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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 3 (d) of the provisional agenda

#### Work on the Globally Harmonized System of Classification and Labelling of Chemicals: 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 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 test guidelines 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.

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\* A/75/6 (Sect.20), para. 20.51.

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). 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	$SI \geq 1.6$
Cat.1A	EC1.6 value $\leq 6\%$
Cat.1B	EC1.6 value $> 6\%$

5. The above newly developed criterion was proposed to be included into Chapter 3.4 at the thirty-ninth session of the Sub-Committee by Japan, and the Sub-Committee agreed to include an item in its programme of work 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 the materials used to establish the proposed criterion met the validation principles of OECD Guideline 34.

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 under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (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 effective concentration (EC) EC1.6 with a 2% cut-off value. 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. At the forty-second session of the Sub-Committee, several countries commented to consider the possibility to include the sub-classification criterion for the LLNA BrdU-ELISA test method to OECD Test Guideline 442B. Considering these comments, Japan discussed with the OECD Secretariat about the revision policy of the test guidelines, and confirmed the positive consideration to include the criteria into related OECD test guidelines, noting the need for (careful) consideration as it may have implications on other test guidelines. In addition, according to the expert comments with regards to the international acceptance of sub-categorization criteria, an additional note was added to clarify the description status of sub-categorization criteria in the OECD test guidelines as follows:

*“Although no sub-categorization criteria have yet been described in OECD test guideline Nos. 406, 429 and 442B, validated sub-categorization criteria may still be accepted by some competent authorities. A competent authority may decide which sub-categorization criteria, if any, should be applied for these test methods.”*

9. In addition, a definition of EC values used for subcategorization criteria of LLNA does not appear in anywhere in OECD test guideline for LLNAs and Chapter 3.4 of GHS document, therefore explanation for EC values was added as a note to the tables.

10. Taken together the above situation of the non-radioisotopic alternatives to LLNA listed in OECD TG442A/B, we propose to add the descriptions of criteria for these methods. In addition, we also propose to add 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 categories 1A/1B by using LLNA: BrdU-ELISA. Then this approach would be helpful to avoid incorrect application of the GHS criteria and incorrect hazard communication for the chemicals.

11. 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 as follows (new text is shown in **bold and underlined**):

“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.”

12. 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’, as shown below:

“Table 3.4.3: Animal test results for category 1

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

13. This new table would also require consequential amendments to renumber the existing tables 3.4.3, 3.4.4, 3.4.5 and 3.4.6, together with the related table references that are provided in:

- paragraph 3.4.2.2.3.2 (currently referring to Table 3.4.3).
- paragraph 3.4.2.2.3.3 (currently referring to Table 3.4.4).
- paragraph 3.4.3.1 (currently referring to Table 3.4.5).
- section 3.4.3.3 (currently referring to Table 3.4.5).
- paragraph 3.4.4.1 (currently referring to Table 3.4.5).
- paragraph 3.4.4.2 (currently referring to Table 3.4.5).
- decision logics 3.4.1 and 3.4.2 (currently referring to refer to the current Table 3.4.5).

14. For the tables on animal test results for sub-category 1A and 1B it is proposed to insert new criteria for the Local lymph node assay: BrdU-ELISA as follows:

- For Animal test results for sub-category 1A:

Assay	Criteria
<b><u>Local lymph node assay: BrdU-ELISA</u></b>	<b><u>EC1.6 value ≤ 6%</u></b>

- For Animal test results for sub-category 1B:

Assay	Criteria
<b><u>Local lymph node assay: BrdU-ELISA</u></b>	<b><u>EC1.6 value &gt; 6%</u></b>

- In addition, it is proposed to insert a 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 17 and 18 to avoid incorrect sub-categorization with these methods.

### Proposed amendments to section 3.4.2.2.3 ‘Animal studies’

15. Amend paragraph 3.4.2.2.3.1 as follows:

“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.”

16. 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
<b><u>Local lymph node assay</u></b>	<b><u>SI ≥ 3</u></b>
<b><u>Local lymph node assay: DA</u></b>	<b><u>SI ≥ 1.8</u></b>
<b><u>Local lymph node assay: BrdU-ELISA</u></b>	<b><u>SI ≥ 1.6</u></b>
<b><u>Local lymph node assay: BrdU-FCM</u></b>	<b><u>SI ≥ 2.7</u></b>
<b><u>Adjuvant Guinea pig test method</u></b>	<b><u>≥30% responding at any intradermal induction</u></b>
<b><u>Non-adjuvant Guinea pig test method</u></b>	<b><u>≥15% responding at any topical induction dose</u></b>

17. In paragraph 3.4.2.2.3.2:

Replace “Table 3.4.3” with ‘Table 3.4.4’ in the text of the paragraph and in the table title ‘Animal test results for sub-category 1A’.

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

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

Under the new Table 3.4.4, insert the following note:

**“Note: The EC3 and EC1.6 values are estimated concentration of a chemical expected to produce positive responses in each assay method.**

**Although no sub-categorization criteria have yet been described in OECD test guideline Nos. 406, 429 and 442B, validated sub-categorization criteria may still be accepted by some competent authorities. A competent authority may decide which sub-categorization criteria, if any, should be applied for these test methods.**

*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.*

18. 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 note:  

*“Note: The EC3 and EC1.6 values are estimated concentration of a chemical expected to produce positive responses in each assay method.*

*Although no sub-categorization criteria have yet been described in OECD test guideline Nos. 406, 429 and 442B, validated sub-categorization criteria may still be accepted by some competent authorities. A competent authority may decide which sub-categorization criteria, if any, should be applied for these test methods.*

*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.*
19. In paragraph 3.4.3.1, replace 'Table 3.4.5' with '**Table 3.4.6**' in the last sentence.
20. 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'.
21. 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'.
22. In paragraph 3.4.4.2, replace 'Table 3.4.5' with '**Table 3.4.6**' in the first sentence.
23. 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**'.
24. 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

25. The Sub-Committee is invited to agree the proposed amendments in paragraph 3.4.2.2.3 of the GHS together with the consequential amendments, as set out in this document in paragraphs 15 to 24 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.

Maeda Y, Takeyoshi M. (2019). Proposal of GHS sub-categorization criteria for LLNA: BrdU-ELISA (OECD TG442B). *Regul Toxicol Pharmacol.* 107:104409.

Organization for Economic Co-operation and Development (OECD). (2010). OECD GUIDELINE FOR THE TESTING OF CHEMICALS No.429, Skin Sensitization: Local Lymph Node Assay. OECD, Paris

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Organization for Economic Co-operation and Development (OECD). (2018). OECD GUIDELINE FOR THE TESTING OF CHEMICALS No.442B, Skin Sensitisation: Local Lymph Node Assay: BrdU-ELISA or FCM. OECD, Paris

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

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