# CLH report

# Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

# International Chemical Identification:

## *2-[N-ethyl-4-[(5-nitrothiazol-2-yl)azo]-m-toluidino]ethyl acetate; Ethanol, 2-[ethyl[3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]phenyl]amino]-, 1-acetate;*

## *C.I. Disperse Blue 124*

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

**Index Number:** **-**

**Contact details for dossier submitter:**

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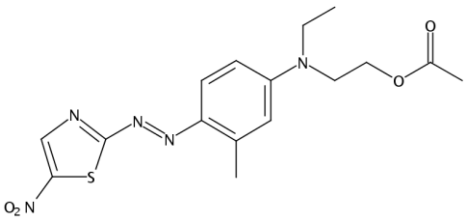
**Version number:** **2.0** **Date:** **September 2019**

# CONTENTS

# 1 IDENTITY OF THE SUBSTANCE

## 1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

| | |
|---|---|
| **Name(s) in the IUPAC nomenclature or other international chemical name(s)** | 2-[N-ethyl-4-[(5-nitrothiazol-2-yl)azo]-m-toluidino]ethyl acetate; Ethanol, 2-[ethyl[3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]phenyl]amino]-, 1-acetate |
| **Other names (usual name, trade name, abbreviation)** | C.I. Disperse Blue 124 |
| **ISO common name (if available and appropriate)** | Not applicable |
| **EC number (if available and appropriate)** | 239-203-6 |
| **EC name (if available and appropriate)** | 2-[N-ethyl-4-[(5-nitrothiazol-2-yl)azo]-m-toluidino]ethyl acetate |
| **CAS number (if available)** | 15141-18-1 |
| **Other identity code (if available)** | - |
| **Molecular formula** | $C_{16}H_{19}N_5O_4S$ |
| **Structural formula** |  |
| **SMILES notation (if available)** | CCN(CCOC(=O)C)C1=CC(=C(C=C1)N=NC2=NC=C(S2)[N+](=O)[O-])C |
| **Molecular weight or molecular weight range** | 377.419 g/mol |
| **Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)** | Not applicable |
| **Description of the manufacturing process and identity of the source (for UVCB substances only)** | |
| **Degree of purity (%) (if relevant for the entry in Annex VI)** | |

The substance 2-[N-ethyl-4-[(5-nitrothiazol-2-yl)azo]-m-toluidino]ethyl acetate is also known as

- Ethanol, 2-[ethyl[3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]phenyl]amino]-, 1-acetate (C.I. Disperse Blue) with CAS no. 61951-51-7 and list no 612-788-9.

This CAS no. however was retrieved and deleted, but is still used by mistake to describe the substance C.I. Disperse Blue 124 (see e.g. ECHAs webpage). Under a regulatory point of view it is necessary to use the CAS and EC numbers given in the above table.

## 1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

| Constituent (Name and numerical identifier) | Concentration range (% w/w minimum and maximum in multi-constituent substances) | Current CLH in Annex VI Table 3.1 (CLP) | Current self-classification and labelling (CLP) |
|---|---|---|---|
| 2-[N-ethyl-4-[(5-nitrothiazol-2-yl)azo]-m-toluidino]ethyl acetate;<br>CAS no. 15141-18-1<br>EC no. 239-203-6 | 100% | **None** | **Acute Tox. 3, Skin Sens. 1** |

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

| Impurity (Name and numerical identifier) | Concentration range (% w/w minimum and maximum) | Current CLH in Annex VI Table 3.1 (CLP) | Current self-classification and labelling (CLP) | The impurity contributes to the classification and labelling |
|---|---|---|---|---|
| Not known | | | | |

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

| Additive (Name and numerical identifier) | Function | Concentration range (% w/w minimum and maximum) | Current CLH in Annex VI Table 3.1 (CLP) | Current self-classification and labelling (CLP) | The additive contributes to the classification and labelling |
|---|---|---|---|---|---|
| **Not applicable** | | | | | |

# 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

## Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling according to the CLP criteria

| | 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 existing entry in Annex VI of CLP | | | | | | | | | |
| Dossier submitters proposal | TBD | 2-[*N*-ethyl-4-[(5-nitrothiazol-2-yl)azo]-*m*-toluidino]ethyl acetate;<br>C.I. Disperse Blue 124 | 239-203-6 | 15141-18-1 | Skin Sens. 1A | H317 | GHS07<br>Wng | H317 | | Skin Sens. 1A; H317: C ≥ 0.001% | |
| Resulting Annex VI entry if agreed by RAC and COM | TBD | 2-[*N*-ethyl-4-[(5-nitrothiazol-2-yl)azo]-*m*-toluidino]ethyl acetate;<br>C.I. Disperse Blue 124 | 239-203-6 | 15141-18-1 | Skin Sens. 1A | H317 | GHS07<br>Wng | H317 | | Skin Sens. 1A; H317: C ≥ 0.001% | |

Table 6: Reason for not proposing harmonised classification and status under public consultation

| Hazard class | Reason for no classification | Within the scope of public consultation |
|---|---|---|
| **Explosives** | Not assessed in this dossier | No |
| **Flammable gases (including chemically unstable gases)** | | |
| **Oxidising gases** | | |
| **Gases under pressure** | | |
| **Flammable liquids** | | |
| **Flammable solids** | | |
| **Self-reactive substances** | | |
| **Pyrophoric liquids** | | |
| **Pyrophoric solids** | | |
| **Self-heating substances** | | |
| **Substances which in contact with water emit flammable gases** | | |
| **Oxidising liquids** | | |
| **Oxidising solids** | | |
| **Organic peroxides** | | |
| **Corrosive to metals** | | |
| **Acute toxicity via oral route** | | |
| **Acute toxicity via dermal route** | | |
| **Acute toxicity via inhalation route** | | |
| **Skin corrosion/irritation** | | |
| **Serious eye damage/eye irritation** | | |
| **Respiratory sensitisation** | | |
| **Skin sensitisation** | **Harmonised classification proposed** | **Yes** |
| **Germ cell mutagenicity** | Not assessed in this dossier | No |
| **Carcinogenicity** | | |
| **Reproductive toxicity** | | |
| **Specific target organ toxicity-single exposure** | | |
| **Specific target organ toxicity-repeated exposure** | | |
| **Aspiration hazard** | | |
| **Hazardous to the aquatic environment** | | |
| **Hazardous to the ozone layer** | | |

# 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Disperse Blue 124 (DB124) has neither been registered under REACH, nor does it have harmonised classification and labelling in Annex VI to the CLP regulation.

Disperse Blue 124 is on the Annex III inventory, a substance list that was produced using publicly available databases with experimental data and by using (Q)SAR model results. According to this analysis, DB124 is indicated as "Suspected carcinogen", "Suspected mutagen", "Suspected persistent in the environment", and "Suspected toxic for reproduction"(ECHA, 2016).

# 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

As justified in section 10.7 below, the dossier submitter (DS) considers that for Disperse Blue 124, classification as Skin Sens. 1A is warranted, while the existing self-classification entries in the C&L Inventory only indicate classification as Skin Sens. 1, i.e. without sub-categorisation. Harmonised classification as Skin Sens. 1A would ensure an adequate perception of the skin sensitisation hazard associated with DB124, *inter alia* by lowering the concentration limit for the classification of mixtures containing DB124 from 1% (Skin Sens. 1) to 0.1% (Skin Sens. 1A). Furthermore, aside from its use in textiles, DB124 may also be used as a colourant in tattoo inks. In fact, it was one of the substances for which use information was requested during the recent public consultation on the restriction proposal for substances in tattoo inks (ECHA, 2018). While restriction option (RO) 1 from that proposal foresees a concentration limit in mixtures of 0.1% for all substances to be restricted, RO 2 would instead apply the individual concentration limits based on the CLP/GHS classification. Under RO 2, therefore DB124 – if included in the final list of substances to be restricted – would receive a ten-fold lower concentration limit, if it had CLH as Skin Sens. 1A as compared to no CLH and relying on the notifiers' classification. Furthermore a harmonised classification as Skin Sens 1A could improve consumer safety if future restriction proposals on the use of the substance (e.g. in textiles) relies on harmonised classifications as Skin Sens. 1A. The harmonised classification would result in even lower concentration thresholds, if the proposed SCL of 0.001% is agreed by RAC and the Commission. (Considerations on a classification proposal on DB106 are in progress.)

# 5 IDENTIFIED USES

Disperse dyes, including DB124 and Disperse Blue 106 (DB106), obtained from DB124 by hydrolysis (cf. section 9), are mainly used to dye or print fabrics made of synthetic fibres such as polyester, nylon, triacetate, cellulose, polyamide, and acrylic fibres (Lacasse and Baumann, 2004). These fibres are used in turn to produce garments that are mostly worn directly on the skin e.g. leggings, bodysuits, suits, dresses, brassieres, tights, and jacket lining (Hausen, 1993; Malinauskiene *et al.*, 2012). Disperse dyes are bound to the fabric with a degree of fixation between 88 and 99%. DB106 and DB124 are commonly used together and in mixtures with other disperse dyes to achieve the final colour during dyeing processes (Hausen, 1993; Le Coz, 2005). Literature for other uses of both disperse blue dyes is rare. DB124 and DB106 appear to play a role in body painting, indicated in one study (Dwyer and Forsyth, 1994). Besides, the use of DB106 as colourant in ultrasound gel was reported (Skalina and Ramesh, 2018).

Numerous human data, published in particular from the 1980s to the 2000s, provide evidence that DB124 and DB106 are "common causes of textile dermatitis" and are 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; Seidenari *et al.*, 1991). Because of these findings the American Contact Dermatitis Society declared disperse blue dyes as the "Contact Allergen of the Year 2000"(Jacob and Ramirez, 2007). Furthermore, the ÖkoTex Standard 100 listed DB124 and DB106 as allergenic dyes, defining a limited value in textiles produced according to this Standard (OEKO-TEX, 2019). For labelling of textiles with the EU Ecolabel DB124 and DB106 "shall not be used for dyeing polyester, acrylic, polyamide, or elasticated or stretchable skin contact garments or underwear (2014/350/EU)". Furthermore, DB124 and DB106 were added to the Restricted Substance List (AAFA, 2019).
Perhaps, as a result of these voluntary initiatives, DB124 and DB106 have rarely been found in clothes and accessories in recent years. This conclusion is based on data from three studies available to the DS, analysing a limited number of textiles from a very large market. Therefore, it cannot be excluded that DB124 and 106

are still used in dyeing processes for clothes, other areas of the textile market or even other fields of application (BVL, 2010; Malinauskiene *et al.*, 2012; Zhou *et al.*, 2014).

## 6 DATA SOURCES

Data were received from the results of a systematic literature screening in databases, including PubMed, Scopus, Web of Science, EMBASE, and Toxnet. Search criterion were: "genetic tox*" OR "genotox*" OR "mutagen*" OR "mutat*" OR "genetical tox*" OR "cancer*" OR "carcinogen*" OR "carcinoma*" OR "metastasis*" OR "metastases" OR "tumor*" OR "tumour*" OR "developmental tox*" OR "fecundity" OR "fertility" OR "fertility disease*" OR "fertility disorder*" OR "ovaries" OR "reproduction toxicity" OR "reproductive toxicity" OR "teratogen*" OR "testis" OR "testes" or "toxicity for reproduction" OR "sperm*" OR "dermat*" OR "allerg*" OR "sensiti*"; "5-Nitro-2-(2-methyl-4-(N-ethyl-N-(2-hydroxyethyl)amino)phenylazo)thiazole" OR "C.I. 111935" OR "C.I. Disperse Blue 106" OR "C.I. Disperse Blue 357" OR "Disperse Blue 106" OR "Disperse Blue 357" OR "EINECS 271-183-4" OR "Miketon Polyester Discharge Blue R" OR "Serisol RD 400" OR "Tersetile Blue CRL" OR "UNII-C48O4" OR "2-(Ethyl(3-methyl-4-((5-nitrothiazol-2-yl)azo)phenyl)amino)ethanol" OR "Ethanol, 2-(ethyl(3-methyl-4-((5-nitro-2-thiazolyl)azo)phenyl)amino)- " OR "Ethanol, 2-(ethyl(3-methyl-4-(2-(5-nitro-2-thiazolyl)diazenyl)phenyl)amino)-" OR "12223-01-7"; and "61951-51-7" OR "Disperse Blue 124" OR "Ethanol, 2-[ethyl[3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]phenyl]amino]-, 1-acetate".
Furthermore, data were retrieved from a public report of NICNAS assessing Disperse Blue 360, DB124, DB106 and Disperse Blue 96 (NICNAS, 2015).

# 7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Physical state at 20°C and 101,3 kPa** | solid | | |
| **Melting/freezing point** | No data available | | |
| **Boiling point** | 545.7±60°C | SciFinder | Predicted value[1], press 760 Torr |
| **Relative density** | 1.35±0.1 g/cm3 | SciFinder | Predicetd value[1], T=20°C, press 760 Torr |
| **Vapour pressure** | 5.80E-12 Torr | SciFinder | Predicted value[1], T=25°C |
| **Surface tension** | No data available | | |
| **Water solubility** | Sparingly Soluble (4.6E-6 mol/L) | SciFinder | Predicted value[1], unbuffered water pH 7.00, T= 25°C |
| **Partition coefficient n-octanol/water** | $\log K_{O/W}$ 2.57±0.5 | SciFinder | Predicted value[1], condition: most basic, T =25°C |

[1] Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2018 ACD/Labs)

# 8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

# 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

DB124 is a thiazolylazo-p-phenylene diamine dye and its structure is identical to that of DB106 (CAS: 12223-01-7, List No.: 602-285-2), except for O-acetylation of the 2-hydroxyethyl group. Acetate esters are sensitive to hydrolysis by esterases, such as carboxyl esterases in human skin (Batz *et al.*, 2013; Fu *et al.*, 2016). Furthermore, Hansson and colleagues (Hansson *et al.*, 1997) showed that DB124 is immediately hydrolysed into DB106 at reduced pH, supporting degradation of DB124 into DB106 on the skin surface. Besides, concomitant allergic reactions to DB106 and DB124 have been detected in many human studies (Lisi *et al.*, 2014; Slodownik *et al.*, 2011; Uter *et al.*, 2001). It is highly probable that DB124 is transformed into DB106 while penetrating the outer human skin, resulting in the same hapten for both disperse blue dyes. Therefore, the DS investigated studies of both dyes for assessment of skin sensitisation.

Table 8: Summary table of toxicokinetic studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| During degradation experiments, aqueous solutions of DB124 and DB106 were treated with a reducing agent, and degradation products were analysed using HPLC. | DB106 formed "immediately" after "adding a few drops of hydrochloric acid" (concentration unknown) to the water solution of DB124 (pH-value not reported), monitored by HPLC. | Study demonstrates DB124 hydrolysis into DB106 after acidification | (Hansson *et al.*, 1997) |

# 10 EVALUATION OF HEALTH HAZARDS

## Acute toxicity

### 10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier.

### 10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

### 10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

### 10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

### 10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

### 10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

### 10.7 Skin sensitisation

Skin sensitisation is an immunological process that has been divided into two phases. During the first phase, the induction, the naive individual becomes sensitised to the allergenic agent accompanied by the production of allergen-specific memory cells. In the second phase, the elicitation, exposure of the sensitised individual to the allergen leads to proliferation and activation of these T-cells, secretion of cytokines and mobilisation of other inflammatory cells resulting in a clinical outcome of allergic contact dermatitis (ECHA, 2017).

Several animal studies are available with DB124 that cover the induction phase and allow placing of the test material into potency groups. Furthermore, a multitude of human studies, including patch test studies and case reports, were found in literature, covering the elicitation phase and indicating previous sensitisation to DB124 in humans.

Based on the results presented in section 9 above, showing that DB124 is immediately hydrolysed into DB106 at reduced pH and it is highly probable that DB124 is transformed into DB106 while penetrating the outer human skin, studies for both, DB124 and DB106 will be used to evaluate if DB124 is a skin sensitiser or not.

There was no Human Repeated Insult Patch Test (HRIPT) or Human Maximization Test (HMT) with DB124 (or DB106) available to the DS.

### 10.7.1 Animal data

Table 9: Summary table of animal studies on skin sensitisation for DB124 and DB106

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance,</th><th>Dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td colspan="6">Key study</td></tr>
<tr><td>Key Study<br>LLNA<br><br>(acc. to (Kimber and Basketter, 1992))<br><br>According to OECD TG 429<br>No information on GLP<br><br>Reliability 2: Reliable with restrictions<br><br>Individual body weights at start of dosing and at scheduled kill not reported, no information for signs on toxicity</td><td>Mice, CBA/Ca, male<br><br>n=4/dose</td><td>DB106<br><br><u>Vehicle:</u> DMSO<br><br><u>Purity:</u> 87%</td><td>Concentrations: 0.25, 0.05, 0.025, 0.1, 0.01, and 0.005% tested in two experiments<br><br><table><tr><th>Tested dye</th><th>EC3 (%)</th></tr><tr><td>DB106 (A)</td><td>0.012</td></tr><tr><td>DB106 (B)</td><td>0.017</td></tr><tr><td>DNCB*</td><td>0.015</td></tr></table></td><td>Positive<br>Extreme sensitiser</td><td>(Betts et al., 2005)</td></tr>
<tr><td colspan="6">Supporting studies</td></tr>
<tr><td>“Biphasic” LLNA<br><br>Non-guideline study<br>No information on GLP<br><br>Reliability 2: Reliable with restrictions<br><br><u>Deviations to OECD TG 429:</u><br>Sensitisation phase: Day 1-3<br>Challenge phase: Day 15-17<br>Instead of monophasic sensitisation protocol;<br>Endpoint analysis: Day 19, instead of two days without treatment;<br>Analysis of cell-count increase using automated cell counter, instead of analysis of $^{3}$HTdR incorporation into DNA;<br>No performance standard;<br>Individual body weights at start of dosing and at scheduled kill not reported;<br>No SI calculation</td><td>Mouse, BALB/c, female<br><br>n=10/dose<br>n=20/ control</td><td>DB124 and DB106<br><br><u>Vehicle:</u> DMSO<br><br><u>Purity:</u> no information for DB106 or DB124 available</td><td>Significant increase in cell-count (%) compared to vehicle control) for concentration (c) of tested dyes is shown:<br><br><table><tr><th>c (%)</th><th>DB124</th><th>DB106</th></tr><tr><td>30</td><td>n.d.</td><td>174</td></tr><tr><td>10</td><td>147</td><td>n.d.</td></tr><tr><td>3.0</td><td>132</td><td>124</td></tr><tr><td>0.3</td><td>116</td><td>82</td></tr><tr><td>0.03</td><td>79</td><td>79</td></tr><tr><td>0.003</td><td>21</td><td>37</td></tr></table><br>10, 3, 0.3, and 0.03% DB124 resulted in a significant increase in ear-thickness by 22, 26, 30, and 4%<br><br>30, 3, 0.3, and 0.03% DB106 resulted in a significant increase in ear-thickness by 26, 13, 17, and 9%</td><td>Positive<br><br>Determi-nation of potency not possible**</td><td>(Ahuja et al., 2010)<br><br>(Ahuja, 2010)</td></tr>
<tr><td>Method developed from FCAT and guinea pig maximisation test (GPMT)<br><br>Similar to OECD TG 406<br>No information on GLP</td><td>Guinea pig, Pirbright White, female<br><br>n=10</td><td>DB124<br><br><u>Vehicle for topical challenge</u>: acetone</td><td>Intradermal injections: 15 mg of DB124 dissolved in 8 ml in FCA/saline (1:1), corresponds to 0.2% (w/v)<br><br>Challenge: 1% in acetone<br><br><table><tr><th></th><th>24 h</th><th>48 h</th><th>72 h</th></tr></table></td><td>Positive<br><br>Strong sensitiser</td><td>(Hausen and Sawall, 1989)</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance,</th><th>Dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Reliability 2: Reliable with restrictions<br><br><u>Deviations:</u><br>Intradermal injections at day 0, 5, and 9 (receiving a total of 4.5 mg per animal), instead of intradermal injections at day 0 and topical induction application at day 6-8,<br>Challenge with open epicutaneous elicitation (day 20)</td>
<td></td>
<td><u>Purity:</u><br>chromato-graphically pure</td>
<td>
<table>
<tr><td>+++</td><td>-</td><td>2</td><td>-</td></tr>
<tr><td>++</td><td>5</td><td>-</td><td>5</td></tr>
<tr><td>+</td><td>2</td><td>1</td><td>2</td></tr>
<tr><td>(+)</td><td>2</td><td>3</td><td>1</td></tr>
<tr><td>-</td><td>1</td><td>3</td><td>2</td></tr>
</table>
</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPMT modified FCA method<br><br>(acc. to (Hausen and Schmalle, 1985))<br><br>Similar to OECD TG 406<br>No GLP<br><br>Reliability 2: Reliable with restrictions<br><br><u>Deviations:</u><br>Intradermal injections at day 0, 5, and 9, instead of intradermal injections at day 0 and topical induction application at day 6-8</td>
<td>Guinea pig Pirbright White, no further information<br><br>n=10</td>
<td>DB106<br><br><u>Vehicle:</u><br>acetone<br><br><u>Purity:</u><br>chromato-graphically pure</td>
<td>Intradermal injection:<br>9 mg dye per guinea pig for the whole procedure in 0.6 ml emulsion FCA/saline (1:1), corresponding to 1.5% (w/v)<br><br>Challenge concentration: 0.001% in acetone<br><br>
<table>
<tr><th></th><th>24 h</th><th>48 h</th><th>72 h</th></tr>
<tr><td>+++</td><td>6</td><td>7</td><td>6</td></tr>
<tr><td>++</td><td>3</td><td>2</td><td>3</td></tr>
<tr><td>+</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>(+)</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td>-</td></tr>
</table>
<br>Reactions for dilutions of 1%, 0.3%, and 0.1% were so strong that no reading could be made.</td>
<td>Positive<br><br>Moderate sensitiser</td>
<td>(Hausen and Menezes Brandao, 1986)</td>
</tr>
</table>

*Pos. control

A significant body of evidence from published literature indicates that DB106/124 induce allergic reactions in animal models. For instance the study of (Betts *et al.*, 2005), comprising a LLNA according to (Kimber and Basketter, 1992) shows that DB106 causes lymph nodes response in mice resulting in very low EC3-values (Experiment A: 0.012% and Experiment B: 0.017%). This well-documented local lymph node assay does not show obvious deviations from OECD TG 429 and indicates that DB106, the hydrolysis product of DB124, causes skin sensitisation with an extreme potency. The DS considers this LLNA as the key animal study.

Ahuja et al. 2010 demonstrated in a “biphasic” LLNA that both DB106 and 124 cause skin sensitisation and have a similar sensitising potency. In their study, the authors used a sensitisation-challenge-protocol and analysed the increase in lymph node cells compared to vehicle control. Very low concentrations (0.003%) of DB124 or DB106 induced a significant increase in cell-count compared to the vehicle control. However, the experimental design deviates from OECD TG 429 and the test was not validated against a LLNA performance standard reference chemical defined in that guideline.

Additionally, in a modified guinea pig maximisation test (GPMT) with open epicutaneous elicitation performed similar to OECD TG 406, the skin sensitising potency of DB124 was analysed (Hausen and Sawall, 1989). At least 66% of the exposed guinea pigs reacted positively after treatment with DB124 (0.2% intradermal induction). This study indicates that DB124 acts as strong sensitiser. Another GPMT similar to

OECD TG 406 resulted in 100% positively reacting animals after DB106 treatment, using an intradermal injection concentration of 1.5% (Hausen and Menezes Brandao, 1986), resulting in a moderate sensitising potency. However, in both GPMTs lower concentrations of DB124 and DB106 for intradermal induction were not tested.

Detailed study summaries for all animal in vivo studies are reported in Annex I.

Furthermore, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) published an assessment of DB124 and DB106. Unpublished study reports submitted by notifiers and summarised by NICNAS give evidence of skin sensitisation with a moderate potency in a Buehler test (according to OECD TG 406) conducted with DB106 (24h after challenge: 16/20 animals with erythema score ≥1; 48h,15/20 animals; 50% topical induction, 50% topical challenge). In an unpublished GPMT of notifiers (according to OECD TG 406), DB106 showed a strong sensitising potency (24h after challenge: 14/20 animals with erythema score ≥ 1; 48h, 12/20 animals; 1% intradermal induction, 50% topical challenge). However, the concentration for topical induction during the Buehler test was 50%, and for intradermal induction during the GPMT was 1%, while fewer concentrations were not tested and an extreme potency cannot be excluded. Another study report of a GPMT performed with DB124 was considered by NICNAS as of low reliability. None of these study reports submitted by notifiers were available to the DS.

### 10.7.2 Human data

A total of 32 reports documenting human patch test data obtained with DB124 and DB106 are available from the published literature (Table 10).

In addition, numerous case reports have been found which document sensitisation of individuals exposed to DB124/106 from various garments. More than 70 relevant case reports are summarised in Table 11. Reports considered as not reliable or not assignable were excluded from further assessment (Carrozza and Nestle, 2000; Corazza *et al.*, 2008; Fuentes Cuesta *et al.*, 2000; Guin *et al.*, 1999; Hansson *et al.*, 1997; Jacob and Ramirez, 2007; Khanna and Sasseville, 2001; Mohamoud and Andersen, 2017; Perez-Crespo *et al.*, 2009; Raccagni *et al.*, 1996; Stante *et al.*, 2006; Ukida *et al.*, 2014).

Table 10: Summary table of human patch test data on skin sensitisation

| No. | Type of data/report | Test substance, relevant information about the study (as applicable) | Test results for DB124/DB106, observation | Results[1], classification | Reference |
|---|---|---|---|---|---|
| **Consecutive dermatitis patients** | | | | | |
| 1 | Patch test from dermatological clinic<br><br>Reliability 2: Reliable with restrictions | 09/2012-08/2014, 1 043 patients were patch-tested; 191 subjects with eczematous eyelid dermatitis were compared with 852 patients suffering of dermatitis in other body areas. Patch testing with SIDAPA[a] series (including DB124, 1%, vehicle not reported) and other haptens | DB124: **1.2%** (12/1043) positive; among those, 6/191 patients with eyelid dermatitis and 6/852 patients without eyelid dermatitis | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Bosco *et al.*, 2016) |
| 2 | Retrospective review of patch test results from dermatological clinics<br><br>Reliability 2: Reliable with restrictions | Electronic patch test database containing demographic information and results from all (3 115) patients tested 01/2006-12/2010. On average, patients were patch-tested for 73 allergens, including DB124, DB106, 1% each (vehicle not reported) were patch-tested. | DB124: **3.4%**<br>DB106: **2.8%**<br><br>Irritant reactions:<br>DB124: 0.8%<br>DB106: 0.6% | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Wentworth *et al.*, 2014) |
| 3 | Patch test analysis from 13 dermatological centres from NACDG[b]<br><br>Reliability 2: Reliable with restrictions | 01/2007- 12/2008, 5 085 patients with suspected allergic contact dermatitis (598 subjects with occupationally related skin condition) were patch-tested with 65 allergens (Chemotechnique Diagnostics), including DB106 (1% in pet.). | DB106: **0.9%** | **Positive**<br>**Low/moderate frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Fransway *et al.*, 2013) |
| 4 | A retrospective chart review of patch tests from hospital<br><br>Reliability 4: Not assignable | Within five years, 427 patients were patch tested for "utilization of TRUE® test versus expanded patch test panels for allergic contact dermatitis" | DB106: **2.3%** | **Positive**<br>No sub-categorisation possible | (Mucci *et al.*, 2012) |
| 5 | Patch test from dermatological clinic<br><br>No time window reported, self-selected volunteers, sensitization rate may be over-represented, volunteers aged 20-27 years | 327 "consecutive patients with eczema" and 205 healthy student volunteers (non-patient population, recruited by advertisement) were patch-tested with modified European baseline series and textile dye allergens, including DB124 and | Consecutive eczema patients<br>DB124: **1.2%**<br>DB106: **1.2%**<br>Healthy volunteers<br>DB124: 1%<br>DB106: 0% | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation | (Li, 2010) |

[1]Frequency and exposure are rated as relatively high or low in line with Tables 3.2 and 3.3 of the ECHA "Guidance on the Applicability of the CLP criteria", where possible.

| No. | Type of data/report | Test substance, relevant information about the study (as applicable) | Test results for DB124/DB106, observation | Results[1], classification | Reference |
|---|---|---|---|---|---|
| | Reliability 2: Reliable with restrictions | DB106, 1% each (vehicle not reported, assumed pet.) | | possible | |
| 6 | Patch tests/consumer tests at Department of Occupational and Environmental Dermatology<br><br>Reliability 2: Reliable with restrictions | 02-12/2005: 982 dermatitis patients were consecutively patch-tested with baseline patch test series, including a textile dyes mix and the eight separate components (DB106 and DB124, both 0.1% in pet. included). 858 patients answered a questionnaire. | DB124: **0.2%** (2/982)<br>DB106: **0.2%** (2/982) | **Positive**<br>**Low/moderate frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Ryberg *et al.*, 2009a) |
| 7 | Descriptive analysis of patch test data to disperse dyes from the IVDK[c]<br><br>Reliability 2: Reliable with restrictions | 07-12/2005, 2 555 patients were consecutively patch-tested with DB124, DB106 (each 0.3% in pet), and Disperse Blue (DB) mix 106/124 (0.35% and 0.2% in pet.), included into 'monitor series' suppl. standard series. Authors analysed two batches of the DB106/124 mix for concentration. | DB106/124 mixes proved to contain an amount of allergen different to the declared one (based on suppliers information). Patch test data for dyes, with reliable concentration:<br>DB124: **0.4%** (8/2 214)<br>DB106: **0.5%** (11/2 215)<br>DB mix 106/124:<br>**0.7%** (19/2 555)<br>6 patients reacted to both, DB124 and DB106<br><br>Irritant reactions: DB124: 3/2 214, DB106: 5/2 215, DB mix 106/124: 2/2 555 | **Positive**<br>**Low/moderate** frequency<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Uter *et al.*, 2007) |
| 8 | Patch test from dermatological clinic<br><br>Reliability 2: Reliable with restrictions | 1995-2001, 1 094 consecutive children (aged: 7 months to 12 years) with suspected contact dermatitis were patch-tested with "pediatric series" of 30 allergens or with 46 allergens; including DB124, DB106, each 1% in pet. | DB124: **1.8%**<br>DB106: **4.0%** | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Seidenari *et al.*, 2005) |
| 9 | Patch test from dermatological clinic, investigation of sensitization to disperse dyes in children<br><br>Reliability 2: Reliable with restrictions | 01/1996-12/2000: 1 098 consecutive children (667 with suspected allergic contact dermatitis and 431 with atopic dermatitis) were patch-tested with "standard patch test series" (including five disperse dyes). Subjects, > 10 years of | DB124: **1.3%** (14/1 098)<br>DB106: **3.0%** (4/134) | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation | (Giusti *et al.*, 2003) |

| No. | Type of data/report | Test substance, relevant information about the study (as applicable) | Test results for DB124/DB106, observation | Results[1], classification | Reference |
|---|---|---|---|---|---|
| | | age, were patch-tested with two additional disperse dyes (including DB106, vehicle or concentration not reported) | | possible | |
| 10 | Patch test analysis from 13 dermatological centres<br><br>Reliability 2: Reliable with restrictions | 3 041 consecutive patients patch-tested from 05/2001-07/2002 using Standard series supplemented with Disperse Blue (DB) mix 124/106 (1% in pet.) | DB mix 124/106: **1.3%** (40/3 041) | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Uter *et al.*, 2003) |
| 11 | Patch test from dermatological clinic<br><br>Reliability 2: Reliable with restrictions | 286 consecutive patients were patch-tested over a period of one year, with standard series (TRUE Tests®) and a textile colour and finish series (Chemotechnique Diagnostics; DB124 and DB106 assumed each 1% pet.) | DB124: **7.3%** (21/286)<br>DB106: **4.2%** (12/286) | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Lazarov *et al.*, 2002) |
| 12 | Patch test from dermatological clinic, investigation on frequency of long-lasting allergic patch test reactions (LLAPTR)<br><br>Reliability 2: Reliable with restrictions | 1995-1998, 798 consecutive patients suspected of having allergic contact dermatitis, were patch-tested with GIRDCA standard series (30 substances, DB124 1% pet.) | DB124: **3.6%** (29/798)<br><br>DB124 identified as a risk factor for LLAPTR | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Mancuso *et al.*, 1999) |
| 13 | Patch test from dermatological clinic time window not reported, short communication<br><br>Reliability 4: Not assignable | Review of 1 012 patients with suspected contact dermatitis and patch-tested with GIRDCA[d] standard series, augmented by a disperse mix and two dark dyes, including DB124 1% (vehicle not reported, assumed pet.) | DB124: **2.2%** (22/1 012) | **Positive**<br>No sub-categorisation possible | (Lodi *et al.*, 1998) |
| 14 | Patch test from dermatological clinic, contact sensitization in children<br><br>Reliability 2: Reliable with restrictions | 1988 -1994: 670 children, six months to 12 years of age (506 with atopic dermatitis and 164 with eczematous lesions) underwent patch tests with European standard series, including DB124 (vehicle or concentration not reported, assumed 1% in pet.) | DB124: **0.7%** (5/670) | **Positive**<br>**Low/moderate** frequency<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Manzini *et al.*, 1998) |
| 15 | Patch test from dermatological | 1990- 1995: 6 203 patients were | DB124: **1.7%** (104/6 203) | **Positive** | (Seidenari *et al.*, |

| No. | Type of data/report | Test substance, relevant information about the study (as applicable) | Test results for DB124/DB106, observation | Results[1], classification | Reference |
|---|---|---|---|---|---|
| | department<br><br>Reliability 3: Not reliable<br><br>Authors show a structure for DB124 not identical to the structure in this dossier | consecutively patch-tested with textile dyes included in standard series, including DB124, concentration or vehicle not reported. | | **High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | 1997) |
| 16 | Patch test from dermatological clinic,<br>evaluation of contact sensitization prevalence to disperse dyes in certain area,<br>short communication<br><br>Reliability 2: Reliable with restrictions | 576 consecutive patients, with various eczemas were investigated over a period of two years. Patch testing with four disperse dyes (DB124 1% in pet.) and GIRDCA standard series was performed. | DB124: **1.9%** (11/576) | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or DB106 not documented<br>No sub-categorisation possible | (Balato *et al.*, 1990) |
| **Selected dermatitis patients** | | | | | |
| 17 | Retrospective analysis including 56 dermatological departments<br><br>Reliability 2: Reliable with restrictions | 2007-2014, 3 207 patients with suspected textile allergy and 95 210 patients as control group were patch-tested with textile and leather dye series, including DB124 and DB106, 0.3% (vehicle not reported) | DB124: **2.3%** (28/1 237)<br>DB106: **2.0%** (25/1 238)<br><br>Irritant reactions:<br>DB124: 9/1 237<br>DB106: 5/1 238 | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Heratizadeh et al., 2017; |
| 18 | Patch test outcome to textile dye mix (TDM) and patch test reactions to single separate dyes with patients allergic to textile dye mix.<br>Consideration for inclusion of the TDM into the international baseline series.<br>Reliability 2: Reliable with restrictions | 03-12/2013, ICDRG[e] representing clinics from nine countries: 2 493 consecutive dermatitis patients were patch-tested with TDM 6.6% in petrolatum, consisting of six disperse dyes, all 1.0% each, and DB106 and DB124 (each 0.3% in pet.). | 3.6% (1.3 – 18.2%; 90/2 493) positive reactions to TDM; 83 positively patch-tested patients were patch-tested with single textile dyes at different concentrations:<br>DB124 (0.3%): **7.2%** (6/83)<br>DB124 (1.0%): **10.8%** (9/83)<br><br>DB106 (0.3%): **7.2%** (6/83)<br>DB106 (1.0%): **15.7%** (13/83) positive | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Isaksson *et al.*, 2015) |
| 19 | Investigations of the patch testing outcome of EECDRG[f] clinics from nine countries to textile dye mix | 01-06/2011, 2 907 consecutive dermatitis patients were patch-tested to TDM 6.6% in pet. (six disperse dyes, each 1.0%, and | 3.7% (108/2 907) positive reactions to TDM, 94 mix-positive patients were tested with single dyes. | **Positive**<br>**High frequency**<br>Previous exposure to | (Ryberg *et al.*, 2014) |

| No. | Type of data/report | Test substance, relevant information about the study (as applicable) | Test results for DB124/DB106, observation | Results[1], classification | Reference |
|---|---|---|---|---|---|
| | (TDM). Consideration for inclusion of the TDM into the European baseline series.<br>Reliability 2: Reliable with restrictions | DB106 and DB124, each 0.3%). | DB124 (0.3%): **5.3%** (5/94)<br>DB124 (1.0%): **8.5%** (8/94)<br><br>DB106 (0.3%): **6.4%** (6/94)<br>DB106 (1.0%): **13.8%** (13/94) | DB124 or 106 not documented<br>No sub-categorisation possible | |
| 20 | Patch test evaluation of clinical features and epidemiology of textile contact dermatitis<br>time window unknown<br><br>Reliability 2: Reliable with restrictions | 277 selected textile dermatitis patients were patch-tested, 154 patients were affected by allergic textile contact dermatitis (non-occupational in 132; occupational in 22 subjects). SIDAPA baseline series, textile series, and suspected garment sample when available were used for patch testing (DB124 and DB106, each 1% in pet. included). | DB124: **54.5%** (84/154),<br>non-occupational: 59.8% (79/132)<br>occupational: 22.7% (5/22)<br><br>DB106: **28.6%** (44/154)<br>non-occupational: 33.3% (44/132)<br>occupational: 0 (0/22)<br><br>39 concomitant reactions between DB124 and DB106 | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Lisi *et al.*, 2014) |
| 21 | Retrospective review of patch tests from department of dermatology<br><br>Reliability 2: Reliable with restrictions | 01/2000-09/2011, a total of 671 patients were patch-tested with textile dye series (DB124 and DB106, each 1%), resins, and standard patch test series (n=620 patients). | DB124: **8.0%** (n=665)<br>DB106: **8.3%** (n=660)<br><br>Irritant reactions<br>DB124: 2.6%<br>DB106: 0.6% | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Wentworth *et al.*, 2012) |
| 22 | Patch tests from general and occupational contact dermatitis clinics at the Skin and Cancer Foundation Melbourne, Australia<br><br>Reliability 2: Reliable with restrictions | 1993-2006, 2 069 patients with suspected textile allergy were tested with extended European baseline series and textile series (including DB124, DB106, each 1% in pet., DB mix 124/106, 1% in pet.) | DB124: **1.0% (**20/2 069)<br>DB106: **1.0% (**21/2 069)<br>DB mix 124/106: **0.3%** (6/2 069)<br><br>three patients reacted to DB124 and DB106 | **Positive**<br>**Low/moderate frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Slodownik *et al.*, 2011) |

| No. | Type of data/report | Test substance, relevant information about the study (as applicable) | Test results for DB124/DB106, observation | Results[1], classification | Reference |
|---|---|---|---|---|---|
| 23 | Patch test from Department of Occupational and Environmental Dermatology, investigation for significance of impurities;<br>Low number of subjects<br>No time window<br><br>Reliability 2: Reliable with restrictions | 21 patients were previously patch-tested in dermatological departments and reacted positively to DB124 and DB106. Patients were patch-tested with purified and commercial DB124 and 106, and with thin-layer chromatography (TLC) strips made from the commercial preparations of dyes. | 12/18 patients reacted positively to DB124 strips, five subjects did not react to main spot;<br>13/21 patients reacted positively to DB106 strips, four subjects did not react to main spot; 11 patients reacted to dilution series of purified DB124 and 106; 15 and 16 patients, respectively, tested positively to dilution series of commercial dyes. | **Positive**<br>Frequency unclear<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Ryberg *et al.*, 2009b) |
| 24 | Patch test analysis from 37 IVDK dermatological clinics<br><br>Reliability 2: Reliable with restrictions | 1998-2002, 696 patients with suspected textile dermatitis were patch-tested with textile dye series, including DB124, DB106, each 1% in pet., DB mix 124/106, 1% in pet. | DB124: **6.5%** (17/263**)**<br>DB106: **7.2%** (19/263)<br>DB mix124/106: **7.7%** (51/659)<br><br>Irritant reactions: DB124: 1/263, DB106: 1/263, DB mix124/106: 1/263 | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Bauer *et al.*, 2004) |
| 25 | Retrospective patch test study from department of occupational dermatology<br><br>Reliability 2: Reliable with restrictions | 01/1996-12/1999, 577 patients with possibility for contact allergy to para or azo dyes were analysed. Patch testing with European standard series and dyes series, including DB124 and DB106 (patch test vehicle or concentration not specified, assumed 1% in pet.) | DB124: **5.0%** (29/577)<br>DB106: **5.9%** (34/577) | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Koopmans and Bruynzeel, 2003) |
| 26 | Patch test analysis from dermatological department<br><br>Reliability 2: Reliable with restrictions | 01/1996-12/2000: 6 478 consecutive patients patch-tested to standard series identified 437 patients allergic to disperse dyes: 130 patients with hand dermatitis (study group) and 307 without hand involvement. Patch testing with Standard series supplemented with azo dyes, including DB124 and DB106, patch test vehicle or concentration not specified | DB124:<br>**49%** (63/130) hand dermatitis patients,<br>**42%** (130/307) no hand involvement)<br><br>DB106:<br>**50%** hand dermatitis patients,<br>**49%** no hand involvement | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Giusti *et al.*, 2002) |

| No. | Type of data/report | Test substance, relevant information about the study (as applicable) | Test results for DB124/DB106, observation | Results[1], classification | Reference |
|---|---|---|---|---|---|
| 27 | Patch test analysis from 31 participating centers, IVDK<br><br>Reliability 2: Reliable with restrictions | 01/1995-06/1999, 1 986 patients were patch-tested to textile dye series, including DB124, DB106, each 1% in pet., DB mix 124/106, 1% in pet. | DB124: **3.0%** (55/1 829)<br>DB106: **3.5%** (64/1 847)<br>DB mix 106/124: **4.7%** (52/1 108)<br><br>46 subjects reacted to both, DB124 and DB106 | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Uter *et al.*, 2001) |
| 28 | Patch test analysis from a dermatological clinic<br><br>Reliability 2: Reliable with restrictions | During 1998, 103 patients with suspected allergic contact dermatitis to clothing were clinically evaluated and patch-tested with Standard series (TRUE Tests®) and textile color & finish series (Chemotechnique Diagnostics), including DB124 and DB106, (vehicle or concentration not reported, assumed 1% in pet.) | DB124: **6.8%** (7/103)<br>DB106: **6.8%** (7/103)<br><br>Purpuric patch tests provoked by DB124, DB106 | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Lazarov and Cordoba, 2000) |
| 29 | Retrospective patch test study from contact dermatitis clinic<br><br>Reliability 2: Reliable with restrictions | 09/1997-07/1999: 788 subjects were patch-tested to either NACDG standard tray or European standard series. 271 patients with clinical suspicion of textile dermatitis were patch-tested with textile series, including DB124 and DB106 (each 1% in pet.). | DB124: **11.8%** (32/271)<br>DB106: **12.2%** (33/271)<br><br>31 patients reacted to both, DB106 and DB124 | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Pratt and Taraska, 2000) |
| 30 | Patch test from dermatological clinic, investigation of disperse dyes at reduced concentrations for patch test evaluation, short communication<br><br>Reliability 2: Reliable with restrictions | 41 patients with textile allergic contact dermatitis and sensitized to one or more disperse dyes (1% in pet.) were patch-tested with disperse dyes at reduced concentrations and disperse dye mix. | 7/8 total reactive patients showed positive reactions to DB125 (0.5% pet.)<br><br>19/23 total reactive patients showed positive reactions to DB124 (0.1% pet.) | **Positive**<br>Frequency unclear<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Sertoli *et al.*, 1994) |

| No. | Type of data/report | Test substance, relevant information about the study (as applicable) | Test results for DB124/DB106, observation | Results[1], classification | Reference |
|---|---|---|---|---|---|
| 31 | Patch test analysis from a dermatological department<br><br>Reliability 2: Reliable with restrictions | 1987-1991: 3 336 patients were investigated for contact dermatitis and patch-tested with European standard series. 159 patients were also tested with 15 textile dyes (DB124 and DB106 included) and five patients with four textile dyes (DB124 not included, Chemotechnique Diagnostics, concentration and vehicle not reported, assumed 1% in pet.) | DB124:<br>**3.8%** (6/159) among all patients tested<br>**26.1%** (6/23) among patients with textile dye dermatitis<br><br>DB106:<br>**9.7%** (16/164) among all patients tested<br>**57.1%** (16/28) among patients with textile dye dermatitis | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Dooms-Goossens, 1992) |
| 32 | Patch test from dermatological department<br><br>Reliability 2: Reliable with restrictions | 10/1987-04/1990: 100 subjects, identified from 2 752 consecutive patients were sensitised to textile dyes GIRDCA standard series and textile industry series, including DB124, 1% in pet. | DB124: **36%** (36/100) | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Seidenari *et al.*, 1991) |
| 33 | Patch test from dermatological clinic<br><br>Reliability 2: Reliable with restrictions | Duration of two years: 145 patients, suspected of having allergic contact dermatitis from textile chemicals, were patch-tested with textile series, including DB124, 1% in pet. | DB124: **8.3%** (12/145) | **Positive**<br>**High frequency**<br>Previous exposure to DB124 or 106 not documented<br>No sub-categorisation possible | (Balato *et al.*, 1990) |

[a]SIDAPA - Italian Society of Allergological Dermatology; [b]NACDG - North American Contact Dermatitis Group; [c]IVDK - Information Network of Departments of Dermatology; [d]GIRDCA – Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali; [e]ICDRG - International Contact Dermatitis Research Group; [f]EECDRG - European Environmental Contact Dermatitis Research Group

Table 11: Summary of the available case reports (reliable, with restriction) on skin sensitisation in relation to wearing garments

| No. | Clinical data/case history | Patch test results/Diagnosis | Ref. |
|---|---|---|---|
| 1 | A 28-year old woman developed eyelid dermatitis after performing "research with focused ultrasound on mice in a horizontal laminar flow hood in which the airflow was towards the user". Blue ultrasound gel dyed with Disperse Blue 106 was used. | DB106 (+) on day three | (Skalina and Ramesh, 2018) |
| 2 | A 37-year-old worker wore blue overalls at work and attended with a 10-month history of a confluent red rash. Patch testing with baseline series and textile dyes was performed. | DB mix 106/124 (+++) for the first patch testing | (Narganes *et al.*, 2013) |
| 3 | A 35-year-old woman had lesion over the incision scar of hip replacement surgery. She wore dark coloured panties made of synthetic materials, for long years. Patch testing with European standard series and therapeutics. | Patch test positive to dispersion mix blue 106/124 (1% in pet.) at 48 hours | (Caliskaner *et al.*, 2012) |
| 4 | A healthy 63-year old woman presented with nonpruritic redness of both breasts of several months' duration in area of her black undergarments. Patch tests with North American series (45 allergens) and clothing series were performed. Replacement of her black brassieres with white ones spontaneously resolved erythema over several months. | DB106 and DB124 (+) on day six | (Wong *et al.*, 2011) |
| 5 | A 42-year-old man, "with a 12-month history of an inflammatory eruption affecting his neck" was described. He "regularly wore dark nylon clothing when refereeing lacrosse matches". Patch testing with standard series, textile series was performed. | DB124 (++) and DB106 (++) | (Walker and Beck, 2005) |
| 6 | A 43-year-old woman had dermatitis under her breast, across her back around her waist. Eczematous eruption occurred 24 hours after wearing a new navy blue lined dress. Patch testing with Skin and Cancer Foundation standard series, textile dye series, and samples of her own blue dress. | Strong positive reactions to DB106 (1%) and a weak positive reaction to the dress lining, at 72 hours; other patch tests were negative. | (Dawes-Higgs and Freeman, 2004) |
| 7 | A 53-year-old woman was seen with a contact dermatitis where a bra and girdle would fit her. Patch testing to a screening series was performed. | DB106 (+) at second reading on day five | (Guin, 2001) |
| 8 | Jan. 1998: A 52-year-old woman presented with eczematous foci and aggregation of petechiae. Topical steroids and skin care products were applied with little effect. Patch tests were performed with standard ointment and textile dye series. Purpuric contact dermatitis exacerbated and generalized after wearing a new blue dress. | Erythematous reaction to DB124 (in pet., concentration not reported), DB106 and mix of DB124/DB106 on day four | (Komericki *et al.*, 2001) |
| Five female workers in a ready-to-wear shop presented with 3-month histories of eczema. The garment suspected was a dark blue smock, introduced as a working uniform in the last 4 months. Patch tests were performed with the Portuguese standard series, including disperse dyes. | | DB106 was identified in smock, using TLC; smock was made of synthetic acetate and polyamide; 5/5 positive reactions to DB124 and DB106 | (Mota *et al.*, 2000) |
| 9 | (Case 1) Age: 34 years, eczema around axillae, neck, upper chest, hands (dorsum) and eyelids | DB106, DB124 positive | |
| 10 | (Case 2) Age: 25 years, eczema around axillae, neck, upper chest, abdominal wall, face | DB106, DB124 positive | |
| 11 | (Case 3) Age: 34 years, eczema around neck, hands (dorsum), antecubital fold, forearm | DB106, DB124 positive | |
| 12 | (Case 4) Age: 34 years, eczema around neck, forearm | DB106, DB124 positive | |
| 13 | (Case 5) Age: 34 years, eczema around neck, fists | DB106, DB124 positive | |
| 788 patients with textile dye allergy were patch-tested to standard series (NACDG or European, DB124 and 106, each 1% in pet. included). Forty patients reacted positively to one or more textile | | 82.5% (33/40) positive reactions to DB106, 80% (32/40) positive reactions to DB124 | (Pratt and Taraska, 2000) |

| No. | Clinical data/case history | Patch test results/Diagnosis | Ref. |
|---|---|---|---|
| dyes. | | | |
| 14 | (Case 1) 51 year-old woman, with dermatitis distributed around anterior and upper inner thighs, lasting for one year | DB106 (+++), DB124 (+++), own textile (+++) | |
| 15 | (Case 2) 50 year-old woman, with dermatitis distributed around axillary folds, waistband, upper inner anterior thighs, lasting for five year | DB106 (+++), DB124 (+++), own textile (+++) | |
| 16 | (Case 3) 78 year-old woman, with dermatitis distributed around upper thighs with widespread id reaction[#], angioedema of lips and tongue and urticarial, lasting for six month | DB106 (+++), DB124 (+++), and own textile (+++) | |
| 17 | (Case 4) 31 year-old woman, with dermatitis distributed around upper inner thighs, neck, chest, lasting for two years | DB106 (+++), DB124 (+++), own textile (+++) | |
| 18 | (Case 5) 71 year-old woman, with dermatitis distributed around thighs, axillary folds, lasting for six month | DB106 (+++), DB124 (+++), own textile (+++) | |
| 19 | (Case 6) 72 year-old woman, with dermatitis distributed around trunk and extremities, lasting for three years | DB106 (++), DB124 (++), own textile (-) | |
| 20 | (Case 7) 45 year-old woman, with dermatitis distributed around chest, axillary folds, upper inner thighs, antecubital fossae, lasting for six months | DB106 (+++), DB124 (+++), and own textile (+++) | |
| 21 | (Case 8) 69 year-old man, with dermatitis distributed around head, neck, scalp, and arms, lasting for six months | DB106 (++), DB124 (++) | |
| 22 | (Case 9) 51 year-old woman, with dermatitis distributed around upper inner thighs, axillary vaults, waistband, face, lasting for 18 months | DB106 (+++), DB124 (+++), and own textile (+++) | |
| 23 | (Case 10) 22 year-old woman, with dermatitis distributed around face, neck, extremities, lasting for one year | DB106 (+), DB124 (+) | |
| 24 | (Case 11) 43 year-old woman, with dermatitis widespread distributed, lasting for two year | DB106 (+++), DB124 (+++), own textile (+++) | |
| 25 | (Case 12) 31 year-old woman, with dermatitis distributed around the arms, legs, back, axillary folds, lasting for five months | DB106 (+++), DB124 (+++), own textile (++) | |
| 26 | (Case 13) 23 year-old woman, with dermatitis distributed around the upper inner thighs, buttocks, forearms, trunk, face, lasting for two years | DB106 (+++), DB124 (+++) | |
| 27 | (Case 14) 55 year-old woman, with dermatitis distributed around the thighs with widespread id, lasting for one year | DB106 (+++), DB124 (+++), own textile (++) | |
| 28 | (Case 15) 88 year-old man, with dermatitis distributed widespread around the trunk and extremities, lasting for one year | DB106 (+++), DB124 (+++) | |
| 29 | (Case 16) 55 year-old woman, with dermatitis distributed around the arms with widespread id reaction, lasting for four months | DB106 (+), DB124 (+) | |
| 30 | (Case 17) 54 year-old woman, with dermatitis distributed around the chest and back, lasting for two months | DB106 (+), DB124 (+) | |
| 31 | (Case 21) 41 year-old man, with widespread dermatitis, lasting for two years | DB106 (+) | |
| 32 | (Case 22) 39 year-old woman, with dermatitis distributed around the face, neck, trunk, and extremities, lasting for six months | DB106 (+), DB124 (+) | |
| 33 | (Case 23) 36 year-old woman, with widespread dermatitis, lasting for six months | DB106 (+++), DB124 (+++) | |
| 34 | (Case 24) 45 year-old woman, with dermatitis distributed around the upper inner thighs and | DB106 (+), DB124 (+) | |

| No. | Clinical data/case history | Patch test results/Diagnosis | Ref. |
|---|---|---|---|
| | groin with widespread id reaction, lasting for one year | | |
| 35 | (Case 25) 43 year-old woman, with dermatitis distributed around the skin, forearms, neck, upper arms, lasting for six months | DB106 (+), DB124 (+) | |
| 36 | (Case 26) 43 year-old woman, with widespread dermatitis beginning over axillary, folds, inframammary area, anterior and inner thighs, lasting for eight years. | DB106 (+++), DB124 (+++), and own textile (+++) | |
| 37 | (Case 27) 58 year-old woman, with dermatitis in areas of the inner thighs, buttocks, lasting for two years | DB106 (+), DB124 (+) | |
| 38 | (Case 28) 44 year-old woman, with dermatitis in areas of genitalia, suprapubic area, periaxillary fold, lasting for two years | DB106 (++), DB124 (++) | |
| 39 | (Case 30) 59 year-old woman, with dermatitis in areas of axillary fold, lasting for three years | DB106 (+), DB124 (+) | |
| 40 | (Case 32) 60 year-old man, with dermatitis in areas of axillary vaults, folds, legs, lasting for two years | DB106 (+), DB124 (+) | |
| 41 | (Case 33) 42 year-old woman, with dermatitis in areas of the trunk and extremities, lasting for two years | DB106 (+), DB124 (+) | |
| 42 | (Case 35) 65 year-old woman, with dermatitis in areas of the eyelids, cheeks, trunk and extremities, lasting for 18 months | DB106 (+), DB124 (+) | |
| 43 | (Case 37) 39 year-old woman, with dermatitis in areas of the upper thighs and chest, lasting for two years | DB106 (+), DB124 (+), blue dress (+) | |
| 44 | (Case 39) 58 year-old woman, with dermatitis in areas of the axillary folds and chest, lasting for 18 months | DB106 (+), DB124 (+) | |
| 45 | (Case 40) 33 year-old woman, with dermatitis in areas of the vulva, mons pubis, inner thighs, supra pubic area, lasting for two years | DB106 (+), DB124 (+) | |
| 46 | A 2-year-old male child that “always had black velvet slippers on and blue pyjamas” presented with skin eruption. Patch test for textile dyes and European series was performed. | DB124 positive at day three | (Baldari *et al.*, 1999) |
| 47 | A 46-year-old woman developed a pruritic eruption. Dermatitis aggravated when wearing dark tights or skirt. Patch testing with European standard and a textile dyes series was performed. | DB106 and DB124 (++) at day two and four | (Pecquet *et al.*, 1999) |
| 48 | A 62-year-old housewife presented in Jan. 1992 with an itchy erythematous oedematous rash, after wearing a new navy-blue 2-piece dress made of 100% polyester for 7 h. “Three years before, she had developed a dermatitis localized to areas similar to those of the current presentation after occasional wearing over a period of 5 months.” Patch testing was conducted with standard series and textile dyes. | Positive reaction (++) to DB124 (1%) at day two and three | (Nakagawa *et al.*, 1996) |
| 49 | A 47-year-old woman developed severe eczema, after two days wearing new black polyester body. Patch testing with European standard series and textile colours and finishes series was performed. | DB106, DB124 (each ++/++) at day two and three, and own piece of garments (++/++) at day two and three | (Dejobert *et al.*, 1995) |
| 50 | A 27-year-old Hindu woman developed eczema on centre of her forehead where she daily applied a bindi spot. Patch testing with European standard series and series of dyes. | Positive patch test reading to DB124, 1% in pet. (++), DB106, 1% in pet. (+), and adhesive material from the bindi disc (++) | (Dwyer and Forsyth, 1994) |

| No. | Clinical data/case history | Patch test results/Diagnosis | Ref. |
|---|---|---|---|
| Six female patients had allergic contact dermatitis from clothing. Duration of clinical features, including erythema, edema, papules and severe pruritus, ranged from eight days to four months. Investigations included patch tests using standard series (Portuguese Contact Dermatitis Group), a textile dye series, two textile resins and pieces cut from the suspected garment (DB106, in 1% in pet.). | | TLC performed in three cases identified DB106 in one garments. Four out of six women reacted positively to DB106 (individual readings not reported). Number of exposures from < 100 to > 100 | (Lisboa *et al.*, 1994) |
| 51 | (Case 2) Years: 39 years, lesions localized around the trunk and abdomen, source of lesion was a black top. | DB106 positive | |
| 52 | (Case 3) Age: 44 years, lesions localized around the waist and tights, source of lesion were black tights. | DB106 positive | |
| 53 | (Case 4) Age: 58 years, lesions localized around the trunk and abdomen, source of lesion were black underwear. | DB106 positive | |
| 54 | (Case 6) Age: 17 years, lesions localized around the waist, thighs and legs, source of lesion were blue trousers. | DB106 positive | |
| Nine women with allergic contact dermatitis after wearing black "velvet" fabrics were patch-tested with five purified disperse dyes. Dyes were isolated from patient's textiles and incorporated in 1% petrolatum for patch testing. | | 8/9 and 9/9 textiles revealed presence of DB124 and DB106, respectively and other disperse dyes in lower yields. | (Hausen, 1993) |
| 55 | (Case 1) Age: 38 years, leggings worn "on several occasions, severe lesions on the thighs and shins" | DB106 (+++/+++), DB124 (+++/+++), and fabric (+++/+++) | |
| 56 | (Case 2) Age: 37 years, "body worn on several occasions, …skin lesions spreading to the arms and legs" | DB106 (++/+++), DB124 (++/+++) at day 1 and 3, fabric not tested | |
| 57 | (Case 3) Age: 32 years, body worn less nine month, "while performing aerobic sports, severe skin lesions where sweat dissolved the black slurry, arms involved too, disability 3 weeks" | DB106 (+++/+++), DB124 (+++/+++) at day 1 and 3, and own fabric (strongly positive) | |
| 58 | (Case 4) Age: 26 years, dress "worn sporadically" (within six month), "severe lesions on the trunk, arms, neck, decollete, emergency treatment necessary" | DB106 (-/++), DB124 (++/+++) at day 1 and 3, fabric not tested | |
| 59 | (Case 5) Age: 25 years, textile was worn "6-7 times in total. severe skin lesions" occurred around trunk and arms "after dancing the whole night" | DB106 (++/++), DB124 (++/+++) at day 1 and 3, fabric not tested | |
| 60 | (Case 6) Age: 27 years, "leggings worn several times, in December 1991; outbreak of severe skin lesions, becoming generalized" | DB106 (+++/+++), DB124 (+++/+++) at day 1 and 3, fabric not tested | |
| 61 | (Case 7) Age: 52 years ,"leggings purchased in November 1991, worn on several occasions; in January 1992, severe skin lesions on the thighs, spreading also to neck and arms, disability 2 weeks" | DB106 (++/+++), DB124 (++/+++) at day 1 and 3 and own fabric (strongly positive) | |
| 62 | (Case 8) Age: 38 years, "leggings purchased in October 1991, worn several times a week, severe skin lesions already by December 1991, burning like sunburn" | DB106 (++/++), DB124 (++/++) at day 1 and 3 and own fabric (strongly positive, lasting for weeks) | |
| 63 | (Case 9) Age: 34 years, "leggings purchased in December 1991; first lesions on the legs, in February and March 1992, worsening after wearing again; pruritus, oedema, eczema" | DB106 (+++/+++), DB124 (++/++) at day 1 and 3 and own fabric (positive) | |
| Four women with allergic contact dermatitis after wearing black "velvet" leggings and bra were reported. Patients were patch-tested with five purified disperse dyes that were isolated from patients own textiles (DB124, DB106, D. Red 1, D. Blue 1, D. Yellow 3). | | DB124 and DB106 were identified in patients' garments using TLC.<br>All four woman reacted positively to DB124 and | (Hausen *et al.*, 1991) |

| No. | Clinical data/case history | Patch test results/Diagnosis | Ref. |
|---|---|---|---|
| | | DB106 | |
| 64 | (Case 1) 53 year-old woman with massive pruritus in areas of the legs and waist after wearing black "velvet" leggings sporadically within four to five month. | DB106 (+++/+++), DB124 (+++/+++) after 24 and 72 hours | |
| 65 | (Case 2) 25 year-old woman with pruritus and eczema around the legs and buttocks after wearing some black trunks | DB106 (++/++), DB124 (++/++) after 24 and 72 hours | |
| 66 | (Case 3) 26 year-old woman with eczema around the thighs after wearing "velvet" leggings | DB106 (0/+/++/++), DB124 (0/+/++/+++) after 24, 48, 72 and 96 hours | |
| 67 | (Case 4) 41 year-old woman with eczema in areas where the bra suits, waist, buttocks after wearing "velvet" leggings and bra | DB106 (++), DB124 (+++) after 24 hours | |
| 68 | (Case 1) "A 67-year-old naval engineer with an erythematous-vesicular palmar dermatitis and itchy erythematous rashes. Rashes were more frequent when he wore overalls". Patch test with GIRDCA standard series was performed. | DB124, 1% pet. (+++) at day two and five | (Massone *et al.*, 1991) |
| 69 | (Case 2) A 50-year-old woman presented with "allergic rhinitis, asthmatic bronchitis, custom jewellery intolerance, and an itchy skin eruption for three years. She often wore blue outer garments and underwear". Patient was patched with GIRDCA standard series. | DB124, 1% pet. (+++/+++) at day two and four. | |
| Nine women with textile dye allergy were investigated from 1980 to 1983. Patch testing with European Standard Series, a textile dye series, pieces of different fabrics, and DB106 (1% in pet.) was performed. | | All nine women patch test reacted positively to DB106 and different textiles. | (Menezes Brandao *et al.*, 1985) |
| 70 | (case 1-4) From 1980 to 1981, four women, aged 36 to 50 years, showed lesions in both axillae, on the sides of the neck, upper back, and inner aspect of the arms after wearing black polyester blouses. | Four out of four women reacted positively to different fabrics (reading from + to +++), DB106 (readings from + to +++), and other dyes | |
| 71 | (Case 5) March 1982, a 57-year-old woman developed a subacute dermatitis of both axillae, the upper back and elbow flexures, shortly after she began to wear two new dark blue and black blouses. | Positive patch test reaction to several clothes (+++) and DB106 | |
| 72 | (Case 6) May 1983, a 39-year-old woman showed "a clinical picture quite similar to that of the 5 preceding patients" (case 1-5), after wearing new black blouse. | Positive patch test reaction to several clothes (+++) and DB106 ("strong") | |
| 73 | (Case 7) A 30-year-old woman presented with "typical blouse dermatitis" around the axillae. | Positive reactions to DB106 and blouses (reading not reported) | |
| 74 | (Case 8) A 41-year-old woman presented with "typical blouse dermatitis" around the axillae and neck. | Positive reactions to DB106 and several blouses and dresses | |
| 75 | (Case 9) A 41-year-old woman presented with "typical blouse dermatitis" around the axillae and waist. | Positive reactions to DB106 and several blouses and dresses | |

[#] "Id reactions describe a secondary immunologic reaction to circulating antibodies or activated T lymphocytes that are directed against microbial antigens derived from non-living organisms" (Ilkit *et al.*, 2012).

A large body of evidence resulting from human reports indicates that DB124 and its hydrolysis product DB106 consistently and repetitively elicit positive reactions in diverse patch tests, in several clinical settings. Human patch test data comprise studies with consecutive or selected dermatitis patients, performed in dermatological clinics analysing the number of patients sensitised to DB124 and/or 106 compared to all patients tested in a certain time-period. In studies with unselected, consecutive dermatitis patients patch testing is generally more standardised. In contrast, for a selected (specific) patient or for worker groups, usually targeted patch testing with special test series is performed. Data for consecutive patients vary between 0.2% and 7.3% positively patch-tested subjects for DB124, and 0.2% and 4.2% positive reactions to DB106, among all patients analysed. Selected dermatitis patients patch-tested positively show frequencies between 1% and more than 50% for both, DB124 and DB106. Among all patch test data available, five studies reported skin irritant reactions in a few tested subjects after treatment with DB124 and DB106. Just as the same number of human patch test studies indicate concomitant reactions between DB124 and DB106.

Furthermore, numerous case reports have been published indicating allergic reactions in patients after wearing clothing containing DB124 and DB106. Reports support that DB124/106 cause allergic contact dermatitis to textiles, especially at sites where garments fit strongly, at areas of friction and sweating, facilitating allergens to migrate out of the textile.

However, in general patch test data or case reports, which aim to determine whether there is a pre-existing sensitization, do not allow for an estimation of exposure levels. Based on exposure models of textile chemicals migrating from fabrics, considering wearing conditions of garments (friction, temperature, and sweating), it has been assumed that humans are externally exposed with dyes from garments with concentrations between 1 ng and 10 µg of dye per $cm^2$ (Heinemann, 2000; Platzek, 2001). Nevertheless, this analysis does not consider textiles not dyed according to the state of the art, for which a higher release of dye is expected. Furthermore, data for DB124 and DB106 exposure from textiles are not available to the DS.

Altogether, most human studies reveal a relatively high frequency of occurrence of DB124 and DB106 skin sensitisation. In several studies, both disperse blue dyes elicit the highest number of positive reactions among the textile chemicals tested.

### 10.7.3 Other studies relevant for skin sensitisation

Table 12 Animal study on skin sensitisation using a mixture of DB124 and DB106

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance, | Dose levels duration of exposure | Results | Reference |
|---|---|---|---|---|---|
| **Supporting studies** | | | | | |
| "Sensitive mouse lymph node assay" (SLNA)<br><br>non-guideline study<br>No information on GLP<br><br>Study reliability 2: Reliable with restrictions<br><br><u>Deviations to OECD TG 429:</u><br>Intradermal injection: Day 1<br>Topical application: Day 6-8, instead of monophasic application;<br>Endpoint analysis: Day 9, instead of two days without treatment;<br>Analysis of lymph node cell number ($SI_n$) after excision of lymph nodes, using automated cell counter;<br>Determination of $^3$HTdR incorporation in lymphocytes after 24 h of cell culture ($SI_p$) was analysed;<br>Individual body weights at start of dosing and at scheduled kill not reported;<br>adjuvant was used | Mouse, BALB/c, female<br><br>n=3/dose | **Mixture of DB124 and 106,** composition not reported<br><br><u>Vehicle</u> for topical application: DMF<br><br><u>Purity:</u> No information | Intradermal injection: 2% in saline/Freund's complete adjuvant (FCA) (1:1)<br><br>Topical application: 10% in DMF<br><br>Results of stimulation index (SI), defined by authors:<br>$SI_n$: 2.91<br>$SI_p$: 2.69<br>$SI_{total}$ ($SI_n$ x $SI_p$) = total LN response: 7.83<br><br>A chemical was regarded as positive (a sensitiser) by the authors if $SI_{total} \geq 3$. | **Positive**<br><br>Determi-nation of potency not possible **<br><br>** | (Ikarashi *et al.*, 1996) |

**According to the Guidance on the Application of the CLP Criteria, Version 5.0; Table 3.5

Ikarashi et al., 1996 performed a "sensitive mouse lymph node assay" addressing the sensitising capacity of a mixture of DB124 and DB106. The authors applied an intradermal injection before topical application with one concentration of several chemicals in mice. Proliferation of lymphocytes was determined after cell isolation from lymph nodes and 24 hours of cell culture following $^3$HTdR incorporation in lymph cells. Results show that a mix of DB124/106 causes increased lymph node cell proliferation in this study design. The test was not performed according to any OECD test guideline. Furthermore, results were obtained with a mixture of DB106 and DB124 and therefore need to be evaluated with care. For a mixture the cut-off in the mouse LLNA should be seen as a threshold for identification of a sensitiser rather than as a threshold for sensitisation (section 3.4.3.2., ECHA 2017). In addition, SCLs are set on the basis of testing of the substance and never on the basis of testing of a mixture containing the sensitising substance (see CLP Annex I, Table 3.4.5). Due to the available animal studies performed with the single substances DB124 and/or DB106, this study is precluded from further assessment.

Sonnenburg and colleagues published a human in vitro assay, named loose-fit coculture-based sensitization assay (LSCA) (Sonnenburg *et al.*, 2012). This assay shows that treatment with DB124 or with DB106 activates CD86 expression of dendritic cell-related cells (Key event 3 of AOP) compared to vehicle control. Nevertheless, this study was not performed according to internationally adopted in chemico/in vitro tests (listed in Table R.7.3-3, Endpoint specific guidance, version 6.0-July 2017) and is precluded from further assessment.

### 10.7.4 Short summary and overall relevance of the provided information on skin sensitisation for Disperse Blue 124

In summary, reliable animal data give strong evidence that DB124, which is almost certainly transformed into DB106 while penetrating the outer human skin, causes skin sensitisation in vivo. During a well-documented local lymph node assay without obvious deviations from OECD TG 429, DB124 hydrolysis product DB106 induces skin sensitisation resulting in very low EC3-values (Experiment A: 0.012% and Experiment B: 0.017%; (Betts *et al.*, 2005)) indicating that DB106 is an extreme sensitiser. Furthermore, in a modified GPMT performed similar to OECD TG 406, at least 66% of the exposed guinea pigs reacted positively after treatment with DB124, using a concentration of 0.2% for intradermal induction (Hausen and Sawall, 1989). This study shows a strong potency of skin sensitisation of DB124, but an extreme potency cannot be excluded as no induction concentration of ≤0.1 % was tested. During another GPMT (similar to OECD TG 406) 100% of tested animals showed positive reactions after DB106 exposure (Hausen and Menezes Brandao, 1986). However, the authors used a concentration of 1.5% for intradermal induction. Therefore results should be taken with care and the possibility of DB106 having a strong or extreme sensitising potency cannot be excluded from this study. In addition, Ahuja et al. 2010 demonstrated in a "biphasic" LLNA that DB124 (and DB106) cause skin sensitisation. Very low concentrations (0.003%) of DB124 (or DB106) induced a significant increase in cell-count compared to the vehicle control. However, the experimental design deviates from OECD TG 429 and therefore, evaluation of the skin sensitisation potency was not possible.

In a "sensitive mouse lymph node assay" a mixture of DB124 and DB106 induced skin sensitisation. However, this study was not performed according to any OECD testing guideline and results are obtained for a mixture of both dyes. Due to the available animal studies performed with the substance DB124 and/or DB106 alone, this study is not considered for further assessment.

A huge human database proves DB124 to be common sources of textile dye allergic contact dermatitis. Results of human patch test studies for consecutive and selected dermatitis patients reveal frequencies between 0.2% and 7.3% positively patch-tested subjects for DB124, and 0.2% and 4.2% positive reactions to its hydrolysis product DB106, among all patients analysed. Selected dermatitis patients, patch-tested positively show frequencies between 1% and more than 50% for both, DB124 and DB106. Furthermore, a huge number of case reports indicate allergic reactions to DB124 and DB106 after wearing clothing containing DB124 and DB106.

Altogether, most human studies reveal a relatively high frequency of occurrence of DB124 and DB106 skin sensitisation. In several studies, both disperse blue dyes elicit the highest number of positive reactions among the textile chemicals tested. Notably, DB124 and DB106 were reported as "common causes of textile dermatitis" (Pratt and Taraska, 2000). Nevertheless, available human data are insufficient for a reliable estimation of exposure levels (and to conclude on potency/SCL setting).

Additionally, in an in vitro assay DB124 (and DB106) activated CD86 expression in dendritic cells, representing a main reaction in key event 3 of AOP for skin sensitisation. This assay was not performed according to any in chemico/in vitro tests with regulatory validation and acceptance (listed in Table R.7.3-3, Endpoint specific guidance, version 6.0-July 2017) and therefore is excluded for further assessment.

Finally, the NICNAS published an assessment of DB124 and DB106. Unpublished study reports of notifiers were summarised, giving evidence of skin sensitisation in a Buehler test and a GPMT (OECD TG 406) conducted with DB106. Another study report for a GPMT performed with DB124 was not sighted by NICNAS. However, notifiers' study reports were not available to the DS and could not be considered for further evaluation. NICNAS concluded that DB124 and DB106 are "very strong sensitisers from animal studies and human data" (NICNAS, 2015).

### 10.7.5 Comparison with the CLP criteria

In Table 13, relevant experiments in animal and human data are compared with CLP criteria, as laid down in the guidance of the Application of the CLP criteria. Only studies with at least reliability 2 are included.

Table 13: Comparison of human and animal data for skin sensitisation of DB124 with CLP criteria

| Reference(s) | Criteria acc. to CLP regulation, as laid out in (ECHA, 2017) | Results | Resulting Classification |
|---|---|---|---|
| **Animal data** | | | |
| LLNA<br><br>(Betts *et al.*, 2005) | Skin Sens. 1A:<br>EC3 > 0.2 - ≤ 2%, Strong sensitiser<br>EC3 ≤ 0.2%, Extreme sensitiser<br><br>Skin Sens. 1B:<br>EC3 > 2%, Moderate sensitiser | EC3 = 0.017% | **Skin Sens. 1A**<br><br>**Extreme potency** |
| GPMT<br><br>(Hausen and Sawall, 1989) | Skin Sens. 1A - Extreme potency:<br>≥ 60% sensitised guinea pigs at ≤ 0.1% intradermal induction<br>Skin Sens. 1A - Strong potency:<br>≥ 30 - < 60% guinea pigs sensitised at ≤ 0.1% intradermal induction or<br>≥ 60% guinea pigs sensitised at > 0.1 - ≤ 1.0% intradermal induction<br><br>Skin Sens. 1B - Moderate potency:<br>≥ 30 - < 60% guinea pigs sensitised at > 0.1 - ≤ 1.0% intradermal induction or<br>≥ 30% guinea pigs sensitised at > 1.0% intradermal induction | ≥ 60% of guinea pigs responded at 0.2% intradermal injection. | **Skin Sens. 1A**<br><br>**Strong potency**<br><br>Extreme potency cannot be excluded |
| GPMT<br><br>(Hausen and Menezes Brandao, 1986) | Skin Sens. 1A - Extreme potency:<br>≥ 60% sensitised guinea pigs at ≤ 0.1% intradermal induction<br>Skin Sens. 1A - Strong potency:<br>≥ 30 - < 60% guinea pigs sensitised at ≤ 0.1% intradermal induction or<br>≥ 60% guinea pigs sensitised at > 0.1 - ≤ 1.0% intradermal induction<br><br>Skin Sens. 1B - Moderate potency:<br>≥ 30 - < 60% guinea pigs sensitised at > 0.1 - ≤ 1.0% intradermal induction or<br>≥ 30% guinea pigs sensitised at > 1.0% intradermal induction | 100% of guinea pigs responded at 1.5% intradermal injection. | **Skin Sens. 1B**<br><br>**Moderate potency**<br><br>Extreme potency cannot be excluded |
| Other LLNA<br><br>(Ahuja *et al.*, 2010) | No criteria for sub-categorisation based on modified LLNA method | Treatment with DB124 (0.003%) results in significant cell count increase, DB124 and DB106 show similar sensitising potencies under testing design in mice. | **Skin Sens. 1** (not suitable for sub-categorisation) |

| Reference(s) | Criteria acc. to CLP regulation, as laid out in (ECHA, 2017) | Results | Resulting Classification |
|---|---|---|---|
| **Human data** | | | |
| Dermatitis patients (unselected, consecutive)<br><br>(Balato *et al.*, 1990; Bosco *et al.*, 2016; Giusti *et al.*, 2003; Lazarov *et al.*, 2002; Li, 2010; Mancuso *et al.*, 1999; Manzini *et al.*, 1998; Seidenari *et al.*, 2005; Uter *et al.*, 2003; Wentworth *et al.*, 2014) | Skin Sens. 1<br>Relatively low/moderate frequency (< 1.0%) and relatively low exposure or<br>Relatively high frequency (≥ 1.0%) and relatively high exposure<br><br>Skin Sens. 1A<br>Relatively high frequency (≥ 1.0%) and relatively low exposure<br><br>Skin Sens. 1B<br>Relatively low/moderate frequency (< 1.0%) and relatively high exposure | Frequency from "relatively low to "relatively high"<br><br>10/13 studies reveal a relatively high frequency<br><br>Exposure unclear | **Skin Sens. 1**<br>(not suitable for sub-categorisation) |
| Selected dermatitis patients<br><br>(Balato *et al.*, 1990; Bauer *et al.*, 2004; Dooms-Goossens, 1992; Giusti *et al.*, 2002; Heratizadeh *et al.*, 2017; Isaksson *et al.*, 2015; Koopmans and Bruynzeel, 2003; Lazarov and Cordoba, 2000; Lisi *et al.*, 2014; Pratt and Taraska, 2000; Ryberg *et al.*, 2014; Seidenari *et al.*, 1991; Slodownik *et al.*, 2011; Uter *et al.*, 2001; Wentworth *et al.*, 2012) | Skin Sens. 1<br>Relatively low/moderate frequency (< 2.0%) and relatively low exposure or<br>Relatively high frequency (≥ 2.0%) and relatively high exposure<br><br>Skin Sens. 1A<br>Relatively high frequency (≥ 2.0%) and relatively low exposure<br><br>Skin Sens. 1B<br>Relatively low/moderate frequency (< 2.0%) and relatively high exposure | Frequency from "relatively low to "relatively high"<br><br>14/15 studies revealed a relatively high frequency<br><br>Exposure unclear | **Skin Sens. 1**<br>(not suitable for sub-categorisation) |

Reliable animal data give strong evidence that DB124 and its hydrolysis product DB106 cause skin sensitisation in vivo. A LLNA according to OECD TG 429 of Betts and colleagues (Betts *et al.*, 2005) proves that DB106 acts as an extreme sensitiser. Furthermore, modified GPMT performed similar to OECD TG 406, indicate that DB124 and DB106 act as skin sensitisers with a strong and moderate potency, respectively (strong potency: > 0.1 - ≤ 1.0% intradermal induction and ≥ 60% animals sensitised, moderate potency: > 1.0% intradermal induction and ≥ 30% animals sensitised, Table 3.7, ECHA 2017). For both modified GPMT performed with DB106 and DB124 the incidences of sensitised guinea pigs (66% for DB124 and 100% for DB106) and the concentration of DB106 used for intradermal induction (1.5%) are very high and results should be taken with care. Because for both dyes concentrations for intradermal injection ≤ 0.1% were not tested during GPMT, an extreme sensitising potency of DB124 and DB106 cannot be excluded.

In a "biphasic LLNA" it is shown that DB106 and DB124 cause skin sensitisation and with comparable potency (Ahuja *et al.*, 2010). However, strong deviations from OECD testing guidelines with respect to the experimental procedure preclude sub-categorisation according to CLP regulation. Notably, already a very low concentration of DB124 (0.003%) resulted in a significant lymph cell response in this "biphasic LLNA", supporting the observation of a significant sensitising effect of DB124.

Available animal data allow classification of DB124 as skin sensitiser with sub-categorisation as Skin Sens. 1A, as laid down in the CLP regulation (Table 3.4.3). Based on the very low EC3 value obtained from (Betts et al, 2005), and because DB106 is a respectable hapten of DB124, DB124 is characterised as an extremely potent skin sensitiser. As a consequence and in line with Table 3.9 of the ECHA Guidance on the Application of the CLP criteria, an SCL of 0.001% (w/v) should be assigned.

There is a substantial body of evidence that DB124 and DB106 are common sources of textile dye allergic contact dermatitis. The majority of patch test studies reveal a relatively high frequency of occurrence of skin sensitisation for DB124 and DB106 in consecutive and selected dermatitis patients (Section 3.4.2.2.3.1, Table 3.2 of the Guidance on the Application of CLP criteria (ECHA 2017) (i.e., ≥ 1.0% for dermatitis patients (unselected/consecutive) or ≥ 2.0% for selected dermatitis patients), which could justify sub-categorisation 1A. Patch test data and case reports do not give information about exposure levels of DB124 and DB106 and besides, exposure data for both dyes are not available to the DS.

In summary, all available studies from animals and humans provide comprehensive data that DB124 acts as skin sensitiser. Furthermore, data are sufficient for sub-categorisation as 1A, according to section 3.4.2.2.1.4 of the CLP regulation. Results suggest that DB124 should be rated an extreme sensitiser assuming that DB124 has the same potency as DB106 supporting an SCL setting of 0.001%.

### 10.7.6 Conclusion on classification and labelling for skin sensitisation

In conclusion, the DS proposes to classify Disperse Blue 124 as an extremely potent skin sensitiser with sub-categorisation as **Skin Sens. 1A (H317 - May cause an allergic skin reaction)** and an SCL of 0.001% (w/v).

## 10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

## 10.9 Carcinogenicity

Hazard class not assessed in this dossier.

## 10.10 Reproductive toxicity

Hazard class not assessed in this dossier.

## 10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

## 10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

## 10.13 Aspiration hazard

Hazard class not assessed in this dossier.

# 11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

# 12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

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# 14 ANNEXES

## 14.1 Annex I

### 14.1.1 Mouse local lymph node assay (LLNA) (Betts *et al.*, 2005), key study

**Study reference:**

Betts C.J., Dearman R.J., Kimber I., and Maibach H.I. (2005): Potency and risk assessment of a skin-sensitizing disperse dye using the local lymph node assay. Contact dermatitis 52 (5), 268-272. DOI: 10.1111/j.0105-1873.2005.00578.x

**Detailed study summary and results:**

Betts and colleagues (Betts *et al.*, 2005) used adult male CBA/Ca strain mice (Harlan, Bicester, Oxfordshire, UK), eight to 12 weeks of age, to perform a LLNA according to the standard protocol described in (Kimber and Basketter, 1992). Disperse Blue 106 (DB106), 87 % pure, was supplied by the Ecological and Toxicological Association of Dye and Organic Pigments Manufacturers (ETAD) via Yorkshire Chemicals PLC, Leeds. Dinitrochlorobenzene (DNCB, CAS: 97-00-7), 98.9% pure, was obtained from Sigma Chemicals (Poole, Dorset, UK).

"Initial experiments were conducted to determine whether DB106 has inherent skin sensitisation potential. For this purpose, a standard LLNA was performed with three relatively high concentrations of the test chemical: 1%, 3% and 10% formulated in DMF vehicle, incorporating the highest non-toxic concentration achievable in this vehicle" (Table 14; Experiment 1). "These data demonstrate clearly that DB106 possesses skin-sensitising activity, with all concentrations of chemical stimulating vigorous LNC proliferation." The authors suppose that with the used dose range maximal proliferation has been achieved resulting in a "lack of a dose–response relationship".
Furthermore, the authors investigated different vehicle and found out that "exposure of control mice to the vehicle DMSO provoked somewhat higher levels of thymidine incorporation than those induced by application of DMF vehicle". However, "despite the increase in background thymidine incorporation the authors observed that topical application of DB106 dissolved in DMSO stimulated marked proliferative responses".
For the main LLNA groups of mice (n = 4) "were exposed topically on the dorsum of both ears to 25 µl of various concentrations" (0.005–0.25%) of DB106 or "to the same volume of vehicle (DMSO) alone, daily for three consecutive days". Measured concurrently was the sensitizing potency of DNCB (0.01–0.25% in DMSO). "Five days after the initiation of exposure, all mice were injected intravenously via the tail vein with 20 µCi of ($^{3}$H)-methyl thymidine ($^{3}$HTdR) in 250 µl of phosphate-buffered saline (PBS). Five hours later, mice were killed, and the draining auricular lymph nodes were excised and pooled for each experimental group. A single-cell suspension of LNCs was prepared by gentle mechanical disaggregation through 200-mesh stainless-steel gauze. Cells were washed twice with an excess of PBS and precipitated in 5% trichloroacetic acid (TCA) at 4 °C" for approximately 12 hours. Then, "pellets were resuspended in 1 ml of 5% TCA and transferred to 10 ml of scintillation fluid (...). Incorporation of $^{3}$HTdR was measured by β-scintillation counting as disintegrations per minute (dpm) per node for each experimental group. In each case, a stimulation index (SI) relative to the concurrent vehicle-treated control value was derived." EC3-values (SI of 3 relative to concurrent vehicle treated controls) were calculated by linear interpolation of dose–response data. Results are shown in Table 14.

Table 14: Local lymph node assay dose–responses to Disperse Blue 106 and DNCB

| | Disperse Blue 106 [dpm/node (SI)] | | | DNCB [dpm/node (SI)]† |
|---|---|---|---|---|
| **Concentration (% w/v)** | **Experiment 1*** | **Experiment 2†** | **Experiment 3†** | |
| 0 | 582 (1) | 924 (1) | 885 (1) | 816 (1) |
| 0.005 | Not done | Not done | 753 (0.9) | Not done |
| 0.01 | Not done | 2352 (2.6) | Not done | 1991 (2.4) |
| 0.025 | Not done | 5031 (5.5) | 4561 (5.2) | 3458 (4.2) |
| 0.05 | Not done | 6073 (6.6) | 8291 (9.4) | 5981 (7.3) |
| 0.1 | Not done | 7590 (8.2) | 8071 (9.1) | 10085 (12.4) |
| 0.25 | Not done | 8483 (9.2) | Not done | 11971 (14.7) |
| 1 | 7889 (13.6) | Not done | Not done | Not done |
| 3 | 9283 (16.0) | Not done | Not done | Not done |
| 10 | 8274 (14.2) | Not done | Not done | Not done |

*DMF vehicle
†DMSO vehicle

### 14.1.2 "Biphasic" LLNA (Ahuja, 2010; Ahuja *et al.*, 2010)

**Study reference:**

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

Ahuja V. (2010): Investigation of the sensitisation potential of various textile dyes using a biphasic mice local lymph node assay (LLNA) and an in vitro loose-fit coculture-based sensitisation assay (LCSA). Dissertation, FU Berlin

**Detailed study summary and results:**

(Ahuja *et al.*, 2010) conducted a LLNA including a "biphasic or sensitization-challenge protocol". Therefore female BALB/c mice (age: seven weeks at the start of the experiment) were shaved over a surface of approximately 2 $cm^2$ on their backs and treated once daily from days one to three with 50 µl of test solution (n = 7–10). DB106 and Disperse Blue 124 (DB124) were purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany. "Animals remained untreated on days four to14. On days 15 to17, mice were treated with 25 µl of the test solution on the dorsum of both ears. Mice were killed on day 19 […], lymph nodes were prepared and various end points analysed. The results were compared to a control group (n = 20) treated with the vehicle alone." The end points investigated included lymph node weight, ear thickness (mm), and ear biopsy weight. Therefore, "the draining auricular lymph nodes were excised and weighed (mg)". Ear thickness (mm) was measured with a spring-loaded micrometer and a section was taken from both ears with a punch of 6 mm diameter and weighed (mg)". Furthermore, the authors analysed lymph node cellularity. The "single cell suspension from a single lymph node was prepared by gentle mechanical disaggregation through stainless steel mesh filter […] and counted (million per lymph node) using an automated cell counter". Results are summarized in

Table 15. For phenotypic determination of lymphocyte subsets, authors stained cells using fluorochrome-conjugated antibodies against CD8a, CD4, CD45R/B220, CD19, CD69, and CD1A. Fluorescence was measured by flow cytometry. Results show a significant decrease in CD4+ and CD8+ cells and an increase in CD19+, CD45+, CD45+/1A+, and CD4+/CD69+ cells after treatment with DB124 and DB106, compared to vehicle control.

Table 15: Cell-count increase, ear thickness, and ear-punch weight measurement (% of vehicle control) measured by biphasic LLNA

| Dye | Cell-count increase | | | | | | Ear thickness | | | | | | Ear-punch weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration (%) | | | | | | | | | | | | | | | | | |
| | **30** | **10** | **3.0** | **0.3** | **0.03** | **0.003** | **30** | **10** | **3.0** | **0.3** | **0.03** | **0.003** | **30** | **10** | **3.0** | **0.3** | **0.03** | **0.003** |
| DB106 | 174 | n. d. | 124 | 82 | 79 | 37 | 26 | n. d. | 13 | 17 | 9 | - | 22 | n. d. | 15 | 17 | 12 | 4* |
| DB124 | n. d. | 147 | 132 | 116 | 79 | 21 | n. d. | 22 | 26 | 30 | 4 | 4 | n. d. | 21 | 22 | 28 | 4* | 4* |

- Concentrations not tested in LLNA

* No significant increase at $p<0.05$ (t-test) between vehicle control and treated animals

### 14.1.3 Method developed from the FCAT and the guinea pig maximization test (Hausen and Sawall, 1989)

**Study reference:**

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

**Detailed study summary and results:**

“Sensitization was carried out by a method developed from the FCAT and the guinea pig maximization test.” DB124 (Yorkshire Chemicals Ltd, Leeds, England) was purified “on preparative thin-layer chromatography (TLC) plates, 0.5 mm thick, silica gel with UV-indicator for 254 and 366 nm” and an eluent of chloroform-methanol (100+3). “After sufficient amounts of the dye had been obtained, the purity was again proven by analytical TLC […].”

Ten female albino guinea pigs of the Pirbright white strain were used for each substance. “An emulsion containing 15 mg of the dye dissolved in 4 ml FCA and emulsified with 4 ml physiologic saline was prepared”, corresponding to 0.2% (w/v) dye emulsion. Six intradermal injections of 0.1-0.15 ml of this emulsion were given in a semicircular arc on the clipped and shaved shoulder area (4 x 6 cm) from left to right, in such a way that the whole amount of the emulsion was used up for the ten animals (including common losses). This procedure was repeated on the 5th and on the 9th day, leaving a gap of two to three cm between the rows of injection. Thus, each animal received a total of approximately 4.5 mg during the whole sensitization procedure. […] Eleven days after the end of the sensitization procedure, open epicutaneous elicitation was done by application of 0.05 ml of the dye dissolved in acetone in a subirritant concentration to the right clipped and shaved flank of the animals”, using a concentration of 1%. The reactions were read after 24 h, 48 h and 72 h (

Table 16). One day before challenge of the sensitized animals a primary irritation study was performed. “Ten guinea pigs were treated with an emulsion of 4 ml FCA and 4 ml physiologic saline in the same manner and at the same intervals as described above, but without the effective dyes. This group was used to determine patterns of irritation. Three different concentrations (10%, 3%, and 1%) of the dye were applied to the flank of all ten animals. The results were read after 24 h.” The irritation threshold of all tested dyes was higher than 10%.

Table 16: Results of sensitizing with disperse (D.) dyes, using the FCA and GPMT

| | | 24 h | | | | | 48h | | | | | 72 h | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sensitised with** | **Challenged with** | **+++** | **++** | **+** | **(+)** | **-** | **+++** | **++** | **+** | **(+)** | **-** | **+++** | **++** | **+** | **(+)** | **-** |
| D. Yellow 3 | D. Yellow 3 | - | - | - | 5 | 5 | - | - | - | 8 | 2 | - | - | - | 3 | 7 |
| D. Blue I | D. Blue I | 1 | 3 | 3 | 2 | 1 | 3 | 2 | 4 | 1 | - | 2 | 6 | 2 | - | - |
| D. Orange 3 | D. Orange 3 | - | - | - | 2 | 8 | - | - | - | 7 | 3 | - | - | 2 | 6 | 1 |
| **DB124** | **DB124** | **-** | **5** | **2** | **2** | **1** | **2** | **-** | **1** | **3** | **3** | **-** | **5** | **2** | **1** | **2** |
| D. Red 1 | D. Red 1 | - | - | - | 8 | 2 | - | - | - | 4 | 6 | - | - | 1 | 7 | 2 |
| D. Blue 3 | D. Blue 3 | - | - | 1 | 1 | 6 | - | 4 | 1 | 2 | 3 | - | 4 | - | 3 | 3 |

+++ Erythema with intense swelling, infiltration and exudation spreading over the test area
++ Erythema and swelling restricted to the test area
(+) discrete erythema covering more than half of the test area and considered as a very weak but positive,
- No reaction

### 14.1.4 Guinea pig maximisation test, slightly modified FCA method (Hausen and Menezes Brandao, 1986)

**Study reference:**

Hausen B.M. and Menezes Brandao F. (1986): Disperse blue 106, a strong sensitizer. Contact dermatitis 15 (2), 102-103. DOI: 10.1111/j.1600-0536.1986.tb01294.x

**Detailed study summary and results:**

Experimental sensitization was carried out using a slightly modified FCA method (Hausen and Schmalle, 1985). DB106 was supplied by the Italian manufacturer as well as by a German chemical company and was purified using preparative thin-layer chromatography plates (solvent system ethyl acetate-chloroform (4+1). The threshold of irritation was determined at a concentration of 10 % (solvent acetone).
Ten guinea pigs (Pirbright white strain) were intradermal injected with 6 x 0.1 ml of an emulsion containing the dye dissolved in 3 ml FCA and 3 ml of N. saline, in a semicircular arc in the shoulder area from the left to the right paw on days one, five, and nine, according to (Hausen and Schmalle, 1985). The authors used 9 mg of the pure dye per animal for the whole procedure (resulting in a 1.5% (w/v) dye emulsion for intradermal induction). Control animals were treated in the same manner with an emulsion of FCA and equal amounts of saline alone. Challenge was performed on the 11th day after the end of the sensitisation procedure by topical application of subirritant doses of the dye. Readings were performed after 24, 48, and 72 hours.
“The reactions obtained on challenge with 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 tests with an additional dilution (0.001%) were performed on the opposite flank. Results are shown in Table 17 (“one animal died during the experiment due to other causes”).

Table 17: Results of sensitization with DB106 using a slightly modified FCA method (challenge concentration 0.001%)

| | **+++** | **++** | **+** | **(+)** | **-** |
|---|---|---|---|---|---|
| **24 h** | 6 | 3 | - | - | - |
| **48 h** | 7 | 2 | - | - | - |
| **72 h** | 6 | 3 | - | - | - |