Committee for Risk Assessment

RAC

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

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CLH-O-0000006911-73-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical 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**

The proposal was submitted by **Germany** and received by RAC on **10 September 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **14 October 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 December 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **10 December 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	2-[*N*-ethyl-4-[(5-nitrothiazol-2-yl)azo]-*m*-toluidino]ethyl acetate; C.I. Disperse Blue 124	239-203-6	15141-18-1	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C ≥ 0,001%	
RAC opinion	TBD	2-[*N*-ethyl-4-[(5-nitrothiazol-2-yl)azo]-*m*-toluidino]ethyl acetate; C.I. Disperse Blue 124	239-203-6	15141-18-1	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C ≥ 0,001%	
Resulting Annex VI entry if agreed by COM	TBD	2-[*N*-ethyl-4-[(5-nitrothiazol-2-yl)azo]-*m*-toluidino]ethyl acetate; C.I. Disperse Blue 124	239-203-6	15141-18-1	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C ≥ 0,001%	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The Dossier Submitter presented the results of animal and human studies on the skin sensitisation properties of 2-[*N*-ethyl-4-[(5-nitrothiazol-2-yl)azo]-*m*-toluidino]ethyl acetate (Disperse Blue 124, DB 124) and 2-(Ethyl(3-methyl-4-((5-nitro-2-thiazolyl)azo)phenyl) amino) ethanol (Disperse Blue 106, DB 106), the hydrolysis product of DB 124.

DB 124 is a thiazolyl azo-p-phenylene diamine dye and its structure is identical to that of DB 106, except for O-acetylation of the 2-hydroxyethyl group. Acetate esters are sensitive to hydrolysis by esterases, such as carboxyl esterases present in human skin (Batz *et al.* 2013; Fu *et al.* 2016). It has been shown that DB 124 is immediately hydrolysed into DB 106 at low pH, which provides supportive evidence that transformation of DB 124 into DB 106 can occur on the skin surface (Hansson *et al.* 1997). In the opinion of the DS, it is probable that DB 124 is transformed into DB 106 while penetrating the outer human skin, resulting in the same hapten for both DB dyes being formed. Therefore, the DS has submitted studies demonstrating the skin sensitising potential of both dyes.

Based on the results of animal and human studies, the DS considers that DB 124 warrants classification as Skin Sens. 1A and, due to its extreme potency, proposed a specific concentration limit (SCL) of ≥ 0,001%.

Comments received during public consultation

Two Member State Competent Authorities (MSCA) supported the DS proposal to classify DB 124 as Skin Sens. 1A, H317, with a SCL of ≥ 0,001%. One noted that although the key LLNA test was performed on DB 106, there is sufficient evidence to consider that DB 124 would also be a strong skin sensitiser based on their very similar potency in the Guinea pig maximisation test (GPMT) (similar to OECD TG 406) performed with these two substances. The likely hydroysis of DB 124 to DB 106 on the skin also supports the classification. This MSCA also remarked that it is not clear whether a SCL of ≥ 0,001% would be sufficient to protect for the occurrence of elicitation of allergic contact dermatitis in humans, since some patients in a study by Ryberg *et al.* (2009) reacted positively in a patch test at a concentration of 0,000001%. In their response, the DS noted that according to the CLP Guidance, SCLs can be set based on the potency outcome from animal testing, predicted on the basis of concentrations for induction of skin sensitisation. Reliable animal data reveal an extreme skin sensitising potency of DB 124 and therefore the proposed SCL of 0,001 % is recommended in accordance with the Guidance. 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 not therefore justify the setting of an even lower SCL.

Assessment and comparison with the classification criteria

Animal studies

In the non-guideline biphasic murine local lymph node assay (Ahuja *et al.* 2010) (reliability 2: Reliable with restrictions), the skin sensitising potency of DB 124 and DB 106 was compared using ear thickness, ear biopsy weight, lymph node weight and lymph node cellularity as the endpoints. Both dyes were administered at the same concentrations on the shaved skin of the back of mice on days 1-3 of the study and then on days 15-17 on the dorsum of both ears have

caused, in comparison with a vehicle control, significant increases in all endpoints demonstrating that the dyes have similar skin sensitizing potency. The design of this study precludes comparison of the effects observed with the classification criteria.

In the key LLNA study (Betts *et al.* 2005), performed according OECD TG 429, the dye DB 106 was found to be an extreme skin sensitiser with the EC3 of 0,012% in a first experiment and 0,017% in a second experiment. Taking into account the results of the Ahuja *et al.* study (2010), it is assumed that DB 124 would show similar potency in LLNA with an EC3 below 0,02%.

In the GPMT study, similar to OECD TG 406 (Hausen and Sawall 1989), intradermal induction with a concentration of 0,2% (w/v) DB 124 caused 70% of the animals to have a positive skin reaction 24 and 72 hours after the challenge with 1% DB 124 in acetone.

In the GPMT study similar to OECD TG 406 (Hausen and Menezes Brandao 1986), intradermal induction with a concentration of 1,5% (w/v) dye DB 106 caused positive skin reactions graded as +++ or ++ in 90% of the animals at 24, 48 and 72 hours after challenge with 0,001% DB 106 in acetone. A differentiation in the intensity of the responses after challenge with DB 106 at higher concentrations (0,1%, 0,3% and 1%) was not possible because the whole flank of the animals became extremely red and swollen. In the pre-testing the threshold for irritation was found at a concentration of 10% using acetone as solvent, therefore all skin reactions observed in the challenges at much lower concentration were due to sensitisation.

The effects observed in the GPMT study (Hausen and Sawall 1989) in which 70% of pigs had a positive reaction after intradermal induction with DB 124 at a concentration of 0,2% (w/v) warrant classification of DB 124 as Skin Sens. 1A, since criteria given in Table 3.4.3 of Regulation 1272/2008 are met (≥ 60 % of animals responding at > 0,1 % to ≤ 1 % intradermal induction dose).

Human studies

The skin sensitisation properties and potency of DB 124 and DB 106 were not evaluated in the Human Repeated Insult Patch Test (HRIPT) or Human Maximization Test (HMT), therefore an induction threshold for skin sensitisation in humans cannot be established.

However, the frequency of skin sensitisation of DB 124 or DB 106 has been assessed in 33 studies with human patch tests using either unselected, consecutive dermatitis patients (16 studies) or selected dermatitis patients (17 studies). In addition, 75 case reports were published demonstrating positive patch test with either DB 124, DB 106 or both. The studies are summarised in the background document.

<u>Patch test studies of unselected, consecutive patients with various types of dermatitis</u>

The frequency of skin sensitisation to DB 124 or DB 106 was relatively high: in the Wentworth *et al.* (2004) study, out of 3115 patients, 3.4% had a positive patch test when exposed to DB 124 and 2.8% to DB 106 patients. In the other 15 studies, summarised in the background document and demonstrating skin sensitisation to one or both dyes, the frequency of positive patch tests with DB 124, DB 106, or DB 106/DB 124 mixture varied between 0,2% in 982 consecutive dermatitis patients (Ryberg *et al.* 2009a) to 7.3% in 286 consecutive dermatitis patients (Lazarov *et al.* 2020). The frequency of skin sensitization in many studies is above 1%, which is considered as a high frequency among selected dermatitis patients according to the recommendation given in table 3.2 of the CLP Guidance (Version 5.0 - July 2017). However, in none of these studies was the level or duration of previous dermal exposure to DB 124 or DB 106 documented. Thus these results do not allow subcategorization of skin sensitising potency.

Patch test studies of selected dermatitis patients

A positive response to DB 124 or DB 106 was observed in all 17 patch test studies of selected dermatitis patients carried out in different dermatological clinics and in different countries, the results of which are summarised in the background document. The frequency of positive response to DB 124 or DB 106 in two studies was ≥ 50% of the tested individuals (Lisi *et al.* 2014; Giusti *et al.* 2002) and > 2% of tested patients in 16 studies[1]. The frequency of skin sensitisation to DB 124 and/or to DB 106 is above 2% and that is considered, in line with the recommendation given in table 3.2 as high. However, in none of these studies was the level or duration of the previous dermal exposures to DB 124 or DB 106 documented. Thus, these results do not allow subcategorization of skin sensitising potential.

The case reports

The positive patch tests with DB 124 and/or DB 106 in over 800 patients demonstrated that these dyes were responsible, either alone or jointly with other substances, for the allergic contact dermatitis diagnosed in these patients.

In the opinion of the RAC, the existing data provide sufficient evidence that DB 124 is a strong human skin sensitiser. However, due to lack of data on the level or duration of exposure it is not possible to prove that the observed cases of allergic contact dermatitis were induced in humans by DB 124 at relatively low exposure or relatively high exposure, therefore human data do not allow for subcategorization of DB 124 based on skin sensitising potency. However, taking into account the animal data that provides evidence that a very low level of exposure was sufficient for induction of sensitisation, RAC considers that DB 124 **warrants classification as Skin Sens. 1A with hazard statement H317**: May cause an allergic skin reaction, and a specific concentration limit of 0,001%.

Specific concentration limit

In setting an SCL for DB 124 it is justified to take into account not only data for this dye, but also data for DB 106, since both are closely structurally related. This is further supported by the confirmed fast hydrolysis of DB 124 to DB 106 at reduced pH (skin pH) (Hansson *et al.* 1997). It is assumed that it is highly plausible that DB 124 is quickly transformed on and in the skin into DB 106, and that DB 106 may be the final hapten in case of dermal exposure to DB 124. It is also noted that both dyes have a very similar skin sensitising potency as shown in the study of Ahuja *et al.* (2010). Therefore, RAC is of the opinion that the study of Betts *et al.* (2005) performed with DB 106 can be used for estimation of the skin sensitising potency of DB 124. Since the EC3 value established for DB 106 (thus that of DB 124), found in this study is below 0,2%, then DB 124 should be considered as meeting the criteria for an extremely potent skin sensitiser and **an SCL of 0,001% (w/v) should be set**.

[1] Heratizadeh *et al.* 2017; Isaksson *et al.* 2015; Ryberg *et al.* 2014; Lisi *et al.* 2014; Wentworth *et al.* 2012; Ryberg *et al.* 2009b; Bauer *et al.* 2004; Koopmans and Bruynzeel 2003; Giusti *et al.* 2002; Uter *et al.* 2001; Lazarov and Cordoba 2000; Pratt and Taraska 2000; Sertoli *et al.* 1994; Dooms-Goossens 1992; Seidenari *et al.* 1991; Balato *et al.* 1990.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).