Committee of Experts on the Transport of Dangerous Goods and on the Globally Harmonized System of Classification and Labelling of Chemicals

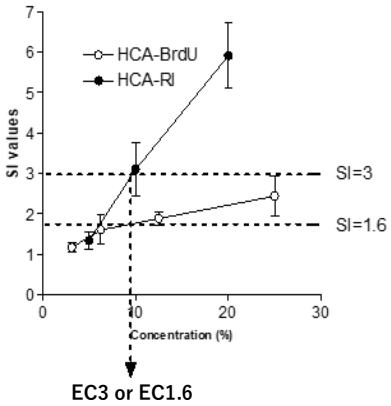
Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals **21 April 2022**

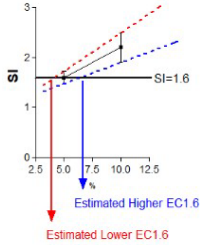
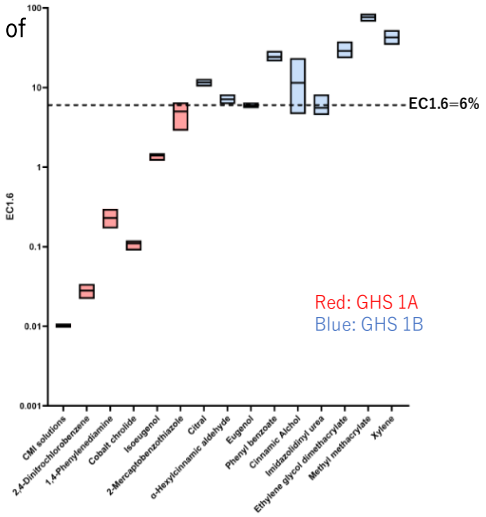
Forty-second session
Geneva, 6-8 July 2022
Item 2 (d) of the provisional agenda
Classification of skin sensitizers using the results of local lymph node assays test methods in accordance with OECD Test Guideline 442B

Japanese response to the comments noted in the peer review report report for sub-categorisation based LLNA_BrdU-ELISA

Transmitted by the expert from Japan

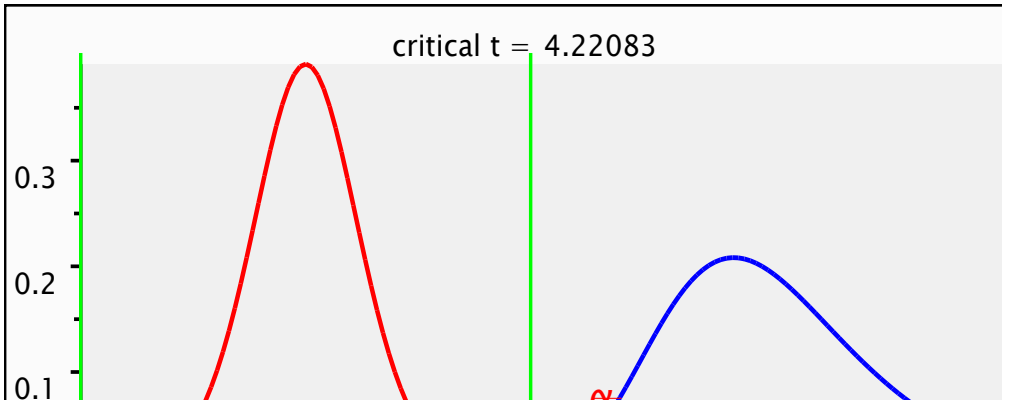
1. This document provides the responses to the comments to the Peer Review Panel (PRP) report for GHS sub-categorisation based LLNA: BrdU-ELISA conducted as the OECD support work for this proposal (see informal document INF.4).

Comments（Paragraph No.）	Response to the PRP comments
27. More importantly, some discussion of the potential for differences in the two nucleotide incorporation endpoints should be presented with respect to potential impact upon discriminating among skin sensitisers to justify the analyses.	Although both RI-LLNA and LLNA: BrdU-ELISA employ the same principle to measure the cell proliferation process by using incorporation of 3[H]-thymidine or BrdU into DNA as indicators, the response curves of these methods are apparently different. And then, there is a difference in the detection process of cell proliferation; RI-LLNA directly detects the scintillation of [3H]-thymidine, whereas LLNA: BrdU-ELISA indirectly detects the chromogenic reaction of incorporated BrdU by immunochemical reaction using enzyme-labelled antibody. The difference of the response curves for these two methods was suggested to be caused by the deference of the detection process. However, the cut-off values for each method were determined based on the agreement between the EC values obtained by each method and the known classification results, so the classification performance of GHS1A/1B of these methods is suggested as the same. **EP2 Effect of differences in the two nucleotide incorporation endpoints**  **Typical response curves of HCA** ・The response curves are apparently different in these two methods. ・Incorporation process of [3H] -thymidine and BrdU are the same. ・RI-LLNA directly detects Scintillation counting of [3H]-thymidine. ・LLNA: BrdU-ELISA indirectly detect incorporated BrdU by chromogenic reaction using immunochemical reaction of enzyme labeled antibody. ・Although the differences in the response curve due to the detection process exist, this would not affect to the performance to categorize GHS 1A/1B because the cut-off % for each method was determined based on the agreement between the EC values obtained by each method and the known classifications results.

Comments（Paragraph No.）	Response to the PRP comments
31. An analysis to determine the range of EC3 values for all of the substances near the putative cut-off should be conducted to best fit the available LLNA:BrdUELISA EC1.6 values with the ranges of RI-LLNA EC3 values.	Based on the data reported by Kobayashi et al. (2020), the maximum and the minimum EC1.6 values estimated from the upper and lower limits of the standard errors at the two data points used for calculating the EC values were plotted for 15 chemicals, and the impact of individual variation on the EC1.6 values was considered. Consequently, the estimated ranges of EC1.6 values were very narrow, then the impact of the variability of the individual animals on the final GHS1A/1B classification was considered as small. EP3 the borderline results, impact of variability of individual animals To confirm the borderline results and individual variability of EC1.6, we tried to estimate the range of EC1.6 for the population from the standard error of the individual SI values in the CBA/J mice from the data used for the peer review.  

Comments（Paragraph No.）	Response to the PRP comments
32. Furthermore, a more sophisticated analyses should be conducted by evaluating not only the mean data driving the single EC3 and EC1.6 values presented for each substance in the tables, but rather an analysis of the impact of variability of the individual animals.	Refer to Para. 31
34. The proposal should include more information on the types of chemicals that can be evaluated using this specific assay.	As described in the ICCVAM Report[1], the applicability domain of LLNA: BrdU-ELISA considered to be the same as that of the RI-LLNA because both test methods are based on the same principle. Also, the applicability domain of LLNA: BrdU-ELISA is described in the TG.
35. Overall, the PRP agreed that the Evaluation Principle 3 has been met. However, the PRP made a number of recommendations for further clarifications on the borderline results, applicability domain and on the impact of variability of individual animals used for the study.	Refer to Para. 31

[1] https://ntp.niehs.nih.gov/iccvam/docs/immunotox_docs/llna-elisa/tmer.pdf

Comments（Paragraph No.）	Response to the PRP comments
36. The majority of PRP considers that the overall number of chemicals used for the analysis to be limited and that further assessment with a statistical tool (i.e., power analysis) to determine whether the number of chemicals was sufficient may be desirable. However, the PRP acknowledges that the proposed criteria is based on the most complete and well-documented dataset available (ICCVAM 2010).	The applicability of the subcategorization criterion for the LLNA: BrdU-ELISA is based on the assumption that EC1.6 and EC3 show a strong correlation between both methods. So, the appropriateness of the sample size (15) in Kobayashi et al., (2020) was analysed by using Power analysis software (GPower, Ver. 3.1). In this case, the correlation coefficient (r) = 0.9077 between EC1.6 and EC1.6 reported in the paper was set as the effect size, and the probability of alpha error was set to 0.001 as the parameter (two-tailed test). As the result, the Power (1-β err prob) was calculated as 0.9993, which is in the range of 0.8-1.0, indicating that the sample size was appropriate. t tests - Correlation: Point biserial model Analysis: Post hoc: Compute achieved power Input: Tail(s)=Two Effect size |ρ|=0.90768 α err prob=0.001 Total sample size=15 Output: Noncentrality parameter δ=8.37654 Critical t =4.22083 Df=13 Power (1-β err prob)=0.99931 

Comments（Paragraph No.）	Response to the PRP comments
50. It was questioned whether the acceptance of the proposal could lead to confusion for risk assessment. For example, if there are data from both the RI-LLNA and the LLNA:BrdU-ELISA, it is not clear which threshold value should be used to support risk assessment (i.e., EC3≤ 2% vs. EC1.6≤ 6% for Cat.1A sensitisers).	Currently, the case that multiple test methods are listed in the same TG (such as OECD TG431[2]), not only for skin sensitization tests, and the GHS classification criteria are also prepared for each test method. Accordingly, the correct selection of classification criteria corresponding to each test method would be necessary. In the case of LLNA, the incorrect application of the GHS subcategorization criteria for RI-LLNA to the data derived from LLNA: BrdU method and the underestimated cases for sensitization potency of chemicals have been reported (Takeyoshi and Nara, 2021). In order to avoid an incorrect application of the GHS classification criteria and incorrect hazard communication for chemicals, the clarification of the categorization criteria for all versions of LLNAs would be desirable., and the GHS classification criteria are also prepared for each test method. Accordingly, the correct selection of classification criteria corresponding to each test method would be necessary. In the case of LLNA, the incorrect application of the GHS subcategorization criteria for RI-LLNA to the data derived from LLNA: BrdU method and the underestimated cases for sensitization potency of chemicals have been reported (Takeyoshi and Nara, 2021). In order to avoid an incorrect application of the GHS classification criteria and incorrect hazard communication for chemicals, the clarification of the categorization criteria for all versions of LLNAs would be desirable.

[2] https://www.oecd-ilibrary.org/docserver/9789264264618-en.pdf?expires=1645763331&id=id&accname=guest&checksum=5B9C43BD1DA635259D08C0C4AD49CE74

Comments（Paragraph No.）	Response to the PRP comments
51. It would be interesting to compare the data with the recently revised allergen classification done by the OECD and see if the correct Cat.1A and 1B classification is supported.	According to the development of OECD GL497[3], the Defined Approaches on Skin Sensitisation (DASS) database was prepared at the OECD, and the LLNA data were reconsidered. As the results of the confirmation of the impact of reconsideration of LLNA dataset on the reliability of the proposed subcategorization criterion in Kobayashi et al. (2020), no modification had been made to affect the proposed criterion was noted. Additional remarks: Difference when refer to DASS database Chemicals / LLNA: BrdU-ELISA / %Tested (SI value) / EC1.6 / GHS sub-category / LLNA-RI / GHS sub-category CMI[a] solution: 0.005 (1.2), 0.05 (5.2) / 0.0095 / 1A / 1A (-) 2,4-Dinitrochlorobenzene: 0.01 (1.1), 0.1 (3.5) / 0.029 / 1A / 1A 1,4-Phenylenediamine: 0.05 (1.2), 0.3 (1.8) / 0.22 / 1A / 1A Cobalt(II) chloride: 0.2 (2.3), 2 (4.7) / 0.10* / 1A / 1A (-) Isoeugenol: 1 (1.5), 10 (4.5) / 1.3 / 1A / 1A 2-Mercaptobenzothiazole: 2.5 (1.5), 5 (1.6), 10 (2.2) / 5.03 / 1A / 1A Citral: 5 (1.1), 25 (2.6) / 11.7 / 1B / 1B α-Hexylcinnamaldehyde: 5 (1.3), 25 (4.2) / 7.07 / 1B / 1B Eugenol: 5 (1.4), 25 (5.3) / 6.03 / 1B / 1B Phenyl benzoate: 3 (1.4), 10 (1.1), 30 (1.8) / 24.3 / 1B / 1B (NA) Cinnamyl alchol: 10 (1.6), 50 (2.4) / 10 / 1B / 1B Imidazolidinyl urea: 5 (1.5), 25 (2.9) / 6.43 / 1B / 1B Ethylene glycol dimethacrylate: 10 (1.1), 50 (2.2) / 28.18 / 1B / 1B Methyl methacrylate: 25 (1.2), 100 (1.8) / 75 / 1B / 1B Xylene: 10 (1.1), 50 (1.7) / 43.3 / 1B / 1B (-) Original table is appeared in Kobayashi et al (2020) as Table 2 using CBA/J mice. (-): not listed in DASS database, (NA) : Changed to (NA, signifies data not available) in DASS database.

[3] https://www.oecd.org/env/guideline-no-497-defined-approaches-on-skin-sensitisation-b92879a4-en.htm

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.

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.