

3.4.2.2.6.1   Delete the second and third sentences "Specific non-test methods…(see 3.4.2.2.7.4)".

3.4.2.2.7.3   Replace "non-stand alone" with "**non stand-alone**"; replace "," with "**or**" after "*in chemico/in vitro* methods" and delete "or low confidence/inconclusive results from defined approaches" in the first sentence; replace "weight-of-evidence" with "**weight of evidence assessment**" in the second paragraph; and delete "(c)   Low confidence/inconclusive results from defined approaches (see 3.4.2.2.4.2)."

3.4.2.2.7.4   Replace "non-stand alone" with "**non stand-alone**".

3.4.2.2.7.5   Replace "non-stand alone" with "**non stand-alone**"; replace "," with "**and**" after "international procedures"; delete "and low confidence/inconclusive results from defined approaches"; and replace "weight-of-evidence" with "**weight of evidence**" in the first sentence.

3.4.2.2.7.6   Replace "weight-of-evidence" with "**weight of evidence assessment**" in the first sentence; and "weight-of-evidence" with "**weight of evidence**" in the second paragraph.

3.4.2.2.7.7   Replace "approach" with "**assessment**" at the end of the paragraph.

Figure 3.4.1

- In the "Tier 2" text box, replace "non-stand alone" with "**non stand-alone**"; insert **"(see 3.4.2.2.4.2 and 3.4.2.2.7.3)"** after "Tier 1"; and delete "and/or low confidence/inconclusive results from defined approaches (see 3.4.2.2.4)".
- In the "Tier 3" text box, replace "weight-of-evidence" with "**weight of evidence**".

3.4.5.2   Decision logic 3.4.2 for skin sensitization:

Amend the text in the central box ("(a) is there evidence in humans…. (see criteria in 3.4.2.2.1 and 3.4.2.2.4)" to read as follows: "**Is there evidence that the substance/mixture fulfils the criteria as described in 3.4.2.2.2.2 to 3.4.2.2.2.8 for substances and in 3.4.3.1 for mixtures**".

3.4.5.3.1   Replace "OECD, 2014" with "**see OECD (2014)**".

3.4.5.3.2.1   In the last sentence, replace "the criteria in 3.4.2.2.2 are provided" with "**the criteria in 3.4.2.2.2 is provided**".

3.4.5.3.2.2   In the last but one sentence, replace "clinical settings and" with "**clinical settings and in general**".

3.4.5.3.2.3   Replace "COIMS" with "**CIOMS**"

3.4.5.3.2.4   Replace "man and/or well documented" with "**humans and/or well documented**" in the fourth sentence

3.4.5.3.2.6   In the last but one sentence replace "weight of evidence" with "**weight of evidence assessment**".

3.4.5.3.2.7   In the first sentence, replace "at DSA (dose per skin area)" with "**at a DSA (dose per skin area)**" and "ruled" with "**ruled out**". In the third sentence, replace "at DSA" with "**at a DSA**". At the beginning of the fourth sentence, replace "but, while classification" with "**However, while classification**".

Amend the last two sentences as follows: "However, a negative test result at a concentration of 100% ~~would indicate that~~ **can justify** no classification ~~is needed~~ (based on this test). ~~However~~**Nevertheless**, negative results at low concentrations may be informative for mixtures containing the substance **at similar and lower concentrations**.".

3.4.5.3.3   Replace the two sentences of 3.4.5.3.3 with:

"**3.4.5.3.3.1   The most common assays used for dermal sensitization testing in animals are the Local Lymph Node Assay (LLNA, OECD Test Guidelines 429 and 442A and 442B), the Guinea Pig Maximization Test (GMT, OECD Test Guideline 406) and the Buehler test (OECD Test Guideline 406). When evaluating the quality of the study, consideration should be given, as relevant, to the strain of the mouse and guinea pig used, the number, age, and sex of the animals, and the test conditions used (e.g., preparation of patch test site, dose level selection, chemical preparation, positive and negative test controls).**

3.4.5.3.3.2   OECD test guidelines for the LLNA include the radioactive assay (OECD Test Guideline 429) and non-radioactive assays (OECD Test Guideline 442A and 442B; LLNA:DA, LLNA:BrdU-ELISA, and LLNA:BrdU-FCM). In these tests, sensitisers are characterised by increasing the group mean Stimulation Index (SI, a measure of lymph node proliferation) in treated groups vs. concurrent vehicle controls by more than a predefined critical value which is different for each form of the LLNA (e.g., $SI \geq 3$ for the radioactive LLNA, $SI \geq 1.6$ for the LLNA:BrdU-ELISA). For sensitisers, sub-categorization is performed based on the effective concentration (EC) causing an increase in SI of exactly the critical magnitude (e.g. the EC3 under OECD Test Guideline 429 is the concentration leading to an exactly threefold increase in group mean SI vs. control).

3.4.5.3.3.3   The respective OECD Test Guidelines for the different LLNA variants specify that a pre-screen test should be undertaken to determine the highest concentration to be tested. If such a test has not been performed and the LLNA was carried out with a test concentration < 100%, a rationale (e.g. based on solubility, local or systemic toxicity, see OECD Test Guidelines 429, and 442A and 442B) needs to be provided that the highest test concentration represents the maximum testable concentration. Otherwise, the reliability of a negative test result has to be considered compromised.

3.4.5.3.3.4   EC values are normally obtained by interpolation between adjacent test concentrations, i.e. between the highest test concentration causing an SI below, and the lowest test concentration causing an SI above the critical value. However, care must be taken when the EC value falls below the lowest concentration tested and can therefore only be estimated by extrapolation, which is associated with additional uncertainty. In some cases, the SI at the highest concentration tested falls only slightly below the critical SI value, which raises the question of upward extrapolation (unless the maximum testable concentration has been applied). These and other issues regarding the reliability of LLNA results are further discussed in Ryan et al. (2007) and Annex 3 of OECD Series on Testing and Assessment No. 336 (Supporting Document to OECD Guideline Document 497), which also provides a highly curated database of Test Guidelines 429 LLNA EC3 values.

3.4.5.3.3.5   Further limitations have been identified for the radioactive and non-radioactive LLNAs. For example, substances containing certain functional groups may interfere with the accuracy of the assay. These limitations as well as the possibility of borderline positive results are described in OECD Test Guidelines 429, and 442A and 442B. Variability in EC values for the same substance may also be the result of the vehicle used. For example, analysis has shown an underestimation of potency (i.e., higher EC3 values) with predominantly aqueous vehicles or propylene glycol (see Jowsey, 2008).

3.4.5.3.3.6   For OECD Test Guideline 406, the concentration of test chemical used for each induction exposure should be systemically well-tolerated using the highest dose to cause mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest non-irritant dose. A positive result in a guinea pig test is defined as a grade 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 observation time-points. A grade of 0.5, which is sometimes reported, is therefore also considered a positive result.".

3.4.5.3.5   Replace "due to the limited mechanistic coverage" with "**due to their limited mechanistic coverage**"; "these methods provides quantitative information," with "**these methods provide quantitative information,**"; "for the purposes of subcategorization into sub-category 1A and subcategory 1B" with "**for the purposes of subcategorization into sub-categories 1A and 1B**"; "weight-of-evidence" with "**a weight of evidence assessment**"; and "non-stand-alone" with "**non stand-alone**".

Delete "UN GHS" and amend the last sentence as follows: "Therefore, **the** 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 **Test Guideline** 442C (~~a~~**A**ppendix III) kinetic Direct Peptide Reactivity Assay (kDPRA) can be used to differentiate between category 1A ~~versus~~ **and** no category 1A."

3.4.5.3.6.1   Replace "3.4.5.3.6.1" with "**3.4.5.3.6.2**"; "or assessment of skin sensitizing potency" with "**or the assessment of skin sensitizing potency**"; and "weight-of-evidence" with "**weight of evidence assessment**".

3.4.5.3.7   Replace paragraph 3.4.5.3.7 with:

"**3.4.5.3.7**   *Guidance on the weight of evidence assessment for classifying substances and mixtures for skin sensitization*

3.4.5.3.7.1   There may be situations where results from tests and/or non-test methods are available but disagree with each other with respect to the classification. In these situations, the tiered approach to classification for skin sensitization requires a weight of evidence assessment consistent with the principles elaborated in sections 1.3.2.4.2 and 1.3.2.4.9 on test data quality and weight of evidence, respectively. In addition, some guidance on the weight of evidence assessment specific for skin sensitisation is provided below which can be applied when the general principles do not result in a conclusion on the classification. It should be noted that human and animal results for a substance obtained at low concentrations may still be informative for classifying a mixture containing the substance at similar or lower concentrations.

3.4.5.3.7.2   Mutual compatibility of study results

3.4.5.3.7.2.1   In cases where results are in disagreement with each other (e.g., not classified vs. category 1, sub-category 1A or 1B; sub-category 1A vs. 1B), a weight of evidence assessment becomes necessary. However, less obvious situations may also occur such as where certain studies may point to not classified or sub-category 1B, while it cannot be excluded that a stricter classification might have resulted under a different dosing regime. For example, a negative HMT result at a dose per skin area of 100 μg/cm^2 cannot exclude that a positive result might have been obtained at e.g., 300 μg/cm^2 (sub-category 1A) or 700 μg/cm^2 (sub-category 1B). The same holds for LLNA test results obtained from tests which have not been carried out using the highest possible test concentration (see OECD test guideline 429 for details).

3.4.5.3.7.2.2   In the following ambiguous cases, study results for substances and mixtures would not be in disagreement with another study result pointing at that stricter classification:

(a)   **A not classified result obtained at a lower test concentration does not exclude the possibility of a sub-category 1B outcome at a higher test concentration. Therefore, a not classified result obtained at a low concentration is compatible with other not classified outcomes, or with category 1 and sub-category 1B outcomes obtained at higher test concentrations.**

(b)   **A not classified result at a very low-test concentration does not even exclude a possible outcome of sub-category 1A at a higher test concentration. Therefore, a not classified outcome obtained at a very low-test concentration is compatible with all possible classification outcomes (i.e., not classified, category 1, sub-category 1A or 1B) obtained at higher test concentrations.**

(c) **A sub-category 1B result at a higher test concentration does not exclude a sub-category 1A outcome at a lower test concentration. Therefore, a Category 1B classification tested at a high-test concentration is compatible with other outcomes of sub-category 1B, or even sub-category 1A, obtained at lower test concentrations.**

3.4.5.3.7.2.3 **If at least one unambiguous study result allows for sub-categorisation of a substance or mixture and all other study results are not in disagreement (see above), then it can be classified into a sub-category. For example, if all study results are in the same sub-category (i.e., sub-category 1A or 1B), or with at least one study permitting sub-categorisation (i.e., either sub-category 1A or 1B) and all other studies classified into category 1 without sub-categorisation, then the substance or mixture can be sub-categorised.**

3.4.5.3.7.3 **Weight of evidence considerations for giving one study result more weight than another.**

3.4.5.3.7.3.1 **Some classifiers or competent authorities may take various approaches to evaluate study results given the required level of expert judgement (see 1.3.2.4.8) required to perform a weight of evidence assessment. Competent authorities may specify their preferred approach in their own guidance. For example, through:**

(a) **Applying a precautionary approach, giving more weight to studies resulting in the stricter classification outcome.**

(b) **Giving human data higher weight than animal or non-test data.**

(c) **Giving certain animal data (e.g., LLNA data) more weight than other animal data (e.g., Buehler test data).**

3.4.5.3.7.3.2 **Often, several results (of the same or different type) may have to be considered in the weight of evidence assessment. There are no generally recognised rules for this situation, however, possible solutions to integrating several results of the same type may include, for example:**

(a) **A precautionary approach where the strictest classification outcome from all studies of sufficient quality is assigned as the overall classification outcome.**

(b) **Averaging the obtained dose descriptors (e.g., LLNA EC3 values) or classification outcomes (no classification, Category, 1, 1A, 1B). A detailed discussion of such approaches can be found in Annex 3 (on LLNA data) and Annex 4 (on HMT/HRIPT data) of OECD Series on Testing and Assessment No. 336 (Supporting Document to OECD Guideline Document 497).**".

Table 3.4.7 Replace "Method described in Annex IV ª" with: "**Method described in Annex IV Genomic Allergen Rapid Detection for assessment of skin sensitizers ª**" in row two under the column "OECD Test Guideline 442E *In vitro* skin sensitization…".

References: Replace "doi" and "DOI" with "**Doi**"; delete "Epub 2016 Dec 10. PMID: 27965148" at the end of the reference entry for Saito K, et al; and delete "PMID: 18498452" at the end of the reference entry for Wright ZM, et al;

- Insert the following reference below the entry for Johansson H., Gradin R., et al":

  "**Jowsey IR, Clapp CJ, Safford B, Gibbons BT, Basketter DA. (2008). The impact of vehicle on the relative potency of skin-sensitizing chemicals in the local lymph node assay. Cutan Ocul Toxicol: 27 (2); 67-75. Doi: 10.1080/15569520801904655.**"

- Delete the references "OECD (2012)"; "OECD 2016a"; and "OECD 2016b".

- Replace "Available at [https://doi.org/10.1787/9789264221444-en]" with "**Doi.org/10.1787/9789264221444-en**"at the end of the reference entry for "OECD (2014)".

- Insert the following reference below the entry for "OECD (2014)":

  "**OECD (2017),** *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 Sensitisation, **OECD Series on Testing and Assessment, No. 256, OECD Publishing, Paris. Doi.org/10.1787/9789264279285-en.**".

- Insert the following reference below the new reference for "OECD (2017)":

  "**Ryan CA et al. (2007): Extrapolating local lymph node assay EC3 values to estimate relative sensitizing potency. Cutan Ocul Toxicol 26(2), 135-45.**"

Action and next steps

14.   The Sub-Committee is invited to consider the proposed amendments listed in paragraph 13 to the proposal in ST/SG/AC.10/C.4/2022/14 (as consolidated in INF.3/Rev.1), with the accompanying consequential amendments to chapters 1.2, 3.2. and 3.3 listed in ST/SG/AC.10/C.4/2022/15 and consolidated in INF.9.

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