Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

2-[*N*-ethyl-4-[(5-nitrothiazol-2-yl)azo]-*m*-toluidino]ethyl acetate; C.I. Disperse Blue 124

EC Number: 239-203-6
CAS Number: 15141-18-1

CLH-O-0000006911-73-01/F

Adopted

10 December 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-[*N*-ETHYL-4-[(5-NITROTHIAZOL-2-YL)AZO]-*M*-TOLUIDINO]ETHYL ACETATE; C.I. DISPERSE BLUE 124

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: 2-[*N*-ethyl-4-[(5-nitrothiazol-2-yl)azo]-*m*-toluidino]ethyl acetate; C.I. Disperse Blue 124
EC number: 239-203-6
CAS number: 15141-18-1
Dossier submitter: Germany

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
10.12.2019	Sweden		Member State	1
Comment received				
The Swedish CA supports classification of DB124 as Skin Sens. 1A, H317, with a SCL of 0.001%.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Support to a classification of DB124 as Skin Sens. 1A, H317, with a SCL of 0.001% has been noted				

Date	Country	Organisation	Type of Organisation	Comment number
11.12.2019	France		Member State	2
Comment received				
We agree with the proposal to classify C.I Disperse Blue 124 as Skin Sens. 1A, H317. Although the LLNA test was performed on C.I Disperse Blue 106, there is sufficient evidence to consider that CI Disperse Blue 124 would also be a strong skin sensitiser based on comparative potency in the GPMT performed with the two substances. The hypothesis that CI Disperse Blue 124 is hydrolysed to CI Disperse Blue 106 on the skin also supports the classification. Based on the "biphasic" LLNA, it seems that CI Disperse Blue 124 may be more irritant that CI Disperse Blue 106. Nevertheless, as no dose-response was observed, it is difficult to make a clear conclusion. In the "biphasic" LLNA, a phenotypic determination was performed in the study with both disperse dyes (page 39). Could you please provide the				

detailed figures in a table? Indeed, %CD69+ and B:T cell ratio could help to differentiate irritant and irritating sensitizers when ear swelling is > 25% (as observed for several doses in the study).

According to Ryberg *et al.*, 2009, some patients reacted to impurities of these two disperse dyes. Could you please clarify whether this has been taken into account in the proposal?

With regard to the SCL, we agree that a SCL of at least 0.001% is necessary. Nevertheless, it is not clear whether this SCL would be enough protective for elicitation. Indeed, according to Ryberg et al., 2009, some patients already reacted to 0.010 ppm of the test material (0.000001%) and 7/21 patients at 0.001%. These data need to be taken into account in the proposal.

Dossier Submitter's Response

Please find below (Table 1) data on the phenotypical characterisation of lymphocyte subsets isolated from lymph nodes of mice after treatment with Disperse Blue 124 (DB124) and Disperse Blue 106 (DB106) according to a sensitisation-challenge protocol of the "biphasic" LLNA (Ahuja *et al.*, 2010). The data reveal that lymphocyte cell surface markers CD4+ and CD8+ were significantly decreased after treatment with DB124 and DB106 at all concentrations tested, while CD19+, CD45+ and CD45+/1A+ cell populations significantly increased after application of DB124 and DB106 (but no increase of CD45+ cell surface marker after application of DB106 at lowest concentration, non-significant increase at the mid concentration, and non-significant increase of DC45+ at lowest concentration of DB124), compared to vehicle control. This regulation of cell surface markers is characteristic of allergens (Ahuja *et al.*, 2010). Furthermore, CD4+/CD69+ cells were significantly increased in mice following treatment with DB124 and DB106 at the three highest concentrations tested. The data support the sensitising potential of DB124 and DB106, but less so their acting as irritants (Homey *et al.*, 1998).

Table 1: Phenotypic analysis (% positive cell population, mean ± SD) of the different epitope markers on lymphocytes obtained from lymph nodes of mice treated with Disperse Blue 124 and 106 according to the protocol of the "biphasic" LLNA (sensitisation-challenge protocol), adapted from (Ahuja *et al.*, 2010).

	CD4+	CD8+	CD19+	CD45+	CD45+/1A+	CD4+/CD69+
Vehicle control	47.7 ± 4.3	20.8 ± 2.1	20.1 ± 2.5	31.4 ± 4.0	21.4 ± 3.4	10.3 ± 1.3
DB124 10%	40.7 ± 4.1*	18.3 ± 1.7*	24.4 ± 3.9*	36.3 ± 3.1*	31.0 ± 2.9*	16.2 ± 4.4*
DB124 3%	37.4 ± 5.5*	15.6 ± 3.4*	41.0 ± 5.0*	40.0 ± 1.3*	42.3 ± 8.7*	13.3 ± 2.0*
DB124 0.3%	39.6 ± 2.4*	18.7 ± 2.0*	34.8 ± 2.0*	34.4 ± 3.5	30.5 ± 3.0*	13.7 ± 1.6*
DB124 0.03%	38.8 ± 3.8*	15.4 ± 2.0*	39.7 ± 5.1*	38.5 ± 4.5*	32.1 ± 5.0*	9.9 ± 0.5
DB124 0.003%	40.6 ± 1.5*	15.5 ± 1.1*	35.8 ± 2.4*	32.3 ± 2.3	30.6 ± 1.6*	10.2 ± 1.2
DB106 30%	39.6 ± 3.3*	17.4 ± 2.4*	26.8 ± 3.5*	36.7 ± 3.6*	32.5 ± 5.1*	14.6 ± 2.3*
DB106 3%	38.4 ± 3.5 *	16.6 ± 3.6*	39.0 ± 3.0*	40.4 ± 2.5*	40.7 ± 8.7*	14.0 ± 1.5*
DB106 0.3%	38.5 ± 3.7*	19.0 ± 2.2*	36.4 ± 5.0*	34.0 ± 4.4	31.1 ± 4.1*	12.1 ± 1.1*
DB106 0.03%	36.0 ± 1.6*	16.3 ± 1.6*	41.1 ± 2.6*	38.3 ± 3.4*	34.9 ± 2.8*	9.8 ± 0.8
DB106 0.003%	40.4 ± 1.2*	15.1 ± 1.3*	34.1 ± 2.1*	31.3 ± 2.0	31.3 ± 1.6*	9.9 ± 0.8

* significant different from vehicle control, $p < 0.05$ (one-way ANOVA followed by post hoc Dunnett test)

Ryberg and colleagues (Ryberg *et al.*, 2009) tested patients, which were identified as DB124 and DB106-sensitised through previous patch testing, with dilution series of commercial and purified DB106 and DB124, as well as with thin-layer chromatography

strips made from the commercial preparations of DB106 and DB124. The objective was to investigate the relevance of impurities in preparations of disp`erse dyes used for patch testing. The authors found that a low number of DB106 and/or DB124-sensitised subjects positively reacted to patch tests with very low concentrations (10^{-6} %) of disperse dyes. According to the Guidance on the Application of CLP criteria, SCLs can be set based on the potency outcome from animal testing, predicted on concentrations for induction of skin sensitisation. Reliable animal data reveal an extreme skin sensitising potency of DB124 and therefore the proposed SCL (0.001 %) is set in accordance with the recommendation of the Guidance for "extreme" skin sensitisers of the highest skin sensitising potency class. In the view of the DE CA, since SCLs are normally based on induction and not elicitation, the additional information that very low concentrations of < 0.001 % of the purified dye were able to elicit an allergic reaction in some pre-sensitised patients does therefore not justify the setting of an even lower SCL.

Furthermore, the authors identified approx. 25 % of the patients, diagnosed as contact allergic to DB106 and DB124, which reacted to other constituents of commercial DB124 or DB106 (as isolated by preparative chromatography). These data might be interpreted as suggesting that the frequency of patch test patients reacting positively to DB106 and DB124 might have been overestimated. There was, however, no information about the characterisation of impurities or putative degradation products. On the other hand, other studies from the literature also recognised that commercial textile dyes were less pure and detected much lower disperse dye concentrations than stated (Hausen and Sawall, 1989; Malinauskiene *et al.*, 2012; Ryberg *et al.*, 2008; Uter *et al.*, 2007). In such cases, an overestimation of the dye concentration causing sensitisation and/or a smaller than expected number of sensitised subjects may result.

References:

Ahuja V., Platzek T., Fink H., Sonnenburg A., and Stahlmann R. (2010): Study of the sensitising potential of various textile dyes using a biphasic murine local lymph node assay. Archives of toxicology 84 (9), 709-718. DOI: 10.1007/s00204-010-0566-0

Hausen B.M. and Sawall E.M. (1989): Sensitization experiments with textile dyes in guinea pigs. Contact dermatitis 20 (1), 27-31. DOI: 10.1111/j.1600-0536.1989.tb03091.x

Homey B., von Schilling C., Blumel J., Schuppe H.C., Ruzicka T., Ahr H.J., Lehmann P., and Vohr H.W. (1998): An integrated model for the differentiation of chemical-induced allergic and irritant skin reactions. Toxicol Appl Pharmacol 153 (1), 83-94. DOI: 10.1006/taap.1998.8535

Malinauskiene L., Zimerson E., Bruze M., Ryberg K., and Isaksson M. (2012): Are allergenic disperse dyes used for dyeing textiles? Contact dermatitis 67 (3), 141-148. DOI: 10.1111/j.1600-0536.2012.02129.x

Ryberg K., Goossens A., Isaksson M., Gruvberger B., Zimerson E., Persson L., and Bruze M. (2009): Patch testing of patients allergic to Disperse Blue 106 and Disperse Blue 124 with thin-layer chromatograms and purified dyes. Contact dermatitis 60 (5), 270-278. DOI: 10.1111/j.1600-0536.2009.01538.x

Ryberg K., Gruvberger B., Zimerson E., Isaksson M., Persson L., Sorensen O., Goossens A., and Bruze M. (2008): Chemical investigations of disperse dyes in patch test

preparations. Contact dermatitis 58 (4), 199-209. DOI: 10.1111/j.1600-0536.2007.01298.x

Uter W., Hildebrandt S., Geier J., Schnuch A., and Lessmann H. (2007): Current patch test results in consecutive patients with, and chemical analysis of, disperse blue (DB) 106, DB 124, and the mix of DB 106 and 124. Contact dermatitis 57 (4), 230-234. DOI: 10.1111/j.1600-0536.2007.01228.x

RAC's response

Thank you very much for comments.

The extent of human and animal data on skin sensitisation of both dyes is very extensive and sufficient for classification therefore it is considered that results of a phenotypic analysis of the different epitope markers on lymphocytes obtained from lymph nodes of mice treated with various disperse dyes (Ajuja *et al.* , 2010) are not essential for making a decision on classification. There are no doubts whether skin reaction in animal and human studies were due to irritation or sensitisation. In addition, since this parameter is not included in the classification criteria and its significance is not as yet clear it was considered that it does not provide significant added value and does not in any way affect conclusion taken.

We agree with the DS in relation to rules taken for setting a SCL based on the level of induction of sensitization observed in animal studies, in line with the Guidance on the Application of CLP criteria.