

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

Transmitted by the expert from Japan

#### Introduction

1. Japan wishes to thank the secretariat and all delegates to consider and discuss this proposal aimed at clarifying the criteria for classification for skin sensitization using animal studies.
2. Currently, three sensitization test methods, two guinea pig prediction tests, the guinea pig maximization test and the Buehler test, known as the official test guidelines (TG) of the Organization of Economic Cooperation and Development (OECD TG406), and the radioisotopic (RI)- LLNA, known as OECD TG429, are suggested as test methods to classify GHS category 1 chemicals. And these three methods can be further applied for GHS sub-categorization 1A/1B to provide information on the sensitization potency of chemicals.
3. OECD TG442B, LLNA: BrdU-ELISA is a reliable sensitization test method using the same principle to the standard radioisotopic LLNA (LLNA-RI), and the method is given scientific justification by the official validation study and the independent peer review by the ICCVAM<sup>1</sup>, so it is applicable to classify GHS category 1. In addition, LLNA: BrdU-ELISA has been used as the conventional skin sensitization test method worldwide and applied also for regulatory purposes in several countries.
4. Recently Japanese researchers re-analysed the existing data of 32 sensitizers used in the peer review process of LLNA: BrdU-ELISA those classified in the 1A or 1B categories of the GHS and attempted to determine optimal criteria 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, showing the preferable performance to GHS sub-categorization using the results of LLNA: BrdU-ELISA (Maeda and Takeyoshi, 2019), showing the correct outcomes (%) for GHS 1A and GHS 1B category chemicals were 92.3 and 84.2 for all 32 chemicals, respectively. When excluding 2-mercaptobenzothiazole which may cause a strain-specific low response in this assay system, the correct outcomes (%) for GHS 1A chemicals was 100.
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 hazard category 1A/1B sensitizers listed in the LLNA performance standard (Kobayashi et al., 2020).

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<sup>1</sup> “Interagency Coordinating Committee on the Validation of Alternative Methods”, National Institute of Environmental Health Sciences. National Toxicology Program, U.S Department of Health and Human Services.

The results revealed that all the GHS 1A or 1B category chemicals could be correctly assigned to the respective 1A and 1B categories using the newly proposed GHS sub-classification criterion.

6. According to the above-mentioned scientific backgrounds, we propose to add a new criterion of the GHS related to the classification criteria of skin sensitizer using the results of animal experiments as follows:

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 $> 6\%$

7. In addition, we also suggest the following revisions under:

(a) 3.4.2.2.3.1, for Category 1: add the words “1.6 or more in Local Lymph Node Assay: BrdU-ELISA.”, and change the wording describe the TGs as “Guidelines 429/442B (Local Lymph Node Assays)”.

(b) 3.4.2.2.3.2 Table 3.4.3: add new criterion for LLNA: BrdU-ELISA as follow;

Local lymph node assay: BrdU-ELISA	EC1.6 value $\leq 6\%$
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(c) 3.4.2.2.3.3 Table 3.4.4: add new criterion for LLNA: BrdU-ELISA as follows:

Local lymph node assay: BrdU-ELISA	EC1.6 value $> 6\%$
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## 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. <https://doi.org/10.1002/jat.3996>.

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

## Proposal

3.4.2.2.3.1 Amend to read as follows (*new text is shown in red*):

“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 local lymph node assay **or 1.6 or more in Local Lymph Node Assay: BrdU-ELISA**. Test methods for skin sensitization are described in the OECD Guideline 406 (the Guinea Pig Maximisation test and the Buehler guinea pig test) and Guidelines 429/442B (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.”

3.4.2.2.3.2 Amend table 3.4.3 as follows (*new text is shown in red*):

Table 3.4.3: Animal test results for sub category 1A

Assay	Criteria
Local lymph node assay	EC3 value $\leq$ 2%
<b>Local lymph node assay: BrdU-ELISA</b>	<b>EC1.6 value <math>\leq</math> 6%</b>
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

3.4.2.2.3.3 Amend Table 3.4.4 as follows (*new text is shown in red*):

Table 3.4.4: Animal test results for sub category 1B

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