EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
European Union Reference Laboratory for Alternative Methods to Animal Testing (ECVAM)

Addendum to the Direct Peptide Reactivity Assay (DPRA) ECVAM Validation Study Report

This document provides additional information to the Working Group in charge of the peer-review of the DPRA validation study to inform its opinion in relation to the results of the study with regards to the study secondary goal b) "*preliminary consideration of the ability of the three tests evaluated in the above mentioned study (including the DPRA) to contribute to subcategorisation of the skin sensitising chemicals, e.g. into Subcategory 1A and Subcategory 1B as adopted in the 3rd revised version of the GHS"* as the Working Group felt that this study goal was not sufficiently addressed in the original report.

Within the GHS classification scheme there is the possibility to refine the evaluation of skin sensitisers on the basis of the potency of the sensitising effect. Skin sensitisers can be assigned to subcategory 1A "strong sensitisers" or to subcategory 1B "other skin sensitisers" using a weight of evidence approach on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in recognized and officially accepted animal tests.

In the case of the LLNA a threshold defined for the EC3 value is used to subcategorise skin sensitisers into subcategory 1A (EC3≤2) or into subcategory 1B (EC3>2).

The DPRA validation study results have been analysed with respect to the potential of the DPRA to contribute to the GHS subcategorisation by evaluating the extent of agreement between the GHS subcategorisation based on LLNA results and the DPRA reactivity class results as represented in the following table.

Table 1 Agreement between the predicted reactivity class and the GHS categories

Chemical	GHS subcategorisation based on LLNA data	Major[A]	P&G	Ricerca	IVMU
Benzoquinone	+ (1A)	HIGH	HIGH	$HIGH_{LYS}$	HIGH
PPD	+ (1A)	HIGH	HIGH	$HIGH_{CL}$	HIGH
Kathon CG	+ (1A)	HIGH	$HIGH_{LYS}$	HIGH	HIGH
Formaldehyde	+ (1A)	LOW	LOW	MODERATE	LOW
Chloramine T	+ (1A)	HIGH	$HIGH_{LYS}$	$HIGH_{LYS}$	HIGH
Chlorpromazine HCl	+ (1A)	MINIMAL	MINIMAL	MINIMAL	MINIMAL
2-MBT	+ (1A)	HIGH	$HIGH_{LYS}$	HIGH	HIGH
Thioglycerol	+ (1B)	$\geq LOW_{CL}$	$MODERATE_{LYS}$	$\geq LOW_{CL}$	$\geq LOW_{CL}$
Imidazolidinyl Urea	+ (1B)	MODERATE	MODERATE	MODERATE	MODERATE
Methyl Methacrylate	+ (1B)	MODERATE	LOW	$\geq MODERATE_{Cl}$	MODERATE
Benzyl Salicylate	+ (1B)	MINIMAL	MINIMAL	MINIMAL	LOW
Benzyl Cinnamate	+ (1B)	MINIMAL	MINIMAL	MINIMAL	MINIMAL
R(+)Limonene	+ (1B)	LOW	LOW	LOW	LOW
Glycerol	- (NC)	MINIMAL	MINIMAL	MINIMAL	MINIMAL
DCNB	- (NC)	MINIMAL	MINIMAL	MINIMAL	MINIMAL
Benzyl Alcohol	- (NC)	MINIMAL	MINIMAL	MINIMAL	LOW
Methyl Salicylate	- (NC)	MINIMAL	MINIMAL	MINIMAL	LOW
Isopropanol	- (NC)	MINIMAL	MINIMAL	MINIMAL	MINIMAL
Dimethyl Isophthalate	- (NC)	MINIMAL	MINIMAL	MINIMAL	MINIMAL
4-PABA	- (NC)	MINIMAL	MINIMAL	MINIMAL	MINIMAL
Xylene	- (NC)[B]	MINIMAL	MINIMAL	MINIMAL	MINIMAL

[A] final predicted reactivity class based on majority voting

LYS subscript or CL subscript corresponds with co-elution for Lysine and co-elution with CYS/LYS
NC: Not Classified
[B] Xylene is a false positive in the LLNA and was assigned to the "no category" by weight of evidence.

Since there are three GHS categories (No Category (NC) and the two subcategories 1A and 1B) while the DPRA can assign the chemicals to four different reactivity classes, for the purposes of this analysis, and based on the definition of the GHS category classes, a minimal reactivity in the DPRA was judged to correspond to a NC in the official classification, low and moderate reactivity to subcategory 1B and high reactivity corresponds to subcategory 1A.

Comparing this subcategorisation to the reference GHS classification of the chemicals resulted in an overall accuracy (cumulative over the 3 labs) of 79.4%. The accuracy for P&G and Ricerca were 81.0% and 76.2% for IVMU as presented in *table 2.*

The chemicals considered by the VMG to fall outside the applicability domain of the DPRA were excluded from the analysis.

Table 2 Overall agreement (cumulative over the three laboratories) of the DPRA reactivity classes with the 3 GHS categories (NC, 1A and 1B) and extent of agreement obtained by each laboratory

Reference classification	**Cumulative**			**P&G**			**Ricerca**			**IVMU**		
	Hi	L-M	Mi	Hi	L-M	Mi	Hi	L-M	Mi	Hi	L-M	Mi
+ (1A) (n=7)	15	3	3	5	1	1	5	1	1	5	1	1
+ (1B) (n=6)	0	13	5	0	4	2	0	4	2	0	5	1
- (NC) (n=8)	0	2	22	0	0	8	0	0	8	0	2	6
Total	15	18	30	5	5	11	5	5	11	5	8	8
Accuracy (%)		79.4			81.0			81.0			76.2	

On the basis of these results the VMG noted that although the DPRA prediction model was not designed specifically for the purpose of classifying chemicals into the GHS categories, there was a significant correlation between the chemical's GHS categories and the reactivity class assigned by the DPRA: chemicals assigned by the DPRA in the HIGH reactivity class were always GHS subcategory 1A substances, chemicals assigned by the DPRA to a LOW or MODERATE reactivity class tend to be GHS subcategory 1B substances and chemicals assigned the MINIMAL reactivity class tend to be not classified (as was also observed in the analysis of the Sensitiser/Non-sensitiser predictions).

Despite the fact that it not possible to conclude on the ability of the DPRA to contribute to a skin sensitizer subcategorisation on the basis of this limited sample size, these results are however encouraging and suggest that the DPRA might have the potential to contribute to such subcategorisation. This will need to be fully assessed on the basis of a larger set of results, and with the possible contribution of other sources of information (eg. in combination with other test methods).