

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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Work on the Globally Harmonized System (GHS):

Classification of skin sensitizers using the results of local lymph node assays (LLNA) 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 informal paper provides an update on the proposal made by Japan to clarify the criteria for classification for skin sensitization using animal studies at the thirty-ninth session of the Sub-Committee.

Background

2. Currently, 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 radio isotopic (RI) LLNA (LLNA-RI), 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. The LLNA: BrdU-ELISA listed in OECD TG 442B (OECD, 2018) is another reliable skin sensitization test method using the same principle as the standard LLNA-RI in TG 429 (OECD, 2010). The LLNA: BrdU-ELISA was scientifically validated and peer-reviewed by ICCVAM in 2010; it is used as a conventional skin sensitization test method worldwide and can be used to classify GHS category 1 versus Not classified.

4. Recently, Japanese researchers re-analysed 32 skin sensitizers data, used in the validation and peer review of LLNA: BrdU-ELISA, that are classified as GHS Category 1A or GHS Category 1B, and attempted to determine optimal criterion for GHS sub-categorization using this method. Consequently, the optimal criterion for the GHS sub-categorization was determined to be 6% when using EC1.6; an estimated concentration to cause SI=1.6 the criterion presents almost equivalent performance to the existing criterion of 2% when using the EC3 with LLNA-RI data, the correct classification outcomes (%) for GHS 1A and GHS 1B category chemicals were 92.3(%) and 84.2(%) for the 32 chemicals, respectively (Maeda and Takeyoshi, 2019). When excluding 2-mercaptobenzothiazole which may cause a strain-specific

low sensitisation response in the LLNA: BrdU-ELISA assay using CBA/JN mouse, the correct classification outcome for GHS 1A chemicals was 100%.

Table 1. Proposed 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. Further examination to confirm the applicability of the proposed GHS sub-categorization criterion to data derived from the commonly used mouse strain was conducted with fifteen chemicals categorized in GHS Category 1A or Category 1B sensitizers listed in the LLNA Performance Standard (OECD, 2010). The results revealed that all GHS Category 1A or Category 1B chemicals could be correctly assigned using the newly proposed criterion. (Kobayashi et al., 2020).

6. It was agreed to include the proposal in the two-year work plan for 2021-2022 at the thirty-ninth session of the Sub-Committee. It was also noted that the work led by Japan should be done in parallel and in coordination with the on-going work on the revision of Chapter 3.4 on non-animal test methods, led by the United Kingdom and the Netherlands.

7. The proposal above was initially presented to the OECD Expert Group on alternative methods for skin sensitization at a meeting held in October 2020. At the December meeting of the GHS Sub-Committee of Experts in December 2020, a request was formulated to subject the LLNA: BrdU-ELISA data to a peer-review by the OECD Expert Group on skin sensitisation. The OECD Secretariat asked support from the Working Party of the National Coordinators of the Test Guidelines Programme (WNT) in April 2021 to carry out this task.

Status report

8. The OECD secretariat kindly coordinated the Peer Review work to evaluate the rationale, validity, and the applicability of the proposed criterion. Japan submitted the proposal and other supporting materials, including the raw data, to the OECD Expert Group Peer Review Panel (PRP) in Q3 2021. The review work was conducted from June 2021 to October 2021 by the PRP, composed of five experts from the OECD Expert Group on Skin Sensitization.

9. The PRP evaluated how the proposed analysis and criterion for GHS sub-categorisation address the principles outlined in the OECD Guidance Document 34 (GD 34) on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. The PRP concluded in the Peer Review Report that the proposed criterion, evaluated based on the materials submitted, meet the validation principles of OECD GD 34.

10. The chairs of the informal working group on the use of Non-Animal Testing Methods Informal Working Group (NATM IWG) contacted Japan to inform us about a NATM IWG discussion on the inclusion of all versions of non-radioactive LLNA test guidelines 442A and 442B in the revisions of Chapter 3.4. On consideration of this, Japan drafted a new text of Chapter 3.4 (animal studies) as shown in the annex to this document.

11. 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 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.

12. In order to avoid incorrect application of the GHS criteria and incorrect risk communication for the hazardous chemicals, a speedy and appropriate revision of the GHS document would be desirable.

13. Japan aims to submit the working document for approval of this proposal to the forty-second session of the GHS Sub-Committee with the finalized OECD Peer Review Report.

References

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Annex

Draft text for Chapter 3.4, section 3.4.2.2.3 (Animal studies)

3.4.2.2.3.1 Amend current paragraph and insert a new table 3.4.3 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 the LLNA: BrdU-FCM is 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)**. **Test results from LLNA: DA and LLNA: BrdU-FCM can only be used to conclude on either classification in category 1 or no classification.** 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.

Table 3.4.3: Animal test results for -category 1

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

3.4.2.2.3.2 Amend and renumber current table 3.4.3 (and related references) as follows:

“3.4.2.2.3.2 Animal test results for sub-category 1A can include data with values indicated in Table 3.4.4 below:

Table 3.4.4: Animal test results for sub-category 1A

Assay	Criteria
Local lymph node assay	EC3 value \leq 2%
Local lymph node assay: BrdU-ELISA	EC1.6 value \leq 6%
Guinea pig maximisation test	\geq 30% responding at \geq 0.1% intradermal induction dose or \geq 60% responding at $>$ 0.1% to \leq 1% intradermal induction dose
Buehler assay	\geq 15% responding at \leq 0.2% topical induction dose or \geq 60% responding at $>$ 0.2% to \leq 20% topical induction dose

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.”

3.4.2.2.3.3 Amend and renumber current table 3.4.4 (and related references) as follows:

“3.4.2.2.3.3 Animal test results for subcategory 1B can include data with values indicated in Table 3.4.5 below:

Table 3.4.5: Animal test results for sub-category 1B

Assay	Criteria
Local lymph node assay	EC3 value > 2%
Local lymph node assay: BrdU-ELISA	EC1.6 value > 6%
Guinea pig maximisation test	≥ 30% to < 60% responding at > 0.1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose
Buehler assay	≥ 15% to < 60% responding at > 0.2% to ≤ 20% topical induction dose or ≥ 15% responding at > 20% topical induction dose

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.”.