Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-[ethyl[3-methyl-4-[(5-nitrothiazol-2-yl)azo]phenyl]amino]ethanol; (Disperse Blue 106)

EC Number: 271-183-4
CAS Number: 68516-81-4

CLH-O-0000007071-84-01/F

Adopted

18 March 2022

18 March 2022

CLH-O-0000007071-84-01/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-[ethyl[3-methyl-4-[(5-nitrothiazol-2-yl)azo]phenyl]amino]ethanol**

EC Number: **271-183-4**

CAS Number: **68516-81-4**

The proposal was submitted by **Germany** and received by RAC on **26 March 2021.**

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 **19 April 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **18 June 2021**.

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 **18 March 2022** by **consensus**.

No current Annex VI entry (CLP, Table 3)

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-[ethyl[3-methyl-4-[(5-nitrothiazol-2-yl)azo]phenyl]amino]ethanol	271-183-4	68516-81-4	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C ≥ 0.001%	
RAC opinion	TBD	2-[ethyl[3-methyl-4-[(5-nitrothiazol-2-yl)azo]phenyl]amino]ethanol	271-183-4	68516-81-4	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C ≥ 0.001%	
Resulting Annex VI entry if agreed by COM	TBD	2-[ethyl[3-methyl-4-[(5-nitrothiazol-2-yl)azo]phenyl]amino]ethanol	271-183-4	68516-81-4	Skin Sens. 1A	H317	GHS07 Wng	H317		Skin Sens. 1A; H317: C ≥ 0.001%	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

2-[ethyl[3-methyl-4-[(5-nitrothiazol-2-yl)azo]phenyl]amino]ethanol (Disperse Blue 106, DB106), is structurally similar to other disperse blue dyes, namely Disperse Blue 124 (DB124). In contrast to DB106, DB124 has an acetylated 2-hydroxyethyl group. This group may be lost by DB124 due to hydrolysis by esterase activity yielding DB106 as the product (Hansson *et al.*, 1997). It has been shown that DB124 is immediately hydrolysed into DB106 at reduced pH, supporting degradation of DB124 into DB106 on the skin surface (Hansson *et al.*, 1997). DB124 has been found to warrant classification as Skin Sens. 1A, H317 with SCL ≥ 0,001 % in the RAC opinion adopted on 10 December 2020.

The DS presented the results of several animal studies conducted with DB106 that cover the induction phase and allow to assess skin sensitising potency of this substance.

There was no Human Repeated Insult Patch Test (HRIPT) or Human Maximisation Test (HMT) performed with DB106, but the DS provided the results of many human patch test studies on consecutive or selected patients with dermatitis and case reports from the literature, covering the elicitation phase and demonstrating occurrence of skin sensitisation to DB106 in humans.

Based on the results of animal and human studies the DS considered that DB106 is an extremely potent skin sensitiser, which warrants classification as Skin Sens. 1A (H317 - May cause an allergic skin reaction) and requires to set a specific concentration limit of ≥ 0.001 %.

Comments received during consultation

Two Member State Competent Authorities (MSCA) commented the proposed classification.

One MSCA supported the proposed harmonised classification of DB106 as Skin Sens. 1A; H317, with an SCL of 0.001% based on animal data (key study LLNA with EC3 = 0.012-0.017 %). Human evidence further supports classification of DB106 as a potent skin sensitiser.

A second MSCA noted that considering both the animal and human data, there is no doubt that DB106 warrants, at least, a classification as Skin Sens 1. In the studies with consecutive patients with dermatitis, half of the patients showed a high frequency of dermal reaction to DB106 and in the other half, the frequency of skin reaction to DB106 was low or moderate.

Studies on selected dermatitis patients, on the contrary, mainly lead to a high frequency of dermal reaction. In experimental animals, the key study, an LLNA, indicated a very high sensitising potency of DB106 with EC3 values of 0.012 % and 0.017 % in the two experiments. Noting that the CLP guidance indicate that "evidence from animal studies is usually much more reliable than evidence from human exposure", a second MSCA agreed with the proposal to classify DB106 to subcategory 1A, based on having an extreme skin sensitising potency as determined by the LLNA. In parallel, this was supported by the other *in vivo* tests, where a conclusion of strong or extreme sensitiser could not be excluded, therefore a second MSCA proposed to set an SCL of 0.001 %.

Assessment and comparison with the classification criteria

Animal studies

In the key LLNA study (Betts *et al.* 2005), the authors performed a pre-test using DB106 formulated in DMF vehicle at concentration of 1 %, 3 %, and 10 % to determine the highest non-toxic concentration of DB106 and the authors investigated different vehicles. For the main study, groups of mice were exposed topically on the dorsum of both ears to 0.25, 0.05, 0.025, 0.1, 0.01, and 0.005 % of DB106 (purity: 87 %) in DMSO, or to the vehicle alone (vehicle control), daily for three consecutive days. The sensitising potency of 2,4-dinitrochlorobenzene (DNCB) (0.01-0.25 % in DMSO) was measured concurrently. Five days after the initiation of exposure, all mice were injected intravenously with (3*H*)-methyl thymidine (3HTdR) via the tail vein. Five hours later, the draining auricular lymph nodes were excised and pooled for each experimental group. Incorporation of 3HTdR was measured in single-cell suspensions of LNCs (Lymph Node Cells). In each case, a stimulation index (SI) relative to the concurrent vehicle-treated control value was derived. The dye DB106 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. Data indicate an extreme sensitising potency of DB106. This well-documented LLNA does not show obvious deviations from OECD TG 429 and is considered to be reliable with restrictions. The DS considers this LLNA as the key animal study.

In the GPMT study, (modified FCA method; Hausen and Menezes Brandao, 1986), guinea pigs were intradermally injected with a 1.5 % (w/v) dye emulsion containing chromatographically pure DB106 dissolved in FCA/saline (1:1), in a semi-circular arc in the shoulder area from the left to the right paw, on days one, five, and nine, according to the method of Hausen and Schmalle (1985). Control animals were treated with an FCA/saline (1:1) emulsion. Eleven days after the end of the sensitisation procedure, challenge was performed by topical application of sub-irritant doses (1 %, 0.3 %, and 0.1 %) of the dye (the threshold of irritation was determined at a concentration of 10 % in solvent acetone). The authors reported that the "reactions obtained on challenge with non-irritant dilutions of 1 %, 0.3 %, and 0.1 % were so strong that no reading could be made because the whole flank of the animals became extremely red and swollen". One week later, after lesions disappeared, further epicutaneous challenge test with an additional dilution (0.001 %) was performed on the opposite flank. Readings after 24, 48, and 72 hours resulted in 100 % positively reacting animals due to DB106 treatment. Since lower concentrations of DB106, ≤ 1.0 % or even ≤ 0.1 %, were not used for intradermal injection, the results of this study using intradermal induction of DB106 at concentration 1.5 % do not provide evidence meeting the classification criteria for subcategory 1A. However, the results indicate a strong or an extreme skin sensitising potency.

In the "biphasic" LLNA (Ahuja *et al.*, 2010) the authors used a sensitisation-challenge-protocol and analysed the increase in lymph node cells compared to vehicle controls. The female mice were treated once daily on their shaved backs from days one to three with 30, 3, 0.3, 0.03 and 0.003 % concentrations of DB106 (no information on purity). On days 15 to 17, mice were challenged with the test solution on the dorsum of both ears. Local lymph nodes were prepared on day 19. The authors investigated the lymph node weight, ear thickness, and ear biopsy weight. Furthermore, single cell suspensions from each single lymph node were counted (million per lymph node) using an automated cell counter. Investigations reveal that treatment with 30, 3, 0.3 and 0.03 % concentrations of DB106 resulted in a significant increase in ear thickness (26, 13, 17 and 9 %, respectively) and enhancement in the ear punch weight (22, 15, 17 and 12 %, respectively), compared to vehicle control. Furthermore, concentrations of 30, 3, 0.3, 0.03 and 0.003 % DB106 increased the cell count by 174, 124, 82, 79, and 37 %, respectively, in comparison to the vehicle control. The results of this "biphasic" LLNA demonstrate that very low

concentrations (0.003 %) of DB106 induced a significant increase in cell-count compared to the vehicle control animals. Since the effective concentration inducing skin sensitisation was well below 2 %, DB106 should be considered as strong skin sensitiser. However, the test material was insufficiently characterised, and this study had not been conducted in accordance with any available OECD testing guideline. Therefore, these experimental data do not allow for conclusion on skin sensitising potency of DB106.

The Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) published an assessment of DB106 (NICNAS, 2015). Unpublished study reports submitted by the notifiers and summarised by NICNAS give evidence that DB106 acts as a skin sensitiser:

- in a Buehler test (according to OECD TG 406) conducted with DB106 (50 % topical induction, 50 % topical challenge) resulted in 16/20 and 15/20 sensitised animals (24 h and 48 h after challenge, respectively; erythema score ≥ 1).
- in an unpublished GPMT of notifiers (according to OECD TG 406), 14/20 and 12/20 animals (24 h and 48 h after challenge, respectively; erythema score ≥ 1) reacted to DB106 (1 % intradermal induction, 50 % topical challenge). The tested induction concentrations during the Buehler test (50 % for topical induction) and GPMT (1 % for intradermal induction), resulted in a moderate and strong sensitising potency of DB106, respectively. However, lower concentrations were not tested, and extreme potency cannot be excluded. None of these study reports submitted by the notifiers was available to the DS (Reliability 4, not assignable) and data were not considered for potency assessment.

Considering only the animal data, DB106 fulfils the criteria given in Regulation (EC) 1272/2008 for classification as Skin Sens. 1A, since in the key LLNA study the EC3 values for BD106, in two experiments were 0.012 % and 0.017 %, both results were well below the criteria of 2 % for subcategory 1A. The other animal studies (Hausen and Menezes Brandao, 1986; Ahuja *et al.*, 2010) also suggest that BD106 has potentially high skin sensitising potency, although due to their specific design, they do not allow for detailed assessment of sensitising potency.

Human studies

According to Regulation (EC) 1272/2008 Annex I: 3.4.2.2.2.1. Human evidence for sub-category 1A can include:

a) positive responses at ≤ 500 µg/cm^2 (HRIPT, HMT – induction threshold);
b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;
c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

Numerous human data, published from the 1980s to the 2000s, provide evidence that DB106 is a common cause of textile dermatitis and is frequently reported to be among the strongest textile dye sensitisers (Hatch and Maibach, 1995; Hausen, 1993; Menezes Brandao *et al.*, 1985; Pratt and Taraska, 2000). As pointed out by the DS in the background document, DB106 is listed in the restriction proposal for placing on the market of textile, leather, hide and fur articles containing skin sensitising substances (ECHA, 2019b) and on the restriction proposal for substances in tattoo inks and permanent make up (ECHA, 2019a).

The skin sensitisation properties and potency of DB106 were not evaluated in the HRIPT or HMT, therefore an induction threshold for skin sensitisation in humans cannot be established.

However, the incidence of skin sensitisation to DB106 has been assessed in 36 studies with human patch tests using either unselected, consecutive dermatitis patients (9 studies) or selected

dermatitis patients (27 studies). In addition, 28 case reports were published demonstrating positive patch test with DB106. The studies are summarised in the background document.

Patch test studies of unselected, consecutive patients with various types of dermatitis

According to Table 3.2 Relatively high or low frequency of occurrence of skin sensitization of the Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures (Version 5.0, July 2017) the incidence of skin sensitisation to a given substance in the dermatitis patients (unselected, consecutive) detected in patch testing at the level of 1.0 % or above is considered as high frequency of contact allergy.

The frequency of skin sensitisation to DB106 was relatively high:

- in the Wentworth study (2014) 2.8 % of consecutive dermatitis patients had a positive patch test with DB106 (1 % in pet.) out of 3 086 patch tested patients;
- in the Mucci *et al.* study (2012) 2.3 % of consecutive dermatitis patients had a positive patch test with DB106 (1 % in pet.) out of 427 tested patients;
- in the Li study (2010) 1.2 % of consecutive dermatitis patients had a positive patch test with DB106 out of 327 tested patients;
- in Lazarov *et al.* study (2002) 4.2 % out of 286 consecutive dermatitis patients had a positive patch test with DB106 (assumed 1 % in pet.);
- in Seidenari *et al.* study (2005) 4.0 % out of 97 consecutive children with dermatitis had a positive patch test with DB106 (1 % in pet.).

However, in none of these studies was the level or duration of previous dermal exposure to DB106 documented. Thus, these results do not allow subcategorization of skin sensitising potency in humans.

In the 4 other studies summarised in the background document and demonstrating skin sensitisation to BD106, the frequency of positive patch tests with DB106 varied between 0.2 % in 982 consecutive dermatitis patients (Ryberg *et al.* 2009a) to 0.9 % in 5 085 consecutive dermatitis patients (Fransway *et al.* 2013). The level or duration of previous dermal exposure to DB106 was not provided in these studies.

Patch test studies of selected dermatitis patients

According to Table 3.2 *Relatively high or low frequency of occurrence of skin sensitization* of the Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures (Version 5.0, July 2017) the incidence of skin sensitisation to a given substance in the selected dermatitis patients detected in patch testing at the level of 2.0 % or above is considered as high frequency of contact allergy.

A positive response to DB106 was observed in 26 out of 27 patch test studies of selected dermatitis patients carried out in different dermatological clinics and in different countries, the results are summarised in the background document. The frequency of positive response in the patch test to DB106 in three studies was ≥ 50 % of the tested selected dermatitis patients (Hatch 2003 and Hatch *et al.* 2003, Giusti *et al.*, 2002; Doomsgoossens, 1992) and > 2 % of tested patients in 20 studies. The frequency of skin sensitisation to DB106 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 DB106 documented. Thus, these results do not allow subcategorization of skin sensitising potential.

The case reports

The positive patch tests with DB106 in 43 patients (see table 8 of Background Document for details) demonstrated that this dye was responsible for the allergic contact dermatitis diagnosed in these patients.

Conclusion

In the opinion of the RAC, the existing data provides sufficient evidence that DB106 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 whether the observed cases of allergic contact dermatitis were induced in humans by DB106 at relatively low or at relatively high exposure, therefore human data do not allow for subcategorization of DB106 based on skin sensitising potency. However, considering the animal data that provides evidence that a very low level of exposure was sufficient for induction of sensitisation, RAC considers that **DB106 warrants classification as Skin Sens. 1A with hazard statement H317: May cause an allergic skin reaction.**

Specific concentration limit

The EC3 value of 0.012 % established for DB106 in key LLNA study (Betts *et al.* 2005) is below 0.2 %, therefore DB106 should be considered as meeting the criteria for an extremely potent skin sensitiser and in line with criteria given Table 3.6 the Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures (Version 5.0, July 2017) an **SCL of 0.001 % (w/v) should be set**.

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).