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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

Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals

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Item 2 (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 and environmental hazards

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

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*

I. Introduction

1. The informal working group on the "Use of non-animal testing methods for the classification of health and environmental hazards" has continued its work on the revision of chapter 3.4 (Respiratory or Skin Sensitization) for skin sensitization for mixtures in accordance with the programme of work for the 2023-2024 biennium[1].

2. This document, together with informal document INF.5, presents for the agreement of the Sub-Committee a revision of chapter 3.4 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 for the classification for skin sensitization for mixtures.

3. These proposals are limited to changes to the classification criteria and guidance for mixtures classified as skin sensitizers to integrate non-animal testing methods, with some additional amendments to section 3.4.5.3 ("Background guidance") in relation to renumbering of the various sections and more specifically to the section on "Guidance on the use of human data" as the group considered that this particular guidance section is relevant for both substances and mixtures. Due to the renumbering of section 3.4.5.3 ("Background

* A/78/6 (Sect. 20), table 20.5.

[1] See ST/SG/AC.10/C.4/86 and informal document INF.16 (forty-third session).

guidance") the references to the background guidance in the criteria for substances have also needed to be amended.

II. Background

4. 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 hazard classification for health hazard and environmental hazard classes in accordance with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 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-sixth session.

5. 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.

6. Following on from the adoption of the revised chapter 3.4 for skin sensitization in relation to substances in December 2022, the group recommenced its discussions in relation to the classification of skin sensitization for mixtures. The group had previously agreed that the revisions 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.

7. The group is very active, both via correspondence and through virtual meetings, to resolve the issues. For example, to date during the 2023-2024 biennium the group has held, or scheduled before the end of the forty-sixth session, thirteen virtual or face to face meetings,

[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.

focussed primarily on completing the group's work on revising chapter 3.4 for the classification of skin sensitization for mixtures. In addition, the group has also considered non-animal testing methods related questions raised by the informal working group on the clarification of the criteria for classification for germ cell mutagenicity and the group's options for the next work. After each meeting, the Netherlands and the United Kingdom, as joint leads, together with the European Commission's Joint Research Centre, 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.

III. Classification criteria and background guidance for mixtures

8. Defined approaches, in vitro and *in chemico* methods were developed and formally validated for identifying sensitizing substances and not mixtures. Nevertheless, they are technically applicable to mixtures. Further, there is limited data indicating whether there is a difference in the predictive capacity between standard animal test methods and defined approaches for the classification of mixtures.

9. The Organisation for Economic Co-operation and Development (OECD) test guidelines for the in vitro and *in chemico* methods and consequently of the defined approaches require upfront consideration to whether such testing will yield results that are predictive of the skin sensitizing properties of the mixture.

10. In addition, some unclarities and concerns regarding the use of defined approaches, in vitro and *in chemico* methods were identified. The informal working group discussed whether the criteria for substances should also be applied for mixtures but no consensus was reached. Therefore, the proposed criteria allow a competent authority to decide which *in chemico*/in vitro test method or defined approach may be accepted for mixtures.

11. As well as proposing completely revised text for the classification of mixtures when data are available for the complete mixture (3.4.3.1), the group also propose amendments to the background guidance section (3.4.5.3) to include guidance on skin sensitizing mixtures.

12. The group also propose to revise the current skin sensitizing substance guidance on the use of human data section (3.4.5.3.2) to include mixtures since that guidance also applies to mixtures and hence their inclusion into that section reduces the need to replicate much of the same text under the proposed new mixtures guidance section.

13. In addition, given that chapter 3.4 also includes classification criteria for respiratory sensitizers, the group considered that it was important to clearly indicate to the reader what hazard the provided guidance relates to. Hence, to help achieve this the group has proposed to insert a new heading ("3.4.5.3.1 Guidance on substances – skin sensitization") under "3.4.5.3 Background guidance". The group also viewed that at some stage in the future when the chapter is revised to include non-animal testing methods for respiratory sensitizers, similar guidance section headings could be used for that hazard class.

14. 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.5 with indication of where the text has changed relative to the tenth revised edition of GHS.

15. Unfortunately, prior to the submission of this document and informal document INF.5, the informal working group were unable to finalise their proposal for paragraph 3.4.5.3.2.3.5, the current draft of which is provided in both documents in square brackets to indicate that it is still under discussion. The informal working group intends to complete this discussion soon and will provide the outcome for the consideration of the Sub-Committee at the July 2024 session.

IV. Action requested

16. 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.5.

Annex

Proposed amendments to chapter 3.4 for skin sensitization

3.4.2.2.5.1 Replace “3.4.5.3.5” with "3.4.5.3.1.5” in the last sentence.

3.4.2.2.5.3 Replace “3.4.5.3.6.2” with "3.4.5.3.1.6.2” in the first sentence and the related footnote 4.

3.4.2.2.7.2 Replace “3.4.5.3.2” with "3.4.5.3.1.2” in subparagraphs (a), (b) and (c). Replace “3.4.5.3.3” with “3.4.5.3.1.3” in subparagraph (d), “3.4.5.3.4” with “3.4.5.3.1.4” in subparagraph (e), and “3.4.5.3.5” with “3.4.5.3.1.5” in subparagraph (f).

3.4.2.2.7.3 Replace “3.4.5.3.5” with "3.4.5.3.1.5” in subparagraph (a).

3.4.3.1 Replace with the following:

> **“3.4.3.1** ***Classification of mixtures when data are available for the complete mixture***
>
> 3.4.3.1.1 In general, the mixture should be classified using the criteria for substances taking into account the tiered approach to evaluate data for this hazard class (see 3.4.3.1.2 and figure 3.4.1). If classification is not possible using the tiered approach, then the approach described in 3.4.3.2 or, if that is not applicable, in 3.4.3.3, should be followed. For supplemental labelling required by some competent authorities, see the note to table 3.4.5 and 3.4.4.2.
>
> 3.4.3.1.2 Care should be exercised in evaluating data on mixtures that the dose used does not render the results inconclusive and that the test methods used to generate such results are appropriate for predicting the skin sensitizing properties of the mixture (see 3.4.5.3.2). Further, for both standard test methods (in vivo, *in chemico*, in vitro) and defined approaches, data can only be used for classification when all ingredients fall within their applicability domain. Specific limitations regarding applicability domains are described in the respective test methods and defined approaches and should be taken into consideration as well as any further information on such limitations from the published literature. A competent authority may decide which *in chemico*/in vitro test method or defined approach may be accepted for mixtures (see 3.4.5.3.2.4 and 3.4.5.3.2.5). A more detailed overview of factors to consider in the classification of mixtures can be found in guidance section 3.4.5.3.2 and the test methods.”.

3.4.5.3 Insert the following new heading beneath “3.4.5.3 Background guidance”: “3.4.5.3.1 *Guidance on substances – skin sensitization”*.

3.4.5.3.1 to 3.4.5.3.2 Current sections “3.4.5.3.1” to “3.4.5.3.2” become new sections “3.4.5.3.1.1” to “3.4.5.3.1.2”.

3.4.5.3.1.2 (former 3.4.5.3.2) Replace with the following:

> “3.4.5.3.1.2 Guidance on the use of human data
>
> 3.4.5.3.1.2.1 This guidance is relevant to substances and mixtures.
>
> 3.4.5.3.1.2.2 The classification of substances and mixtures 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. Guidance for evaluating human

evidence and the criteria in 3.4.2.2.2 is provided by some competent authorities (e.g., ECHA Guidance on the Application of the CLP Criteria, 2017). Further valuable information which should be considered for classification purposes (e.g., on use of appropriate concentrations and vehicles, as well as mixture evaluation) is also available (see U.S. Consumer Product Safety Commission (U.S CPSC), 2013; European Society of Contact Dermatitis guidance, 2015; Frosch et al., 2015).

3.4.5.3.1.2.3 When evaluating existing data, its quality should be taken into consideration. Criteria for a "well conducted" study would include validated outcomes, relevant dosing and route of administration and use of appropriate controls. Special attention should be applied to ascertain that exposure to the relevant substance or mixture is established with sufficient reliability. Studies should, where applicable, be carried out according to national and/or international test guidelines and according to good laboratory practice (GLP), compliance with good clinical practice (GCP), and good epidemiological practice (GEP) (U.S. CPSC, 2013; Hoffman, 2019; Alba, 2020; World Health Organization, Council for *International* Organizations of Medical Sciences (WHO CIOMS), 2009).

3.4.5.3.1.2.4 Positive data from well-run epidemiological studies (in accordance with WHO CIOMS guidelines, 2009) can be used for classifying substances and mixtures 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 or a mixture.

3.4.5.3.1.2.5 When using human epidemiological data for classification, consideration should be given to available data from a number of sources: (a) well-conducted clinical and diagnostic studies; (b) epidemiological studies, either general population studies or occupational studies; (c) cross-reactivity data; (d) case histories. Positive data from well-run epidemiological studies (which should also comply with WHO CIOMS guidelines, 2009) can be used for classifying substances and mixtures for skin sensitization. The incidence and severity of sensitization in occupational epidemiological studies may be higher than in general population studies due to the higher exposure levels (both in time and concentration). The exposure, the incidence and the severity in the study populations should be taken into account especially when deciding on the subcategory (see 3.4.2.2.2).

3.4.5.3.1.2.6 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; Frosch et al., 2015). Importantly, due consideration needs to be given to the appropriate selection of vehicle, test material composition, 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~~/~~ or diagnostic studies in humans and/or well-documented episodes of allergic contact dermatitis may be used to classify substances and mixtures for skin sensitization, when it can be assumed with sufficient confidence that the tested substance or mixture was indeed the most likely cause for induction of sensitization. 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 or mixture. On the other hand, negative results from such tests are not sufficient to prove that the test substance or mixture should not be classified as a skin sensitizer.

3.4.5.3.1.2.7 For some substances and mixtures, predictive patch test data in human volunteers are available (e.g. Strickland et al., 2023). Two test designs for predicting whether the substance or mixture will induce sensitization are

the Human Maximization Test (HMT) and the Human Repeated Insult Patch Tests (HRIPT).

3.4.5.3.1.2.8 Positive data from predictive patch testing (HRIPT or HMT) showing allergic contact dermatitis caused by the test substance or mixture can be used to classify for skin sensitization. These studies are generally conducted in controlled clinical settings and in general the study outcome is considered more reliable the larger the test panel size. Criteria for evaluating these data are provided in 3.4.2.2.2.2 and 3.4.2.2.2.3. When evaluating the data from HRIPT, consideration should be given to the appropriate use of vehicle as this can affect the outcome of testing (Johansen et al., 2015; Frosch et al., 2015).

3.4.5.3.1.2.9 The HMT is no longer in use, due to ethical concerns about its potential to create adverse health consequences for the person being tested. In cases where such data exist, they can nevertheless be used for classification.

3.4.5.3.1.2.10 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 or mixture does not have skin sensitizing 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 assessment. 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).

3.4.5.3.1.2.11 For example, negative results from substances or mixtures tested in a predictive patch test at a DSA (dose per skin area) of < 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 out, 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 or mixtures tested at a DSA ≥ 500 μg/cm^2 suggest that classification might not be needed. However, 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% (i.e. the undiluted substance or mixture) can justify no classification (based on this test). Nevertheless, negative results at low concentrations may be informative for classification of mixtures containing the substance or mixture at similar or lower concentrations.

3.4.5.3.1.2.12 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 substance~~s~~ or mixture, as well as factors such as the exposure situation, bioavailability, individual predisposition, cross-reactivity and preventive measures taken.”.

3.4.5.3.3 to 3.4.5.3.6 Current sections “3.4.5.3.3” to “3.4.5.3.6” become new sections “3.4.5.3.1.3” to “3.4.5.3.1.6”. Renumber the paragraphs within each section accordingly.

3.4.5.3.1.5 (former 3.4.5.3.5) Replace “criteria” with “methods” in the second sentence and insert “for this purpose” at the end of the second sentence.

3.4.5.3.1.6.1 (former 3.4.5.3.6.1) Replace “3.4.5.3.6.2” with “3.4.5.3.1.6.2”.

3.4.5.3.2 (new) Insert the following new section after 3.4.5.3.1.6 (former 3.4.5.3.6) to read:

"3.4.5.3.2 *Guidance on mixtures – skin sensitization*

3.4.5.3.2.1 General considerations

3.4.5.3.2.1.1 Mechanistic information in the OECD document on the Adverse Outcome Pathway for skin sensitization can be helpful in understanding the value of the individual *in chemico* and in vitro methods compared to the in vivo methods (see OECD (2014)).

3.4.5.3.2.1.2 Most of the standard animal test methods, defined approaches, in vitro and *in chemico* methods were developed and formally validated for identifying sensitizing substances and not mixtures. Nevertheless they are technically applicable to mixtures (see 3.4.3.1.2). However, there is limited data indicating whether there is a difference in the predictive capacity between standard animal test methods and defined approaches for the classification of mixtures. Sometimes, standard animal tests (see 3.4.2.2.3) on mixtures are required by competent authorities or applied voluntarily and the results are internationally accepted for classification. Therefore, the results of standard animal test methods can be used for the classification of mixtures. The defined approaches were first introduced in OECD Guideline 497 in 2021 without a clear statement on the applicability of the defined approaches for mixtures (see also 3.4.5.3.2.4.1). Human data can also be used for the classification of mixtures (see 3.4.5.3.2.2).

3.4.5.3.2.2 Guidance on the use of human data

See the guidance on the use of human data in 3.4.5.3.1.2 which is also applicable to mixtures.

3.4.5.3.2.3 Guidance on the use of standard animal data

3.4.5.3.2.3.1 Animal tests have been developed to identify sensitizing substances and not mixtures. Therefore, the results obtained on mixtures need to be evaluated with care. The following considerations can be relevant for mixtures because of dilution effects, in particular for borderline cases, but can also be applicable for substances.

3.4.5.3.2.3.2 For example, a stimulation index of three or more in the radioactive local lymph node assay (LLNA) (OECD Test Guideline 429) should be seen as a regulatory threshold for identification of a sensitizing mixture rather than as a threshold for sensitization as such. If a sensitizing substance is present at a low concentration in a mixture, a stimulation index of three may not be reached in the LLNA, but the substance in that mixture may still act as a sensitizer at population level. For this reason, a conclusion on the absence of sensitizing potential of a mixture based on the negative outcome in a test must be taken with great caution.

3.4.5.3.2.3.3 Where the mixture is tested undiluted, contains sensitizing ingredients and there is an increase in positive animals (Buehler, guinea pig maximisation test (GPMT)) or in the response (LLNA) which does not fulfil the criteria for a positive result, an overall weight of evidence assessment is required including the indicators included in Tier 3. This should also include available data on the sensitizing ingredient(s) regarding their potency, bioavailability, accumulation in the skin and interaction with the other ingredients. When the result is inconclusive, where applicable the bridging principles should be applied, otherwise the ingredient-based approach should be followed according to the tiered approach for mixtures (see 1.3.2.3).

3.4.5.3.2.3.4 Test data on a mixture takes into account effects of possible interactions of its components. For instance, it is known that the presence of a vehicle may significantly influence the skin sensitizing potency, by altering the

penetration of the sensitizing component(s) through the skin, (Basketter et al. 2001, Dearman et al. 1996, Heylings et al. 1996) or through other mechanisms involved in the induction of sensitization (Cumberbatch et al. 1993; Dearman et al. 1996). These mechanisms may differ between animals and humans. Especially where differences are known or suspected that could lead to the underestimation of sensitization, negative outcomes may not be reliable.

[3.4.5.3.2.3.5 If the classification based on standard animal test(s) with a mixture is inconsistent with the classification based on the concentration and potency (e.g. from standard animal test(s) or human data) of a sensitizing ingredient, additional considerations may need to be taken into account for the classification of the mixture (see OECD Test Guideline 429). This could include, for example, test concentrations, difference in vehicle and purity of the test material.]

3.4.5.3.2.3.6 Where the mixture contains corrosives or potent irritants resulting in unacceptable irritation in the pilot study with the mixture, either a dilution has to be used or the results may be a false positive. If a dilution is tested, the lower tested dose of the potential sensitizer(s) in the mixture may lead to false negative results for classification. In such cases, where applicable the bridging principles should be applied, otherwise the ingredient-based approach should be followed according to the tiered approach for mixtures (see 1.3.2.3), unless evidence is provided that the negative result is not caused by the dilution. This could for example be shown by testing the mixture without the corrosive or irritant ingredients at the actual concentration. Also, the validity of a well conducted LLNA on a mixture with a negative outcome can scientifically be confirmed by spiking the test mixture with another sensitizer (positive control) at different concentrations, or by showing a dose-response relationship.

3.4.5.3.2.4 Guidance on the use of defined approaches

3.4.5.3.2.4.1 Defined approaches may not have been formally validated for mixtures according to international procedures. Several defined approaches require upfront consideration to whether such testing will yield results that are predictive of the skin sensitizing properties of the mixture (see 3.4.5.3.2.4.5). This upfront consideration could include a comparison of the classification based on the results of a defined approach with existing classifications of similar mixtures. Where the comparison shows that the defined approach is predictive of certain types of mixtures, the outcome of the defined approach can be used for other mixtures of the same type for classification.

3.4.5.3.2.4.2 *In chemico* and in vitro methods used in defined approaches do not account for dermal penetration. Therefore, results from defined approaches may lead to false positive predictions compared to the standard animal tests that account for dermal penetration.

3.4.5.3.2.4.3 Also, it is necessary to exercise care when evaluating whether the dose used will yield results that are predictive of the skin sensitizing properties of the mixture. For example, in some *in chemico* and in vitro methods, the limited solubility of the ingredients of the mixture or limited stability of any suspension formed in the exposure medium or solvent may not allow testing at a dose that corresponds to the test requirements. In such a case, no valid outcome can be obtained for a negative result. Also, where the mixture is tested at lower concentrations in the in vitro methods due to the presence of cytotoxic ingredients, a positive result can be used for classification. However, a negative result is considered inconclusive as the concentration of the sensitizing ingredient(s) could have been too low unless evidence is provided that the negative result is not caused by the dilution. In such cases, where applicable the bridging principles should be applied, otherwise the ingredient-based approach should be followed according to the tiered approach for

mixtures (see 1.3.2.3). Approaches to address cytotoxicity are suggested in the relevant OECD test guidelines 442D and 442E.

3.4.5.3.2.4.4 In some methods, e.g. in silico predictions in the defined approaches for skin sensitization listed in OECD Guideline 497, all ingredients have to be assessed individually and the outcome from the in silico component of the defined approach is considered positive, if one ingredient is positive. However, it is noted that this may provide overly conservative or false positive predictions, as the in silico methods currently do not take into account the concentration at which the ingredient is present in the mixture.

3.4.5.3.2.5 Guidance on the use of non stand-alone *in chemico*/in vitro methods

3.4.5.3.2.5.1 Individual *in chemico*/in vitro methods such as those reported in OECD test guidelines 442C, 442D and 442E, due to their limited mechanistic coverage, cannot be used on their own to conclude on Category 1 or no classification. In addition, although some of these methods provide quantitative information, these cannot be used for the purposes of subcategorization into sub-categories 1A and 1B since the methods have not been validated according to international procedures for this purpose. Nevertheless, such quantitative information may be accepted by a competent authority when used in a weight of evidence assessment 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, 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 (Appendix III) kinetic Direct Peptide Reactivity Assay (kDPRA) can be used to differentiate between sub-category 1A and no sub-category 1A.

3.4.5.3.2.5.2 *In chemico*/in vitro methods may not have been formally validated for mixtures according to international procedures. Several *in chemico*/in vitro methods require upfront consideration to whether such testing will yield results that are predictive of the skin sensitizing properties of the mixture (see 3.4.5.3.2.4.5). This upfront consideration could include a comparison of the classification based on the results of an *in chemico*/in vitro method with existing classifications of similar mixtures. Where the comparison shows that the *in chemico*/in vitro method is predictive of certain types of mixtures, the outcome of the *in chemico*/in vitro method may be used for other mixtures of the same type for classification.

3.4.5.3.2.5.3 *In chemico*/in vitro methods do not account for dermal penetration. Therefore, results from *in chemico*/in vitro methods may lead to false positive predictions compared to the standard animal tests that account for dermal penetration.

3.4.5.3.2.5.4 Also, it is necessary to exercise care when evaluating whether the dose used will yield results that are predictive of the skin sensitizing properties of the mixture. For example, in some *in chemico* and in vitro methods, the limited solubility of the ingredients of the mixture or limited stability of any suspension formed in the exposure medium or solvent may not allow testing at a dose that corresponds to the test requirements. In such a case, no valid outcome can be obtained for a negative result. Also, where the mixture is tested at lower concentrations in the in vitro methods due to the presence of cytotoxic ingredients, a positive result can be used for classification. However, a negative result is considered inconclusive as the concentration of the sensitizing ingredient(s) could have been too low unless evidence is provided that the negative result is not caused by the dilution. In such cases, where

applicable the bridging principles should be applied, otherwise the ingredient-based approach should be followed according to the tiered approach for mixtures (see 1.3.2.3). Approaches to address cytotoxicity are suggested in the relevant OECD test guidelines 442D and 442E. ”

3.4.5.3.7 Current section “3.4.5.3.7” becomes new section “3.4.5.3.3”. Renumber the paragraphs within the section accordingly.

Insert the following references:

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* *References:*

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