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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**

**Forty-second session**

Geneva, 6-8 July 2022

Item 2 (d) of the provisional agenda

**Classification of skin sensitizers using the results of local lymph node assays test methods**

**in accordance with OECD Test Guideline 442B**

Clarification of the criteria for classification for skin sensitization using animal studies

 Transmitted by the expert from Japan

 Introduction

1. This document provides the outcome of the work undertaken by Japan to clarify the criteria for classification for skin sensitization using animal studies. Japan wishes to take the opportunity to thank the secretariat and all the experts considering our proposal to clarify the criteria for classification for skin sensitization using animal studies.

**Discussion**

2. In Chapter 3.4 of the Globally Hamonized System of Classification and Labelling of Chemicals (GHS), three skin sensitization test methods are listed as test methods to classify GHS category 1 chemicals. These include two guinea pig prediction tests (the guinea pig maximization test and the Buehler test), known as the official test guidelines (TG) 406 of the Organization of Economic Cooperation and Development (OECD), and the radioisotopic (RI) Local Lymph Node Assay (LLNA), known as OECD TG 429 (OECD, 2010). These three methods can be further applied for GHS sub-categorization 1A/1B to provide information on the skin sensitization potency of chemicals (United Nations, 2021).

3. Currently, three non-radioisotopic alternatives to the LLNA (OECD TG429), LLNA: DA, LLNA: BrdU-ELISA and LLNA: BrdU-FCM, are listed in OECD TG as TG 442A and 442B (OECD, 2018). Although these non-radioisotopic alternatives of the LLNA are scientifically validated and can be used to classify GHS category 1 versus “Not classified”. However, the current status of their applicability to the GHS classification is not clearly described in the GHS.

4. In addition, Japanese researchers investigated about the applicability of the data derived from LLNA: BrdU-ELISA to GHS sub-categorization and attempted to determine optimal criterion for GHS sub-categorization using this method (Maeda and Takeyoshi, 2019). And the newly developed GHS sub-categorization criterion was further confirmed to be applicable to the commonly used mouse strain (Kobayashi et al., 2020).

Table 1: Newly developed GHS sub-categorization criterion for LLNA: BrdU-ELISA

|  |  |
| --- | --- |
| Category | Criterion |
| Cat.1 | SI ≥ 1.6 |
| Cat.1A | EC1.6 value ≤ 6% |
| Cat.1B | EC1.6 value > 6% |

5. Japan proposed to include the above newly developed criterion in Chapter 3.4 at the thirty-ninth session of the Sub-Committee. It was agreed to include it in the two-year work plan for 2021-2022. Then Japan led the work in parallel and in coordination with the on-going work led by the United Kingdom and the Netherlands.

6. Meanwhile, the OECD secretariat kindly coordinated the peer review work to evaluate the rationale, validity, and the applicability of the proposed criterion, and the review work was conducted from June 2021 to October 2021 by the Peer Review Panel (PRP), composed of five experts from the OECD Expert Group on Skin Sensitization. The PRP concluded in the peer review report (see informal document INF.4) that the proposed criterion, evaluated based on the materials submitted, met the validation principles of OECD Guidance Document 34. Japanese responses to comments from the PRP are provided in informal document INF.5.

7. As for the additional information, Japanese researchers conducted the survey on the OECD eChemPortal to find skin sensitisation test data obtained by the LLNA: BrdU-ELISA registered in the REACH (Takeyoshi and Nara, 2021), and they found an issue of incorrect application of the existing sub-categorisation criteria for LLNA-RI to the data derived from the LLNA: BrdU-ELISA, using EC1.6 with a cut-off of 2%. The survey reported that some chemicals predicted based on EC1.6 range from 2% to 6% may be underestimated for their sensitization potential. In order to avoid incorrect application of the GHS criteria and incorrect hazard communication for the chemicals, a speedy and appropriate revision of the GHS would be desirable. Application of the sub-categorisation criterion will also allow for making correct classification for existing data that have been obtained and accumulated from using LLNA: BrdU-ELISA.

8. Taken together the above situations of the non-radioisotopic alternatives to LLNA listed in OECD TG442A/B, we propose to add the descriptions of the criteria for these methods. In addition, we also propose to add the sub-categorization criterion using the data derived from LLNA: BrdU-ELISA listed in OECD TG442B. This approach would achieve a clarification of the GHS categorization when using the non-radioisotopic alternatives to LLNA, and the further sub-categorization of GHS 1A/1B sub-categories by using LLNA: BrdU-ELISA. Then this approach would be helpful for avoiding incorrect application of the GHS criteria and incorrect hazard communication for the chemicals.

9. To provide clarity on the classification of skin sensitization in Category 1 using animal studies, it is proposed to amend paragraph 3.4.2.2.3.1 of the GHS (see proposal in paragraph 13 below.

10. To assist classifiers and users of the GHS, it was also considered appropriate to present this information in a table format. Therefore, it is proposed to insert a new Table 3.4.3 (on animal study test results for category 1) under 3.4.2.2.3.1 (see proposal in paragraph 14).

11. This new table would also require consequential amendments to renumber the existing tables, together with the related table references that are provided in 3.4.2.2.3.2, (table on animal test results for sub-category 1A), 3.4.2.2.3.3 (table on animal test results for sub-category 1B); 3.4.3.3 (table on “Cut-off values/concentration limits of ingredients of a mixture classified as either respiratory sensitizer or skin sensitizer that would trigger classification of the mixture”), 3.4.4.1 (table on label elements for respiratory or skin sensitization) and decision logics 3.4.1 and 3.4.2. The required consequential amendments are listed in paragraphs 15 to 20 below.

12. For the tables on animal test results for sub-category 1A and 1B it is proposed to insert new criteria (“EC1.6 value ≤ 6%” and “EC1.6 value **>**  6%” respectively)for the Local lymph node assay: BrdU-ELISA (See proposal in paragraphs 15 and 16 below).

 In In addition, it is proposed to insert a new note beneath each of these tables providing additional information that currently, there is no validated and internationally agreed criteria for subcategorization of skin sensitizers for LLNA: DA and LLNA: BrdU-FCM as detailed in paragraphs 15 and 16 to avoid incorrect sub-categorization with these methods.

**Proposed amendments**

*(new text is shown in bold, underlined)*

13. Replace paragraph 3.4.2.2.3.1 with:

“3.4.2.2.3.1 For Category 1, when an adjuvant type test method for skin sensitization is used, a response of at least 30% of the animals is considered as positive. For a non-adjuvant Guinea pig test method a response of at least 15% of the animals is considered positive. For Category 1, a stimulation index of three or more is considered a positive response in the **radioisotopic** local lymph node assay **(LLNA). For the non-radioactive modifications to the LLNA, a stimulation index of 1.8 or more in the LLNA: DA, 1.6 or more in the LLNA: BrdU-ELISA, and 2.7 or more in the LLNA: BrdU-FCM are considered positive.** Test methods for skin sensitization are described in the OECD Guideline 406 (the Guinea Pig Maximisation test and the Buehler guinea pig test) and **in Guidelines 429/442A/442B (Local Lymph Node Assays).** Other methods may be used provided that they are well-validated and scientific justification is given. The Mouse Ear Swelling Test (MEST), appears to be a reliable screening test to detect moderate to strong sensitizers, and can be used as a first stage in the assessment of skin sensitization potential.”.

14. After paragraph 3.4.2.2.3.1 insert the following new table:

**“Table 3.4.3: Animal test results for category 1**

|  |  |
| --- | --- |
| **Assay** | **Criteria** |
| **Local lymph node assay** | **SI ≥ 3** |
| **Local lymph node assay: DA** | **SI ≥ 1.8** |
| **Local lymph node assay: BrdU-ELISA** | **SI ≥ 1.6** |
| **Local lymph node assay: BrdU-FCM** | **SI ≥ 2.7** |
| **Adjuvant Guinea pig test method**  | **≥ 30% responding at any intradermal induction dose** |
| **Non-adjuvant Guinea pig test method**  | **≥ 15% responding at any topical induction dose** |

15. In paragraph 3.4.2.2.3.2,

 Replace ‘Table 3.4.3’ with ‘**Table 3.4.4**’ in both the sentence and the table title ‘Animal test results for sub-category 1A’.

 Under the first column ‘Assay’, after the table entry for ‘Local lymph node assay’, insert a new table entry: ‘**Local lymph node assay: BrdU-ELISA**’.

 Under the second column ‘Criteria’, for the new entry for ‘**Local lymph node assay: BrdU-ELISA**’, insert ‘**EC1.6 value ≤ 6%**’.

Under the new Table 3.4.4, insert the following footnote:

“***Note: As for the LLNA: DA and LLNA: BrdU-FCM, there are currently no validated and internationally agreed criteria for subcategorization of skin sensitizers. Therefore, these test methods can only be used to conclude on either classification in category 1 or no classification***.”

16. In paragraph 3.4.2.2.3.3,

 Replace ‘Table 3.4.4’ with ‘**Table 3.4.5**’ in both the sentence and the table title ‘Animal test results for sub-category 1B’.

 Under the first column ‘Assay’, after the table entry for ‘Local lymph node assay’, insert a new table entry: ‘**Local lymph node assay: BrdU-ELISA**’.

 Under the second column ‘Criteria’, for the new entry for ‘**Local lymph node assay: BrdU-ELISA**’, insert ‘**EC1.6 value > 6%**’.

Under the new Table 3.4.5, insert the following footnote:

“***Note: As for the LLNA: DA and LLNA: BrdU-FCM, there are currently no validated and internationally agreed criteria for subcategorization of skin sensitizers. Therefore, these test methods can only be used to conclude on either classification in category 1 or no classification.***”

17. In section 3.4.3.3, replace ‘Table 3.4.5’ with ‘**Table 3.4.6**’ in both the sentence and the table title: ‘Cut-off values/concentration limits of ingredients of a mixture classified as either respiratory sensitizer or skin sensitizer that would trigger classification of the mixture’.

18. In paragraph 3.4.4.1, replace ‘Table 3.4.6’ with ‘**Table 3.4.7**’ in both the last sentence and the table title: ‘Label elements for respiratory or skin sensitization’.

19. In section 3.4.5.1 ‘Decision logic 3.4.1 for respiratory sensitization’, last box replace ‘Table 3.4.5’ with ‘**Table 3.4.6**’.

20. In section 3.4.5.2 ‘Decision logic 3.4.1 for skin sensitization’, last box replace ‘Table 3.4.5’ with ‘**Table 3.4.6**’.

 Action requested

21. The Sub-Committee is invited to agree the proposed amendments in paragraph 3.4.2.2.3 of the GHS and the related consequential amendments, as set out in paragraphs 13 to 20 above.

 Annex

 References

Kobayashi T, Maeda Y, Kondo H, Takeyoshi M. (2020). Applicability of the proposed GHS sub-categorization criterion for LLNA: BrdU-ELISA (OECD TG442B) to the CBA/J strain mouse. J Appl Toxicol.; 1-5.

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Takeyoshi M, Nara S. (2021). Registration status of skin sensitisation data derived from the Local Lymph Node Assay (LLNA): BrdU-ELISA in REACH. Arch Toxicol. May;95(5):1857-1858.

United Nations (2021). Globally Harmonized System of Classification and Labelling of Chemicals (GHS) 9th revised edition. Chapter 3.4 Respiratory or skin sensitization. United Nations Publications. New York. pp. 153-161.