CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl]sulphonyl] phenyl]azo]naphthalene-2,7-disulphonate; [1]

and

Reaction products of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid, coupled twice with diazotized 2-[(4-aminophenyl)sulfonyl]ethyl hydrogen sulfate, sodium salts; [2]

and

disodium 4-amino-5-hydroxy-3,6-bis{[4-(vinylsulfonyl) phenyl]diazenyl}naphthalene-2,7-disulfonate; [3]

EC Number: 241-164-5 [1]
- [2]
- [3]

CAS Number: 17095-24-8 [1]
- [2]
100556-82-9 [3]

Index Number: 607-RST-VW-Y

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Contents

1 IDENTITY OF THE SUBSTANCES

The substance tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl]sulphonyl]-phenyl]azo]naphthalene-2,7-disulphonate [1] was registered in single joint submission as a mono-constituent substance using the numerical identifier EC-No. 241-164-5 and CAS-No. 17095-24-8. However, the substance is described more appropriately as an UVCB substance. Therefore, ECHA initiated discussions with all concerned registrants. The outcome of these discussions is a split of the joint registration. There will be seperate registrations of:

- [1] tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl] sulphonyl]phenyl]azo]naphthalene-2,7-disulphonate; EC-No. 241-164-5, CAS-No. 17095-24-8
- [2] Reaction products of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid, coupled twice with diazotized 2-[(4-aminophenyl)sulfonyl]ethyl hydrogen sulfate, sodium salts; List no 701-365-5.

Substance [1] is a mono-constituent substance while substance [2] is a UVCB substance having substance [1] as a constituent in relevant concentrations. Information on the identity of the tested substances (UVCB versus mono-constituent substance) can be found in the confidential Annex. The balance of the registered substances will fall under the registration of substance [2] while only a minority is identified as substance [1].

The name “Reactive Black 5” is commonly used in the market to describe a commercially available dye, which was identified as a mono-constituent substance with the chemical name and identifiers of substance [1], i.e. tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl] sulphonyl]-phenyl]azo]naphthalene-2,7-disulphonate; and the numerical identifiers EC no 241-164-5 and CAS no 17095-24-8. Though, as described above, the registered substances using these identifiers are mostly UVCB substances which contain subtance [1] in relevant quantities.

Therefore the Name “Reactive Black 5” was formerly used interchangebly for the monoconstituent substance [1] and the UVCB substance [2]. As the manufacturers of „Reactive Black 5“ did not differentiate between the monoconstituent and the UVCB substance as it was not relevant for marketing purposes it is impossible to retroactively identifiy which substance was refereed to when the name “Reactive Black 5” or any of the associated trade names was used in studies or the literature. Thus, it was decided to also use the name „Reactive Black 5 (RB5)” for both the mono-constituent and the UVCB substance in this CLH report. However in certain cases, as with the radioactive labbelled “Reactive Black 5” probably the monocosntituent substance was addressed. In most other cases, were comercially available products or industrial grade substances are addressed most likely the UVCB substance [2] was on hand and was tested.

Disodium 4-amino-5-hydroxy-3,6-bis{[4-(vinylsulfonyl) phenyl]diazenyl}naphthalene-2,7-disulfonate [3] will be formed under basic conditions from Tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl] sulphonyl]phenyl]azo]naphthalene-2,7-disulphonate [1]. The Bis-Vinyl substance [3] is considered to be more reactive compared to Tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl] sulphonyl]phenyl]azo]naphthalene-2,7-disulphonate [1] as it is able to undergo 1,4 addition reactions, like Michael additions.

To the current knowledge, the mechanistic pathway of skin and respiratory sensitisation includes four molecular key events. The first one consists of the covalent binding of the substance to proteins, the formation of a hapten.

The ability to bind to proteins requires a functional group capable of forming covalent bonds with amino acid residues present in the proteins. Skin and respiratory sensitisers thus either need to be themselves electrophilic or require conversion into electrophilic species in order to react with nucleophilic amino acids. For “Reactive Black 5”, it is assumed that the activation of the sulphoxyethylsulfonylphenyl group yielding the vinylsulphonylphenyl group is a prerequisite and protein binding occurs, for instance, via Michael-addition of the vinyl group to cysteine residues. This transformation to an electrophile is in fact

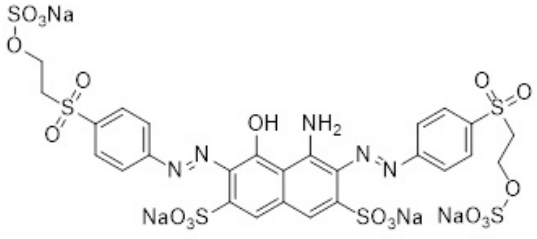
the mechanistic basis of the textile dyeing process, where the activated bis-vinyl form reacts with amino acid residues of cellulose fibres and thus forms a covalent and stable link to the textile.

In good agreement with this, only the activated bis-vinyl form triggers profiler alerts in the OECD QSAR Toolbox (version 4.4) for: a) protein binding by OECD and by OASIS through Michael addition of polarised alkenes forming polarised alkenesulfones and b) protein binding potency by GSH “highly reactive”.

It is therefore considered valid to include the activated substance [3] in this assessment and treat the three substances as a group with principally the same properties.

1.1 Name and other identifiers of the substance Tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl]sulphonyl] phenyl]azo]naphthalene-2,7-disulphonate [1]

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)	tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl]sulphonyl] phenyl]azo]naphthalene-2,7-disulphonate
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	241-164-5
EC name (if available and appropriate)	tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy) ethyl]sulphonyl]phenyl]azo]naphthalene-2,7-disulphonate
CAS number (if available)	17095-24-8
Other identity code (if available)	-
Molecular formula	$C_{26}H_{21}N_5Na_4O_{19}S_6$
Structural formula	
SMILES notation (if available)	[Na+].[Na+].[Na+].[Na+].NC1=C(\N=N\C2=CC=C(C=C2)S(=O)(=O)CCOS([O-])(=O)=O)C(=CC2=C1C(O)=C(\N=N\C1=CC=C(C=C1)S(=O)(=O)CCOS([O-])(=O)=O)C(=C2)S([O-])(=O)=O)S([O-])(=O)=O
Molecular weight or molecular weight range	991,8 $g \cdot mol^{-1}$
Information on optical activity and typical ratio of (stereo) isomers (if	-

applicable and appropriate)	
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)	not relevant for the entry

1.1.1 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 (CLP)**	**Current self-classification and labelling (CLP)**
tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl]sulphonyl]phenyl]azo]naphthalene-2,7-disulphonate; CAS no 17095-24-8, EC no 241-164-5	-	-	**Skin Sens. 1; H317** **Resp. Sens. 1; H334** Acute Tox. 3; H302 Skin Irrit. 2; H315 Eye Irrit.2; H319 Aquatic Chronic 3;H412

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 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
-				

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 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
-					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	**Purity**	**Impurities and additives (identity,%, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
-				

1.2 Name and other identifiers of the substance 'Reaction products of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid, coupled twice with diazotized 2-[(4-aminophenyl)sulfonyl]ethyl hydrogen sulfate, sodium salts' [2]

Table 6: 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)	Reaction products of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid, coupled twice with diazotized 2-[(4-aminophenyl)sulfonyl]ethyl hydrogen sulfate, sodium salts
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	-
EC name (if available and appropriate)	-
CAS number (if available)	-
Other identity code (if available)	-
Molecular formula	Not applicable
Structural formula	Not applicable
SMILES notation (if available)	Not applicable
Molecular weight or molecular weight range	Not applicable
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	Reaction of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid and two equivalences of diazotized 2-[(4-aminophenyl)sulfonyl]ethyl hydrogen sulfate in closed batch processes.
Degree of purity (%) (if relevant for the entry in Annex VI)	not relevant for the entry

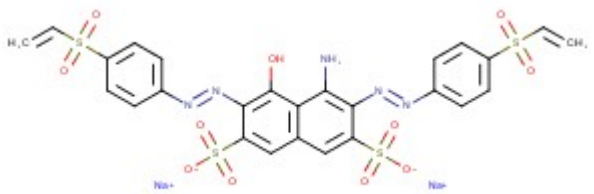
1.2.1 Composition of the substance

Table 7: 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 (CLP)**	**Current self-classification and labelling (CLP)**
tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy) ethyl]sulphonyl]phenyl]azo]naphthalene-2,7-disulphonate	Please refer to the confidential Annex		
Please refer to the confidential Annex			

1.3 Name and other identifiers of the substance 'Disodium 4-amino-3,6-bis{[4-(ethenylsulfonyl)phenyl] diazenyl}-5-hydroxynaphthalene-2,7-disulfonate [3]

Table 8: 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)	Disodium 4-amino-3,6-bis{[4-(ethenylsulfonyl)phenyl] diazenyl}-5-hydroxynaphthalene-2,7-disulfonate
Other names (usual name, trade name, abbreviation)	RB5 bis-vinyl
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	-
EC name (if available and appropriate)	Not available
CAS number (if available)	100556-82-9
Other identity code (if available)	
Molecular formula	$C_{26}H_{19}N_5Na_2O_{11}S_4$
Structural formula	
SMILES notation (if available)	[Na+].[Na+].NC1=C(\N=N\C2=CC=C(C=C2)S(=O)(=O)C=C)C(=CC2=C1C(O)=C(\N=N\C1=CC=C(C=C1)S(=O)(=O)C=C)C(=C2)S([O-])(=O)=O)S([O-])(=O)=O
Molecular weight or molecular weight range	752 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not available
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)	-

1.3.1 Composition of the substance

Table 9: 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)
Disodium 4-amino-5-hydroxy-3,6-bis{(E)-[4-(vinylsulfonyl) phenyl]diazenyl}naphthalene-2,7-disulfonate; CAS no 100556-82-9	-	-	Skin Sens. 1; H317 Resp. Sens. 1; H334

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 10: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	607-RST-VW-Y	tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl] sulphonyl]phenyl]azo] naphthalene-2,7-disulphonate; [1] Reaction products of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid, coupled twice with diazotized 2-[(4-aminophenyl)sulfonyl]ethyl hydrogen sulfate, sodium salts; [2] disodium 4-amino-3,6-bis{[4-(ethenylsulfonyl)phenyl] diazenyl}-5-hydroxynaphthalene-2,7-disulfonate; [3]	241-164-5 [1] - [2] - [3]	17095-24-8 [1] - [2] 100556-82-9 [3]	Resp. Sens. 1A Skin Sens. 1	H334 H317	GHS08 Dgr	H334 H317	-	-	-

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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)		
Resulting Annex VI entry if agreed by RAC and COM	607-RST-VW-Y	tetrasodium 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulphonatooxy)ethyl] sulphonyl]phenyl]azo] naphthalene-2,7-disulphonate; [1] Reaction products of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid, coupled twice with diazotized 2-[(4-aminophenyl)sulfonyl]ethyl hydrogen sulfate, sodium salts; [2] disodium 4-amino-3,6-bis{[4-(ethenylsulfonyl)phenyl] diazenyl}-5-hydroxynaphthalene-2,7-disulfonate; [3]	241-164-5 [1] - [2] - [3]	17095-24-8 [1] - [2] 100556-82-9 [3]	Resp. Sens. 1A Skin Sens. 1	H334 H317	GHS08 Dgr	H334 H317	-	-	-

Table 11: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class 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	Harmonised classification proposed	Yes
Skin sensitisation		
Germ cell mutagenicity	Hazard class 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

The substances have not yet been subject to any measures regarding harmonised classification on the level of the European Union.

Of the two substances that were formerly refered to as “Reactive Black 5” the UVCB substance [2] is registered under REACH with a total tonnage band of 100-1000 tonnes per annum, while the mono constituent substance [1] is registered at a tonnage of 10-100 tonnes per annum. RB5 bis-vinyl [3] is registered with a total tonnage band of 10-100 tonnes per annum.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Respiratory sensitisers should be subjected to harmonised classification pursuant to Article 36(1) of Regulation (EC) 1272/2008 and do not require justification.

Skin sensitisation is not an endpoint with obligatory harmonised classification, but because of its link to respiratory sensitisation, it is considered essential that individuals handling “Reactive Black 5” (either as a substance as such [1] or as constituent in [2]) or RB5 bis-vinyl [3] are sufficiently protected against both the risks arising from inhalation as well as dermal exposure. In addition, there are differences in self-classification (see Table 2). Together with the huge annual tonnage and wide-spread use of the substances this highlights the importance of a sufficient protection against the risks of dermal exposure to “Reactive Black 5” ([1] or [2]) and RB5 bis-vinyl [3] on an EU-wide scale.

5 IDENTIFIED USES

The substance is used as a colouring agent for textiles and black toner particles.

6 DATA SOURCES

This report has been created based on the data submitted by the lead registrant in the REACH registration dossier for the substance that was formerly only identified as “Reactive Black 5” and or RB5 bis-vinyl [3]. Since then the registration for “Reactive Black 5” has been split into two separate joint submissions for the substances [1] and [2], but currently both registrations still contain the same data set. As explained in Chapter 1 it is not possible to unambigiously state which data has been generated on what substance. It is therefore assumed that most data has been generated on substance [2], as most of the registrants have joined this submission. In addition, further relevant data were retrieved from a literature search in PubMed, Web of Science, Embase, Wiley and Google Scholar (last search January 2020).

7 PHYSICOCHEMICAL PROPERTIES

Table 12: Summary of physicochemical properties of Reaction products of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid, coupled twice with diazotized 2-[(4-aminophenyl)sulfonyl]ethyl hydrogen sulfate, sodium salts [2].

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid; dark brown to black powder, odourless	Huber, V.; 2010; report no. PS 20090683.03-Hu	No guideline followed
Melting/freezing point	Melting point >180 °C.	Naing, K. and Klais, O.; 1998; report no. 19981078,1092	The test substance melts under substance decomposition. According to OECD Guideline 102
Boiling point			Waiver: The test substance melts under substance decomposition.

Property	Value	Reference	Comment (e.g. measured or estimated)
Relative density	1.21 at 20°C	Huber, V.; 2010; report no. 20090683.01	According to EU Method A.3
Vapour pressure	<10E-5 hPa at 20 °C	Smeykal, H.; 2010; report no. 20090683.02	According to EU Method A.4
Surface tension	35.254 mN/m at 20°C	Weihrather, A.; Probst, D.; 2019; unpublished data	According to OECD Guideline 115
Water solubility	> 551 g/L at 20°C and pH ca. 4	Huber, V.; 2010; report no. 20090683.04	According to EU Method A.6, flask method
Partition coefficient n-octanol/water	log Pow < -4.34 at 20°C	Huber, V.; 2010; report no. 20090683.05	According to EU Method A.8
Granulometry			Waiver: Study scientifically unjustified
Stability in organic solvents and identity of relevant degradation products			The test substance has an extremely high water solubility and a very low solubility in organic solvents. Stability in organic solvents is not relevant for the use of the test substance.
Dissociation constant	pKa at 22 °C: 1 to 2.89	Praschak, I. and Mühlberger, B.; 2000; report no. PI 687 DK	according to OECD Guideline 112
Viscosity			The test substance is a solid.

Table 13: Summary of physicochemical properties disodium 4-amino-3,6-bis{[4-(ethenylsulfonyl)phenyl] diazenyl}-5-hydroxynaphthalene-2,7-disulfonate; Reactive Black 5 Bis-Vinyl [3]

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	black odourless powder	Certificate of Analysis, 2009	
Melting/freezing point	substance decomposes prior to melting; calculated melting point 350 °C	Lead registration dossier; submitted: 08.03.2016	EPI Suite Results - MPBPVP (v1.43)
Boiling point	Substance decomposes prior to boiling; estimated boling point: 1202.41° C	Lead registration dossier; submitted: 08.03.2016	Adapted Stein and Brown Method
Relative density	D_4^{20}= 1.363	Jungheim (2012)	Method A3., displacement method

Property	Value	Reference	Comment (e.g. measured or estimated)
Vapour pressure	The melting point is above 300 Deg C and hence the study has been omitted in accordance with Column 2 of Annex VII Section 7.5	Lead registration dossier; submitted: 08.03.2016	Estimated value can be found in the refered registration dossier. The validity of these values cannot be guaranteed.
Surface tension	The surface tension of the aqueous solution at 20°C is below 33.7 mN/m.	Jungheim (2012)	The test item has hence surface-active properties. 1g/L solution measured at 20 °C according to plate tensiometer method A.5
Water solubility	Value not corrected for purity: 93.4 g/L; value corrected for purity: 65.5 g/L	Esser (2013)	At 20 °C
Partition coefficient n-octanol/water	Log Kow = -1.73 (calculated) Log Kow = -3.73 (calculated, corrected for measured water solubility)	Lead registration dossier; submitted: 08.03.2016	EpiSuite modul KOWWIN Program (v1.68), 2012, value corrected using measured water solubility instead of the calculated water solubility of 664.7 mg/L
Stability in organic solvents and identity of relevant degradation products	Not available		
Dissociation constant	Not available		
Viscosity	Not applicable		

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 14: Summary table of toxicokinetic studies for RB5.

Method	Test substance	Route of administration (dose)	Sampling times; Method of detection	Results	Reference
Toxicokinetics (absorption, distribution, metabolism) Rat, Wistar Rat (male), Animals per group: Exhalation: 3 Blood/plasma levels:5 Excretion: 5 Excretion i.v: 3 GLP compliant guideline study (EPA OPPTS 870.7485) with derivations: metabolism determined in separate study	Bisphenyl-U-^{14}C labelled RB5 [1] Purity and composition see confidential Annex	- Oral (10 mg/kg bw) - Intravenous (1 mg/kg bw)	Blood: 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24, 32, 48, 72, 96, 120, 144, 168 h Urine (in polyethylene bottles): 0-2, 2-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h Faeces (in glass vessels): 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h Organ/tissue: directly after sacrifice - 7 days after test item administration Exhaled air: continuous aspiration at 0.2 m³/h Measuring radioactivity (Liquid scintillation counting)	Distribution: Blood: 0.25 µg eq./mL Liver: 0.18 µg eq./g Kidneys: 0.10 µg eq./g All other organs: <0.1 µg eq./g In total 0.28% of the administered dose; with mean values of 0.13% in the blood and 0.10% in the liver Excretion (over 7 days): *Oral administration:* mainly via faeces: 83.71% renal excretion: 14.79%, biphasic: $t_{1/2}$ (I) = 4-7 h, $t_{1/2}$ (II) = 40-69 h *Intravenous administration:* Mainly renal: 51.72 % - bi-phasic; Faecal excretion: 26.5 % No excretion by exhalation Mean absorption rate: 28.6 % (determined comparing renal excretion after oral vs i.v. administration)	Hoechst AG (1989)
Metabolism and excretion Rat, Wistar Rat (male), 5 animals tested GLP compliant guideline study (EPA OPPTS 870.7485) with	Bisphenyl-U-^{14}C labelled RB5 [1] Purity and exact composition see	- Oral (10 mg/kg bw)	Urine and faeces: 0-24 h, 24-48 h, 48-72 h HPLC-UV, HPLC-^{14}C, TSP-HPLC-MS pooled from all animals treatment for cleavage of conjugates: acetylase; enzyme cleavage with beta-glucoronidase/arylsulfatase	**Excretion**: 85% excretion via faeces, 15% excretion via urine within 24 h. no unchanged test item excreted **Metabolites**: Urine: 6 metabolites were separated, two main metabolites identified. 8 % of total radioactivity: sulfate-ester 4 % of total radioactivity: N-acetylate **Faeces:** About 17 % of the radioactivity remained unextracted, 76% of total radioactivity: sulfate-	Hoechst AG (1988)

Method	Test substance	Route of administration (dose)	Sampling times; Method of detection	Results	Reference
derivations: exhalation determined in separate study	confidential Annex			ester amount decreased over time, N-acetylate increased over time	
Toxicokinetics (absorption, distribution, metabolism) Rat, Wistar Rat (male) Animals per group: Exhalation: 2 Blood/plasma levels:5 Excretion: 5 Excretion i.v: 3 guideline study (OECD TG 417)	1,4,5,8-naphthyl-^{14}C – labelled RB5 [1] purity and exact composition see confidential Annex	- Oral (10 mg/kg bw)	Blood : 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24, 32, 48, 72, 96, 120, 144, 168 h Urine (in polyethylen bottles): 0-2, 2-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h Faeces (in glass vessels): 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h Organ/tissue: directly after sacrifice - 7 days after test item administration Exhaled air: continuous aspiration at 0.2 m^3/h Measurement of radioactivity: Liquid scintillation counting	**Distribution:** Kidneys: 0.1228 µg eq./g Spleen: 0.0332 µg eq./g subcutaneous fat: 0.0174 µg eq./g Lungs: 0.013 µg eq./g All other organs: < 0.01 µg eq./g In sum 0.0344% of the administered dose **Excretion:** Faeces: 95.58%; $t_{1/2}$(I): 4.9 h, $t_{1/2}$(II): 75 h Renal excretion: 1.22% + 014%; $t_{1/2}$(I): 5 h, $t_{1/2}$(II): 71 h, Bi-phasic elimination no excretion by exhalation	Hoechst AG (1991)
Metabolism and excretion Rat, Wistar Rat (male), 5 animals tested GLP complient guideline study (EPA OPPTS 870.7485) with derivations: exhalation determined in separate study	1,4,5,8-naphthyl-^{14}C – labelled RB5 [1] purity and exact composition see confidential Annex	- Oral (10 mg/kg bw)	Urine and faeces: 0-24 h, 24-48 h, 48-72 h Liquid scintillation counting, HPLC, NMR, GC-MS; pooled from all animals treatment for cleavage of conjugates: enzyme cleavage with beta-glucoronidase/ arylsulfatase	**Excretion:** 72 hours following administration: 108.8% excretion via faeces 0.3% excretion via urine Nearly 99% were excreted within first 24 hours. **Metabolism:** No unchanged test item was found in urine or faeces (rapid and total metabolization) All excreted metabolites were even more polar than the test substance itself. Urine: At least 3 metabolites were separated, but no match with one of the reference compounds was found. Faeces: At least 7 metabolites were detected including the main metabolite. Due to the high amount of coextracted material, no clear identification could be made.	Hoechst AG (1992)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Overall, 4 studies are available, all performed in rats with radioactive labelled Reactive Black 5 (substance [1]). The 2 toxicokinetics and 2 metabolism studies are not sufficient to determine the complete metabolic profile, as only 2 of at least 6 urinary and at least 7 faecal metabolites were identified (a sulfate ester and an N-acetylated form). However, the studies indicate that the majority of the dye is metabolised and mainly excreted via the faeces.

Differences exist between molecules labelled either at the naphthalene or the phenyl rings. About 99 % of the administered dose is excreted via the faeces within 24 h when the naphthalene ring is labelled, and a bi-phasic excretion profile is shown for the phenyl ring-labelled substance with half-lives between 7 to 9 h and 40 to 69 h, respectively. In addition, total excretion via faeces was 80 to 88 % within 7 days, the remaining radioactivity is excreted via the urine. In conclusion, it appears that the sulfoxyethylsulfonylphenyl group has a longer residence time than the naphthalene group. The difference in faecal excretion between the differently labelled moieties indicates further (probably reductive) cleavage and different metabolic fates of the napthtalene group and the sulfoxyethylsulfonylphenyl groups.

Based on the known degradation profile for “Reactive Black 5” (substance [1]), it can be assumed that the dye remains unchanged in the acidic environment of the stomach. In the more basic setting of the intestines, it most likely undergoes hydrolysis yielding the vinyl sulphone (RB5 bis-vinyl [3]) as seen in the abiotic degradation studies performed.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

Not assessed in this report

10.2 Skin corrosion/irritation

Not assessed in this report

10.3 Serious eye damage/eye irritation

Not assessed in this report

10.4 Respiratory sensitisation

According to Annex I, sections 3.4.1.1 and 3.4.2.1.2.1 of the CLP regulation “respiratory sensitiser means a substance that will lead to hypersensitivity of the airways following inhalation of the substance” and “in this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered” (EC, 2008). However, there is still uncertainty regarding the exact mechanism on how substances cause respiratory sensitisation. Based on currently available knowledge, it is assumed that respiratory sensitisation can occur not only via inhalation of the substances, but as well via dermal exposure (ECHA, 2017).

Since there is no validated and universally accepted *in vitro* or *in vivo* test method to identify respiratory sensitisers, testing for this endpoint is currently not a standard information requirement under REACH. Thus, an identification of substances as respiratory sensitisers can only be derived from human observations in exposed populations.

10.4.1 Human Data

After the first report of occupational asthma caused by reactive dyes in textile plant workers in 1978 by Alanko

and the deadly course of an asthma attack in one worker employed in a dyeworks in Leicester (Stern, 1985), in the following decade more and more case reports as well as detailed occupational health investigations of workers exposed to reactive dye powders have been published with the clear conclusion that many reactive dyes are causative agents for respiratory sensitisation leading to occupational asthma. Among the reactive dyes in focus, RB5, which had a widespread use, was reported in many cases as a causative agent for respiratory or nasal symptoms such as asthma, rhinitis or cough. Allergic reactions to RB5 have been confirmed through bronchial provocation tests, skin Prick tests (SPT), radioallergosorbent tests (RAST) and based on increased specific IgE levels.

The main data pointing to the respiratory sensitisation potential of RB5 result from three comprehensive health investigations among dyehouse employees in the United Kingdom (UK), Korea and Sweden (Table 15). Beside these comprehensive studies, overall evidence is strengthened by manifold occupational case reports listed in Table 16.

It is noted that in many of the listed reports different trade names are used instead of the name “Reactive Black 5”. By comparing chemical structures and colour indices, the following synonyms for “Reactive Black 5” have been identified: Levafix Black E-B (Ringenbach 1985, Thorén 1996, Docker 1987), Dimaren Black K-3B (Docker 1987), Remazol Black B (Luczynska 1986, Docker 1987, Estlander 1988, Nilson 1993), and Black GR (color index BK 5, Park 1989,1991, Hong 1992). While it is not possible to unambigiously identify any of these commercial products with either substance [1] or [2], it is assumed, that in most cases substance [2] was described.

The first case control study is an investigation of workers employed in dyehouses of Leicester and Manchester, UK, perfomed in 1985 by Docker et al. The authors designed a questionnaire in order to identify all dyehouse employees showing respiratory symptoms and being concomitantly exposed to reactive dye powders. From a total of 414 workers, 74 employees (17.8 %) were identified with work-related respiratory and nasal symptoms. 49 (11.8 %) had respiratory symptoms (with and without additional nasal symptoms). These 49 employees were clinically assessed in order to explore the nature and causative agents of their symptoms, the severity of the symptoms and their relation to work. Blood samples for immunological analysis were taken from all employees and from 20 workers from a different industry with no possibility of exposure to reactive dyes as control group.

In order to draw conclusions on the sensitisation potential of the dye powders, workers were grouped into 4 groups depending on their frequency of exposure ranging from almost all day (group 1), daily to frequently (group 2), regularly small quantities (group 3) and only previous, without current dye exposure (group 4). The reactive dyes in use were identified through lists supplied by the dyehouses. Reactive dyes to which at least 70 workers were commonly exposed were chosen for the immunological tests and were grouped according to their colours. The black pool contained three dyes (Dimaren Black K-3B, Levafix Black E-B, Remazol Black B) which according to the colour index are only different trade names for RB5. Even if chemical structures of the reactive dyes thus should be the same for all three designations (trade names), typical purities of such dye powders are in the range of approximal 50-80 % and different trade names may contain different additives or impurities along with the main dye.

Blood sera from all employees were tested for specific IgE using RAST. Tests were perfomed when there was an indicated possibility of exposure derived from the lists of dyes in use in each factory. For the black dye pool, RAST was perfomed with sera from 67 employees in Manchester and 112 in Leicester with 2 and 12 positive results, respectively. This correlates with a frequency of 3 % (Manchester) and 10.7 % (Leicester). However, not all RAST-positive workers were symptomatic.

The clinical assessment of the 49 employees with respiratory symptoms revealed that in 21 cases (5 % of all workers) their symptoms were attributed to allergic reactions to one or more reactive dyes. These symptoms included “typical“ occupational asthma (9 patients), asthma of short duration after exposure (4 patients) and only nasal symptoms (8 patients). Comparison of employees with work-related symptoms and asymptomatic ones showed no differences in age, duration of employment, smoking status, as well as no significant difference among the different exposure groups.

SPT with the black pool were perfomed on 19 of the allergic employees with 6 (1.4 % of all workers, 3.3 % among RB5 exposed workers) positive results. 5/13 allergic workers from the black dye pool were RAST-positive, corresponding to 1.2 % of all workers, 2.8 % among RB5 exposed workers, and a rate of symptomatic

patients of 36 % (5/13) among all RAST-positives.

Overall, allergic reactions were strongly associated with increased levels of specific IgE (detected by RAST) indicating an IgE-mediated pathway of sensitisation. This is supported by one case in the study, which gave a positive RAST result against RB5, but at the time of the survey was asymptomatic. Shortly after the study, this patient developed occupational asthma, and the symptoms disappeared after relocation to a workplace without dye exposure. In addition, in another case of an asymptomatic worker in Leicester with RAST and SPT-positive tests, these test results could be attributed to a history of occupational respiratory symptoms in a prior work place where the patient had been exposed to dye powder. High levels of specific IgE as observed in these studies indicate that sensitisation has occurred, but elicitation of an allergic response is not solely based on the presence of specific IgE.

Overall, this study demonstrates the ability of RB5 and other reactive dyes to cause respiratory sensitisation in workers exposed to the dye powders and indicates an IgE-mediated pathway of asthma development. However, it has to be noted that none of the workers was exposed to only one dye and in many cases allergic reactions against several dyes were observed. Additionally, the study does not provide any insight into exact exposure doses and thus does not allow to draw conclusions on dose-response relationships.

The second comprehensive study was perfomed by Park and co-workers in 1991 on all employees of one Korean dye company. In similarity to the study by Docker et al, workers with lower respiratory tract symptoms (78, 25 %) were identified through a questionnaire among the 309 employees. Sera were taken from all employees and additionally from 11 volunteers without any exposure to reactive dyes. All were tested in a RAST for specific antigens against a RB5-HSA conjugate. In addition, total levels of IgE were quantified and cross-reactivity between RB5 and Orange 3R were investigated in a RAST inhibition assay.

78 workers (25 %) reported work-related respiratory symptoms. Amongst them a methacholine bronchial challenge was performed showing non-specific bronchial reactivity in 38, which is typically occurring in correlation with occupational asthma. 20 workers with strong reactions in this challenge were then assessed with a bronchoprovocation test with the reactive dye considered by the patients as the causative agent for their symptoms. 5 workers underwent bronchial challenge with an RB5 solution and all showed a clear positive response, 2/5 showed an early response pattern and 3/5 a dual response (early response and long-lasting symptoms). It has to be noted that these tests were performed without a placebo control group and thus false-positive responses caused by psychogenic factors cannot be ruled out completely.

Among all workers, SPT and RAST were positive for RB5 in 25 and 52 cases, respectively, corresponding to a frequency of 8 % or 17 %. The relatively high ratio of workers with a positive RAST, but negative SPT is explained by the authors by the use of free dye instead of dye-HSA conjugate in SPT. However, comparing total levels of specific IgE antibodies (to RB5-HSA), high concentrations were found not only in symptomatic employees (23/78 patients, 30 % among symptomatic, 7.4 % among all workers) but also in 26 asymptomatic ones, while in the control group no increased levels were detected. A similar ratio (36 %) of symptomatic patients among RAST-positives was reported by Docker et al (1985). This demonstrates that exposure to dye powder triggers in some cases sensitisation (IgE formation), but this does not nessessary result in elicitation of respiratory symptoms or skin reactions afterwards.

On the other hand, there are also several cases where patients with clear positive response in the bronchoprovocation test had negative results in RAST/SPT. Such findings were also reported by the same authors in a previous study on 9 asthmatic patients for RB5 and other reactive dyes (Park et al., 1989). In the view of the authors, a negative RAST result despite the presence of clinical symptoms could occur either due to a too low sensitivity of the RAST method applied (yielding false negatives) or indicating that beside an IgE-mediated allergic reaction also other mechanisms are involved.

Contrary to the study by Docker (1985), Park and co-workers found an increased prevalence of specific IgE in current or former smokers when compared to non-smokers. In the RAST inhibition experiments, RB5 (free dye) was inhibited most efficiently by its HSA-conjugate demonstrating that hapten formation is crucial for effective antibody binding. In a less pronounced manner also the orange 3R-HSA conjugate was able to inhibit antibody binding demonstrating cross-reactivity between the two dyes which might be due to the formation of some shared epitopes due to their structural similarity.

The third comprehensive study comprising 15 textile plants of varying size with attached dyehouses was

conducted in Sweden (Nilsson et al., 1993). Employees with work-related skin or respiratory symptoms (within the last 5 years) were identified through interviews with the company physician and nurses, management representatives and trade union occupational safety officers. Interviews were also perfomed with employees who were present when the authors visited the dyehouses. Among a total of 1142 employees, 229 workers were employed in the dyehouses and laboratory departments. They were grouped into exposure groups depending on the frequency of dye exposure (similar to the study of Docker (1985)). 162 of them were allocated to those exposed to dye powders (exposure groups 1-3). 67 workers employed at the dyehouses were not exposed to dye powders.

10 employees (6 % of exposed workers) showed work-related respiratory or nasal symptoms. Rhinitis was reported in 8 workers, asthma in 6 workers und bronchitis in 7 workers, and most workers reported several symptoms. In 5 workers showing symptoms, these could be correlated with an IgE-mediated allergy to RB5 through positive SPT and/or RAST. In two out of these five, non-specific bronchial reactivity was identified in a metacholine challenge test. 5 out of 10 symptomatic workers showed additional skin symptoms such as dermatitis, urticarial or Quincke oedema, the latter two are indicating that also immediate type 1 immune response were seen. Overall, on the basis of the anamnestic and immunologic data, RB5 seems to be the causative agent for allergic reactions in at least five cases out of 162 exposed workers (3.1 %).

Table 15: Summary table of human case-control studies on respiratory sensitisation.

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
Case-control study Dyehouses in Manchester (22) and Leicester (30) 49/414 workers had respiratory symptoms and were further clinical assessed	RB5, three different trade names tested pooled: Dimaren Black K-3B/ Levafix Black E-B/ Remazol Black B (in all cases most likely substance [2]) No information on purity of the test substances Dye powders used without purification	1. Questionnaire (all 414 workers handling reactive dyes), Blood samples taken from 405 employees. 179 workers exposed to RB5, Co-exposure of workers to other reactive dyes 2. 49 workers showing symptoms were clinically assessed along with a control group of 20 employees from different industries with no possible exposure to reactive dyes Tests on RB5: Skin Prick Test on 19 symptomatic workers, RAST on 179 workers exposed to RB5	Clearly positive results for RB5 1. Questionnaire: 18 % work related resp. or nasal symptoms, 12 % resp. (+/- additional nasal) symptoms 2. Clinical assessment results (for RB5): RAST-positives (related to all employees exposed to black dye at a site): Manchester 2/67 (3 %), Leicester 12/112 (10.7 %), Overall 14/179 (7.8 %) SPT-positives (performed on 19 allergic employees): 6/19 RAST-positives (performed on 13 allergic employees): 5/13 Comparison of employees with work-related symptoms and asymptomatic ones showed no differences in age, duration of employment, smoking status or exposure frequency (4 groups: exposed all-day, daily, regularly, currently no exposure, but exposed in the past).	Docker et al. (1987)
Case-control study (dye-producing industry, Korea) 309 workers of dye-producing factory	RB5 (trade name Rifazol black GR) (most likely substance [2]) No information on purity of the test substance Dye powder used as obtained (i.e. without purification)	All employees (309): 1. Questionnaire 2. Clinical Test: SPT, RAST, RAST inhibition (cross-reactivity of different dyes), specific IgE level, bronchoprovocation Co-exposure of workers to other reactive dyes also produced within the company: Rifacion orange HE 2G (O-20), Rifacion red HE 313, Rifacion navy blue HER, Rifafix yellow 3 RN, Rifafix red BBN, Rifazol brilliant orange 3R (O-16)	Confirmed allergic reactions to RB5 correlated with occupational exposue to reactive dye powder 1. Questionnaire: 78 (25.2 %) of the workers had work-related lower respiratory tract symptoms (cough, sputum, chest tightness, or shortness of breath); 3 of them with additional skin symptoms Bronchial challenge: 13/78 asthmatic responses to bronchial challenge with several reactive dyes (incl. RB5) IgE was more frequently present in symptomatic employees (30 % of 78 workers) than in asymptomatic ones (17 %) IgE was increased in 100 % of symptomatic smokers (46 workers) RB5 specific results: Bronchial challenge: 5 tested: 5/5 positive (2/5 early reaction, 3/5 dual (early/late reaction) SPT positive: 25/309 (8.1 %) RAST positive : 52/309 (16.8 %)	Park et al. (1991b)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
			RAST inhibition: Orange 3R showed cross reactivity with RB5, inhibition only effective with HSA-dye conjugate (and not free dye)	
Case-control study Textile plants in Sweden 1142 employees, 162 exposed to reactive dyes	RB5 (trade name Remazol black B) (most likely substance [2]) No information on purity of the test substance	1. Interviews in 15 textile plants in western Sweden (1142 employees) 2. Clinical investigations of 162 workers exposed to reactive dyes (RB5 among others) Tests: Spirometry, Metacholine Challenge Test, IgE level, RAST, RAST inhibition, SPT and Patch test RAST, SPT and patch test perfomed with 9 suspected commercial reactive dye powders (brought in by the patients)	RB5 identified as causative agent for occupational respiratory allergy which is sometimes accompanied by skin symptoms 1. Interviews: 162/1142 workers employed in dyehouse and laboratory departments exposed to dye powder 2. Clinical investigations Workers with respiratory symptoms: -10 (6 %) of exposed workers had work-related respiratory or nasal symptoms: 8 rhinitis, 6 asthma, 7 bronchitis - in 5/10 workers (3 % of all workers) asthma was confirmed by spirometry (FEV_1 < 80 % predicted; 2 persons) or metacholine challenge (3 persons) - 5/10 had additional skin symptoms SPT positive (RB5): 5 RAST positive (RB5): 4 Patch test: all negative IgE level slightly elevated in 5 patients	Nilsson et al. (1993)

Table 16: Summary table of human case reports on respiratory sensitisation.

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
Occupational case report on 4 patients developing asthma after working with reactive dyes	RB5 (trade name Remazol B) (most likely substance [2]) No information on purity of the test substance	Description of work history and medical observations	In 1 of the 4 patients the occurrence of asthma attacks was especially correlated with exposure to RB5 powders at work.	Ringenbach (1985)
Occupational case report on 1 patient	RB5 (trade name Levafix black E-B)	Patient developed rhinitis and cough and 1 year later eczema on hands and front of	Positive for RB5, skin allergy developed later than respiratory symptoms	Thorén et al. (1986)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
working in a reactive dye producing company	(most likely substance [2]) No information on purity of the test substance	the neck after company started to produce reactive dyes (RB5 among others) Tests: SPT, RAST, IgE-level	SPT: mild positive with human serum albumin (HSA)-conjugated to RB5, strong positive with non-conjugated RB5 RAST: negative IgE level: elevated Symptoms disappeared after exposure had ceased	
Specific IgE measurements in blood sera of allergic dye house operatives	RB5 (trade name Remazol Black B) (most likely substance [2]) No information on purity of the test substance	For all 6 patients, controls of same age, same pattern of exposure and smoking status but without symptoms were investigated as well, and 6 controls without any exposure, Co-exposure of workers to other reactive dyes Test: RAST, IgE quantification	Six of the tested individuals had respiratory symptoms (not further described) associated with exposure to reactive dyes 3/6 patients tested with RB5, all three positive Comparison of RAST with dye-HSA conjugates and free dyes demonstrates better correlation of symptoms with results from dye-HSA conjugates demonstrating importance of hapten fomation	(Luczynska and Topping, 1986)
Occupational case reports on 5 patients, all employed in dye houses or textile plants being exposed to dye powders (RB5 among others)	RB5 (Remazol Black B) (most likely substance [2]) Dye powder used as obtained without purification TLC analysis of RB5 dye powder indicated at least ten different impurities which were not quantified. The dye powder contained 9 µg/g water-soluble chromium.	5 cases of occupational eczema, urticarial and respiratory disease 3/5 patients had respiratory symptoms (2/5 asthma), of these three all had skin symptoms in addition (eczema and/or urticaria) 2/5 patients exhibited skin symptoms only (eczema) Tests: patch, scratch chamber and prick test, nasal challenge (only for patients showing respiratory symptoms)	2 patients demonstrated clear allergic reactions to RB5 Patch test: 2/5 patients positive (one with only skin symptoms, 1 with both skin and respiratory symptoms) The patient with resp. symptoms was also: - Scratch- or prick-tested (RB5): positive - Nasally challenged (RB5): positive 1/2 positive patients was also positive against chromium (and probably exposed to both/sensitised separately) 4/5 patients could not continue their work due to severe allergic reactions.	(Estlander, 1988)
Occupational case report on 9 patients,	RB5 (trade name Rifazol black GR)	9 patients with asthmatic symptoms	Clearly positive for RB5 - SPT: 9/9 positive to RB5	(Park et al., 1989)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
all exposed to reactive dyes during their work (dye industry, Korea) Exposure to RB5 and other reactive dyes	(most likely substance [2]) No information on purity of the test substance	Measurements: IgE level, RAST, RAST inhibition (cross-reactivity), Prick skin test, Bronchial challenge with methacholine and reactive dyes Methods of bronchoprovocation: FEV1 and maximum mid-expiratory flow measured before and 10 min after inhalation of test solutions (serial increments of antigen concentration (0.01, 0.1, 1.0, 2.5 mg/ml) every 10 minutes until 20 % decrease of FEV1 was recorded; pulmonary function test was performed every 9 or 10 hours after challenge	-RAST 8/9 positive for specific IgE antibodies to RB5-HSA conjugate - Bronchoprovocation with RB5 performed on 4 patients: 3/4 showed dual response, 1/4 immediate response - RAST inhibition: no cross-reactivity of other tested dyes on RB5	
Study on relevance of specific IgG and IgG4 antibodies in dye-exposed workers 309 employees of a dye factory and 63 unexposed patients as control	RB5 (trade name Rifazol black GR) (most likely substance [2]) No information on purity of the test substance	Measurement of RB5-HSA specific IgG and IgG4	IgG formation in response to (also single) exposure Prevalence of IgG is not associated to work place (office, laboratory, dye processing station) or duration of dye exposure, but is indicative of whether exposure has occurred IgG detected in 23 % of exposed workers, IgG4 in 14 % IgG prevalence was significantly higher in smokers, workers with specific IgE, and workers with respiratory symptoms	Park andHong (1991)
Study on specificity of IgE antibodies	RB5 (trade name Rifazol black GR) (most likely substance [2]) No information on purity of the test substance	Specificity of IgE antibody by RAST and RAST inhibition (tested on blood sera from 4 patients of Park 1991 with high IgE levels)	IgE response to RB5-HSA conjugates and cross-reactivity with orange 3R differed from one patient to another	Hong andPark (1992)
Study on prevalence of specific IgE and IgG in workers of a dye factory and four	RB5 (trade name Rifazol black GR) (most likely substance [2])	All 176 workers: 1. Questionnaire 2. Prevalence of specific RB5- IgG (by ELISA) and RB5-IgE (by RAST)	Prevalence of occupational asthma: - key factory: 11/81 (14 %) - neighboring: factory 1: 3/24 (13 %) + factory 2: 3/22 (14 %)	Park et al. (1991a)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
neighbouring factories 1 key factory (81 workers, producing reactive dyes (RB5 among others) and 4 neighbouring factories (75 workers, no production of reactive dyes/RB5)	No information on purity of the test substance		Prevalence of IgE and IgG, respectively: - key factory (81 workers): 19 (23 %), 40(49 %) - neighboring factories: 1 (24 workers): 12 (50 %), 4 (17 %) 2 (22 workers): 10 (45 %) , 4 (18 %) 3 (29 workers): 12 (41 %), 7(24 %) 4 (20 workers): 5 (25 %), 0 → prevalence of IgG could be an indicator of exposure to reactive dyes (level decreases with distance to key factory); while allergy seems IgE mediated	
Study of relevance of SPT and specific IgE measurements in diagnosis of occupation asthma	RB5 (trade name Rifazol black GR) (most likely substance [2]) No information on purity of the test substance	Comparison of SPT and specific IgE levels (measured by ELISA) in a) 42 patients with occupational asthma against reactive dyes (positive in bronchial challenge), 33/42 positive for RB5 b) 93 exposed workers without symptoms (no asthma, negative in bronchial test) and c) 16 unexposed controls (no asthma, negative in bronchial test)	SPT and IgE measurements complement each other, sensitivity and specificity is higher in SPT, medium to high positive predictivity rate, high negative predictivity rate 83.3 % of asthmatic patients showed positive results in SPT or ELISA (or both) SPT: - patients: 32/42 positive (76.2 % sensitivity); - exposed (non-symptomatic): 8/93 positive (8.6 %, → 91.4 % specificity) - unexposed controls: all negative → positive predictivity (real positives (allergic and positive tests, 32) among all positive tests (32 + 8)): 32/40 (80 %) → negative predictivity (real negatives (non-allergic and negative test, 85) among all negative tests (85 + 10)): 85/95 (89.5 %) Specific IgE (by ELISA): - patients: 22/41 (53.7 % sensitivity) (no data on 1 asthmatic) - exposed (non-symptomatic): 13/93 positive (14 %, → 86 % specificity) - unexposed controls: all negative → positive predictivity (real positives (allergic and positive tests) among all positive tests): 22/35 (62.9 %)	Park et al. (2001b)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
			→ negative predictivity (real negatives (non-allergic and negative tests) among all negative tests): 80/99 (80.8 %)	
Study on long-term occupational asthma	RB5 (trade names Remazol black GR (Black SF-GR) and Remazol black B (Black B)) (most likely substance [2]) No information on purity of the test substance	11 patients with occupational asthma to reactive dyes (10 diagnosed by positive bronchial challenge with RB5) were re-evaluated (pulmonary function test and a methacholine bronchial provocation) for symptoms after 2-6 and 11-16 yrs avoidance of dyes, in the second re-evaluation additionally SPT performed All patients obtained medical treatment after diagnosis on their asthma severity basis, all patients stopped smoking after diagnosis.	Lung functions (FEV_1 %) did not recover even after long-term avoidance of exposure Skin reactivity almost disappeared in second examination <u>Initial investigation, lung function:</u> 3/11 patients had normal FEV_1 (≥ 80 %) 8/11 patients had FEV_1 < 80 % (56 - 79 %) <u>First follow-up examination:</u> 3/11 patients had normal FEV_1 (≥ 80 %) 8/11 patients had FEV_1 < 80 % (54 - 79 %) <u>Second follow-up examination</u> 3/11 patients had normal FEV_1 (≥ 80 %) 8/11 patients had FEV_1 < 80 % (49 - 79 %) No significant changes in geometric mean of PC20-methacholine challenge between initial and first, or first and second evaluation. Skin Prick Test - A/H ratio: <u>Initial examination:</u> 0 (negative) - 3/11 (Black B), 1/11 (Black SF-GR) >0 - 1 (+) - 4/11 (Black B), 4/11 (Black SF-GR) >1-2 (++) – 2/11 (Black B), 5/11 (Black SF-GR) >2 (+++) – 2/11 (Black B), 1/11 (Black SF-GR) <u>Seond examination:</u> 0 (negative) – 10/11 (Black B), 8/11 (Black SF-GR) <0-1 – 1(11) (Black B), 3/11 (Black SF-GR)	Park et al. (2007)

10.4.2 Animal Studies

As already stated above, there are no validated animal studies for the identification of respiratory sensitisers. However, available results from animal studies can be included in a weight of evidence evaluation. For RB5 there are only few reported animal studies (Table 17). Three studies were performed in guinea pigs. In these studies, no changes in lung functions after inhalative challenge with RB5 were reported.

Sarlo and Clark (1992) used a tiered approach for respiratory sensitisation with tier 1 and 2 *in vitro* tests and tier 3 and 4 *in vivo* tests in guinea pigs. In the first *in vivo* model, induction was performed by intradermal injection of the test substances followed by subcutaneous and intratracheal challenge. In the next tier, induction was perfomed via inhalation. In both tests no change in pulmonary/respiratory function was observed, but a positive response in the cutaneous anaphylaxis test (tier 3) and a dose-dependent formation of antibodies (tier 4), demonstrating an immune response in correlation with RB5 exposure.

In addition, in a modified local lymph node assay (LLNA) reported by (Dearman et al., 2013) resulted in a clearly positive outcome and dose-dependent profileration of lymphocytes was detected. The study deviated from OECD test guideline (OECD TG) No 429 in the choice of animal strain: BALB/c mice were used instead of CBA/Ca mice as recommended in the guideline. However, the authors performed further tests on these two mouse strains using two other reference chemicals and a broad concentration range in order to demonstrate that the BALB/c strain is of sufficient sensitivity for characterising responses in the LLNA assay.

The LLNA is a validated and accepted test method to determine the skin sensitisation potential of chemicals (OECD TG No. 429). However, to current knowledge, all low molecular weight respiratory sensitisers are also skin sensitisers and thus should give positive results in skin sensitising test methods. Positive results in the LLNA are expected for respiratory sensitisers, but a positive result is not automatically correlated with respiratory sensitisation potential.

Table 17: Summary table of animal studies on respiratory sensitisation for RB5.

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**Evaluation parameters**	**Results**	**Reference**
Non-guideline study, *in vivo* testing on guinea pigs	Guinea pig, Pirbright-White (HOE DHPK (SPFLac)), m/f, Animals used per group: 2 for intradermal tolerance 4 for inhalation of a non-irritant concentration 8 for control (treated with vehicle only) 8 in test group: 8	Test material information: RB5 (Remazol Schwarz B - Pt. 7/88) [2], constituent; solid: particulate/powder, purity of test substance: see Confidential Annex Induction: intradermal 1 %, 5 %, 30 % Challenge, 3 weeks after induction: inhalation (over 15 min) Male: 150, 210 mg/m^3 Female: 140, 180 mg/m^3	Allergic reactions in the test groups are assessed by changes of lung function parameters (respiratory rate, tidal volume, inspiration time, expiration time, minute volume, peak expiratory flow and relaxation time) compared to the material control groups.	Negative No significant changes of lung function parameters.	Hoechst AG (1993)
Non-guideline study, *in vivo* testing on guinea pigs Injection model to assess chemical immunogenicity (indicated by the authors as Tier 3 of respiratory sensitisation study)	Guinea pig, Hartley, f, 10/group	Test substance RB5 (Reactive Black B [2], obtained from Hoechst-Celanese, no information about purity) Induction: subcutaneous 6.7x10^{-3} M, 6.7x10^{-4} M, 6.7x10^{-5} M 2/week (4 weeks) Challenge: subcutaneous (SC) 1 week after induction (400 µL of same concentration as used in induction), after another week: intratracheal (IT), 100 µL of 500 µg/mL (No testing on IgE)	Evaluation: Serum collection: 7 days after SC challenge Resp. evaluation: 8 days after SC challenge Skin reaction: 10 days after SC Challenge, 48h after IT challenge Evaluation after IT challenge: visual (changes in breathing pattern, depicted as exaggerated diaphragmatic), immediately after challenge for a 10 min period Active cutaneous anaphylaxis (ACA) testing (48 h after challenge): tissue fixed antibodies ELISA and passive cutaneous anaphylaxis (PCA): for detection of circulating IgG antibodies	Ambiguous No respiratory reaction after IT challenge. But positive in the ACA test (9-10 /10). Slight increase in antibody titers (IgG measured by ELISA). No allergic antibody (IgG) was detected by PCA in sera from treated animals at the high and mid dose; minimal allergic antibody was detected at the 6.7*10^{-5} M dose.	Sarlo andClark (1992)

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, duration of exposure	Evaluation parameters	Results	Reference
Non-guideline study, *in vivo* testing in a guinea pig inhalation model to address questions about relevant routes of chemical exposure and allergenicity (Tier 4 of respiratory sensitisation study)	Guinea pig, Hartley, f, 8/group	Test substance RB5 (Reactive Black B [2], obtained from Hoechst-Celenese, no information about purity) Induction: inhalation 5 days; 3 hr/day, 1, 5, 10, 100 mg/m³ Dye aerosol Challenge: inhalation (30 min) (No testing on IgE)	Evaluation (after challenge): respiratory rate and breath peak height: continuously monitored (before and during challenge) Other: Passive cutaneous anaphylaxis (PCA) testing ELISA	No change in pulmonary function, Dose-dependent IgG antibody production Animals exposed to 1, 5, 10, and 100 mg/m³ dye did not exhibit any change in pulmonary function during or after inhalation challenge with Dye-guinea pig serum albumin conjugate (dye-GPSA). Animals exposed to 1 and 5 mg/m³ dye did not produce detectable antibodies. Animals exposed to 10 and 100 mg/m³ dye did produce IgG and IgG1a allergic antibodies to dye-GPSA as measured in the ELISA and PCA tests.	Sarlo andClark (1992)
LLNA (guideline with changes: other strain than recommended in the guideline	BALB/c mice, n=4	Test substance: RB5 (Remazol Schwarz B [2], 70 % pure) C = 5,10, 25 % in dimethyl formamide (DMF) (25 µL), initiation: daily dermal application for 3days, challenge after 5 days with i.v. 20 µCi of ^{3}H-methyl thymidine	Lymph nodes	Positive, Dose-dependent proliferation of lymphocytes, SI >3 in all three tested concentrations	Dearman et al. (2013)

10.4.3 In Vitro Studies

There are few *in vitro* studies available on RB5, summarised in Table 21. The studies by Wass 1990 and Sarlo 1992 reveal that the binding of RB5 to proteins or peptides is only occurring after basic activation (or metabolization) of the sulfoxyethylsulfonyl group yielding the activated bis-vinyl form [3]. As the covalent binding to proteins is the first key event in sensitisation by low molecular weight chemical allergens (respiratory as well as skin), these studies underpin the inclusion of the bis vinyl form besides substances [1] and [2] in this report. However, read-across to other sulfoxyethylsulfonyl dyes known as respiratory sensitisers is limited as - according to the results from Park 2001 - the formation of specific IgE epitopes does not only rely on the type of reactive group alone but is also strongly influenced by the structure of the chromophore.

Recently, the OECD adopted a battery of *in vitro* test methods (OECD Test Guideline No. 442 C,D and E) for evaluation of the skin sensiting potential of chemicals. The OECD TG 442C test method is an *in chemico* evaluation using the Direct Peptide Reactivity Assay (DPRA). Here, the ability of compounds to bind covalently to proteins, which is assumed as the first molecular initiating event leading to (skin) sensitisation, is assessed through a reactivity measurement towards synthetic peptides containing either lysine or cysteine residues. DPRA studies by Lalko 2012 and 2013 performed with a range of known respiratory and skin sensitising chemicals led to two major conclusions: firstly, all respiratory sensitisers tested gave positive test results in the DPRA, supporting the assumption that in both, induction of skin as well as respiratory sensitisation, binding to proteins is a molecular key event. Secondly, for the majority of respiratory sensitisers tested, a prevalence for binding to lysine residues when compared to cysteine residues was observed, while chemicals known as skin sensitisers “only” bind to both amino acids without any clear preference for one or the other. Among the respiratory sensitisers studied by Lalko et al, also “Reactive Black 5” was investigated with clear positive results and a slight preference for lysine over cysteine (ratio ~1.2).

Table 18: Summary table of other studies relevant for respiratory sensitisation.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vitro study on reactivity with lysine bearing peptide	RB5 (Remazole black B)	Reactivity to react in aqueous solution, at 37 °C and neutral pH (reaction time 10 min) with lysine-bearing peptide was monitored using HPLC	No binding occurred for RB5 (score* 0) while binding was observed for other well-known sensitisers such as isocyanates (scores 5-10) *percentage of reacted sample divided by 10, rounded to the nearest integer: 10 = 100 % binding, 0 no binding Study suggests that activation of the dye (in dying process typically done under alkaline conditions) is required to form covalent bonds with proteins	Wass andBelin (1990)
Optimized RAST for detection of specific IgE	RB5 (Remazole black B)	Preparation of dye-protein conjugates for RAST, screening for optimal pH, molar ratio between dye and protein and different proteins	Dye-conjugates in general gave much better results than direct dyeing, HSA is a suitable carrier, Optimal of conjugation conditions reported: 7 dye haptens per HSA, pH 8.8, 20 °C, 1 h Comparison of protein reactivity varied between blood sera from different patients indicating antibody specificity is dependent not only on the nature of the hapten but also on individual immune response factors	Wass et al. (1990)
In vitro binding to protein (Tier 2 of multi modal approach)	RB5 (Remazole black B)	Reacted under alkaline conditions with guinea pig serum albumin, quantification of reaction by optical density measurement	Result: positive (molar ratio 20:1 dye:protein) → confirms that covalent protein binding requires basic activation	Sarlo andClark (1992)
Study on IgE epitopes for vinyl-reactive dyes	Vinyl reactive dyes: RB5 and Remazole Orange 3R, Vinylsulphone and Procion Red-MX5B (dichlorotriazine reactive group, naphthalene chromophore)	Measurements: Dye-HSA specific IgE by ELISA; inhibition of specific IgE, gel electrophoresis, immunoblotting	Both, chromogenic and reactive group contribute to the formation of specific IgE epitope, -epitopes of HSA-dye complex are heterogeneous, -intact protein structure of HSA is important (no formation of IgE after denaturation)	Park et al. (2001a)
OECD 442C - in chemico skin sensititization	Black GR reactive dye (RB5, purity 55 %) among others	Comparison of known respiratory sensitiser to known skin sensitiser in DPRA	RB5: Both strong: 99.5 % lysine and 77.3 % cysteine depletion Majority of respiratory sensitisers had a higher reactivity towards lysine.	Lalko et al. (2012)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Modified DPRA	Black GR reactive dye (RB5, purity 55 %) among others	Peroxidase Peptide Reactivity Assay (PPRA) as a refinement to the DPRA	RB5 was also positive here, but selectivity to lysine was lost for all respiratory sensitisers	Lalko et al. (2013a)
DPRA	Black GR reactive dye (RB5, purity 55 %) among others	DPRA with known respiratory sensitisers on Lysine, Cysteine, Histidine, Arginine and Tyrosine bearing peptides; Competitive DPRA with different lysine:cysteine ratios	Ratio for RB5 with slight preference for lysine over cysteine was confirmed (85 % lysine depletion, 82 % cysteine depletion), RB5 has no reactivity towards other aminoacid residues investigated Majority of respiratory sensitisers had a higher reactivity towards lysine, an exception are isocyanates which show preference for cysteine.	Lalko et al. (2013b)

10.4.4 Short summary and overall relevance of the provided information on respiratory sensitisation

Beside manifold occupational case reports (Table 16), case-control studies (Table 15) among dyehouse workers in England (Docker 1987), Korea (Park 1991) and Sweden (Nilson 1993) revealed clear evidence for an increase in cases of respiratory sensitisation amongst workers exposed to RB5 dye powder.

The study by Docker et al. identified workers with respiratory symptoms among all dyehouse employees in Leicester and Manchester which were – according to a questionnaire - exposed to reactive dye powders. From a total of 414 workers, 49 employees (12 %) were identified with work-related respiratory symptoms. The clinical assessment revealed that the symptoms in 21 cases (5 % of all workers) were attributed to allergic reactions against reactive dyes. Among the allergic employees, 19 or 13, respectively, were tested in SPT and RAST against the black dye pool which consisted of a mix of RB5 from three different brands. With these tests, an allergy against RB5 was confirmed in 6 (SPT) and 5 (RAST) patients, respectively, corresponding to an overall frequency of 1.4 or 1.2 % of all exposed workers and 2.8 or 3.3 % of dye-exposed workers. It has to be noted that also several employees without symptoms gave a RAST positive result, so that the overall frequency of positive RASTs (among all employees) was even higher with 3 % in Manchester and 10.7 % in Leicester. This indicates that sensitisation had occurred in a higher number of employees, even though not all workers with a RAST positive test were symptomatic.

Interestingly, comparison of employees with work-related symptoms and asymptomatic ones showed no differences in age, duration of employment, smoking status, as well as no significant difference between the different exposure groups (workers had been grouped into 4 groups depending on their frequency of exposure ranging from almost all day (group 1), daily to frequently (group 2), regularly small quantities (group 3) and only previous, without current dye exposure (group 4)). Even though the study did not provide information about exact exposure doses, the occurrence of respiratory symptoms in exposure groups even with rare contact to dye powders indicates a high sensitising potential of RB5.

The study by Park and coworkers on all employees of one Korean dye company (309 employees) identified and clinically assessed 78 workers who showed symptoms of the lower respiratory tract (25 %). 38 of these 78 employees showed non-specific bronchial reactivity. 5 symptomatic workers were bronchially challenged with an RB5 solution and all showed a clear positive response, 2/5 showed an early response pattern and 3/5 a dual response (early response and long-lasting symptoms). It has to be noted that these tests were performed without a placebo control group and thus a false-positive response caused by psychogenic factors cannot be completely ruled out.

Among all workers, SPT and RAST were positive for RB5 in 25 (8 %) and 52 (17 %) cases, respectively. In good agreement with the findings of Docker et al. (1997), increased total levels of specific IgE antibodies (to RB5-HSA) were found not only in symptomatic employees (23/78 patients, 30 % among symptomatic, 7.4 % among all workers) but also in 26 asymptomatic ones, while in the control group no increased levels were detected. This demonstrates that exposure to the dye powder triggers in some cases sensitisation (RB5-specific IgE formation), but this does not nessessarily result in elicitation of respiratory symptoms or skin reactions afterwards.

On the other hand, there are also several cases where patients with a clear positive response in the bronchoprovocation test had negative results in RAST/SPT. This could either be attributed to a too low sensitivity of the tests performed or indicating that beside an IgE-mediated allergic reaction also other mechanisms could be involved. The authors hypothesised that non-IgE mediated pathways may rely on specific IgG antibodies. Two follow-up studies using blood sera from the same study (Park and Hong, 1991) as well as another investigation analysing blood sera from workers of one factory, where dye powders are produced and three factories located close by (Park et al., 1991a), however demonstrated that specific IgG seems to be formed in response to dye exposure (even to single exposure), but the correlation with asthmatic symptoms is poor. They therefore concluded later that IgG measurement could be used as an indicator for whether an exposure has taken place or not, but not as an indicator for an allergic response.

In the third comprehensive study, examinations were performed in 15 textile plants with attached dyehouses of varying size in Sweden (Nilsson, 1993). Among a total of 1142 employees, 229 workers were employed in

the dyehouses and laboratory departments, 162 of which were exposed to dye powders.

10 employees (6 % of exposed workers) had work-related respiratory or nasal symptoms (rhinitis, asthma, bronchitis). Among these, symptoms could be correlated to an IgE-mediated allergy to RB5 through positive SPT and/or RAST in 5 workers. In two out of these five, non-specific bronchial reactivity was observed in a metacholine challenge test. In one half of the symptomatic workers additional skin symptoms such as dermatitis, urticarial or Quincke oedema occured. Overall, on the basis of the anamnestic and immunological data, RB5 seems to have been the causative agent for allergic reactions in at least five cases.

From the three case-control studies, the overall frequency of occupational asthma induced by RB5 among the employees exposed to dye powder seems to be in the range of 1.2 – 3 % (frequency of symptomatic workers with confirmed allergy through a positive RAST or SPT result). The comparison with the unexposed control groups clearly showed that IgE formation was correlated to dye exposure. In all three studies, the overall sensitisation rates indicated by high levels of specific IgE antibodies were even higher, but only 30 - 50 % of RAST/SPT positives actually showed respiratory symptoms.

In order to elucidate to which extent a positive (or negative) result in SPT or the presence of specific IgE antibodies are predictive for the development of allergic symptoms, or able to diagnose an allergy, Park and co-workers performed SPT and ELISA tests on 42 patients with occupational asthma against reactive dyes (Park et al., 2001b). Their allergy was confirmed through bronchoprovocation tests with the respective causative agents (33 cases with RB5). In addition, 93 dye-exposed workers without clinical symptoms and negative bronchoprovocation challenge served as the negative (non-allergic) control group.

The authors compared test results of SPT and ELISA (specific serum IgE) and determined: a) sensitivity as the fraction of positive test results among confirmed allergic patients, b) specificity as the fraction of negative test results among confirmed non-allergic, but exposed workers, c) positive predictivity as the fraction of positive test results in allergic patients among all positive tests, and d) negative predictivity as fraction of negative test results within non-allergic, exposed workers among all negative tests. Overall, SPT yielded better correlations between test results and presence or absence of allergic symptoms than measurement of specific IgE. However, for both tests negative specificity and predictivity were high with 86 % and 80.8 % (specific IgE) and 91.4 % and 89.5 % (SPT), respectively. This means that a negative test result is a good predictor for the absence of an allergy.

Sensitivity and positive predictivity were in a similar range for SPT (76.2 % and 80 %), but the measurement of specific IgE yielded only values of 54 % and 63 %, respectively. This is in good agreement with the findings in the three case control studies (Docker et al., 1987; Nilsson et al., 1993; Park et al., 1991b) which detected a higher frequency of RAST-positives than of workers with allergic symptoms. This demonstrates that a positive RAST or ELISA (both measuring specific IgE) alone ist not sufficient to forecast the occurrence or severity of an allergic reaction and requires combination with clinical history or other assessments. However, also in the study by Park and co-workers (2001b), comparison with 16 unexposed subjects demonstrates that the formation of IgE is correlated to the dye exposure. It is uncertain to which extent asymptomatic workers with specific antibodies have a higher risk of developing allergic reactions later on as one of the workers investigated in the study by Docker (1987) did.

The level of exposure was not measured in any of the studies. It is thus not possible to draw conclusions about dose-response relationships. However, the development of occupational respiratory symptoms is also reported in exposure groups with less frequent contact or exposure to low amounts (even if not exactly quantified) of dye powders indicating a high respiratory potency.

In addition, in many cases symptoms were so severe that continuation of work associated with RB5 exposure was not possible and relocation to other working places without dye exposure was neccessary. This also may result in an underestimation of the overall frequency from the above studies as some workers who developed symptoms might have changed their job without contacting official bodies.

All three comprehensive studies were perfomed with workers employed in the dyeing industry. Thus, beside RB5, workers were exposed to other (reactive) dyes in use as well as to dyeing process related chemicals such as acids or bases. It was also reported that some of the workers also had positive SPT/RAST results for other dyes. However, tests on cross-reactivity showed variable patterns among the workers and no pattern of “typical” cross-reactivity. From the anamnestic and immunological data, RB5 could be identified as the

causative agent in the majority of cases.

The general evidence that RB5 is a causative agent for respiratory hypersensitivity is additionally supported by many case reports of workers mostly employed in the dye industry such as in textile plants or dye-producing companies (Table 16). In most of these case reports, allergy was confirmed to be IgE-mediated through RAST, SPT, or patch tests.

Bronchial provocation tests with RB5 were performed in studies by Estlander (1988): 1 patient; Park et al. (1989): 4 patients, Park et al. (2001b): 33/42 patients positive, and Park et al. (2007): 3/11 patients positive. The latter study investigated whether lung functions of patients with severe asthmatic symptoms recover after long-time avoidance of the reactive dyes and medical treatment. After the initial diagnosis of occupational asthma, the patients were re-examined twice, once after 2 - 6 years and once 11 - 16 years later. Beside SPT, a pulmonary function test as well as metacholine bronchial provocation were performed. Overall, the lung functions of the patients recovered neither in the first, nor in the second examination and non-specific airway hyper-responsiveness to methacholine also did not improve even though several of the patients were on medications. Interestingly, skin reactivity examined by SPT disappeared almost completely over time.

The exact mechanism leading to respiratory hypersensitivity is still not fully understood. However, to current knowledge it is assumed that a Th2-type immune response is triggered which results in the production of cytokines such as IL-4 and IL-5, and IgE antibodies (ECHA, 2017).

To the current knowledge, the mechanistic pathway of respiratory sensitisation includes four molecular key events. The first one consists of the covalent binding to proteins, the formation of a hapten. This molecular event is shared in principle with skin sensitisers and even though there are no validated *in vitro* tests available concerning respiratory sensitisation, it is assumed that respiratory sensitisers will yield positive results in tests for skin sensitisers such as the recently adopted OECD Test No. 442C (OECD, 2019): the Direct Peptide Reactivity Assay (DPRA). Lalko and co-workers tested a range of respiratory and skin sensitisers in the DPRA assay (Lalko et al., 2013a; Lalko et al., 2013b; Lalko et al., 2012). It is noteworthy that in these studies RB5 was used as a positive control for respiratory sensitisers. RB5 gave positive results with lysine and cysteine-bearing peptides with a slight preference for lysine over cysteine (ratio ~1.2). A similar pattern was found for several other respiratory sensitisers contrary to the tested skin sensitisers. The authors thus conclude that a ratio of lysine- to cysteine-binding in DPRA could be used to differentiate skin and respiratory sensitisers. So far, mechanistic knowledge is still limited (for review see Basketter et al. (2017)).

The ability to bind to proteins requires a functional group capable of forming covalent bonds with amino acid residues present in the proteins. Similar to skin sensitisers, respiratory sensitisers thus either need to be themselves electrophilic or require conversion into electrophilic species in order to react with nucleophilic amino acids. For RB5, it is assumed that the activation of the sulphoxyethylsulfonylphenyl group yielding the vinylsulphonylphenyl group is a prerequisite and protein binding occurs, for instance, via Michael-addition of the vinyl group to cysteine residues. This transformation to an electrophile is in fact the mechanistic basis of the textile dyeing process, where the activated bis-vinyl form reacts with amino acid residues of cellulose fibres and thus forms a covalent and stable link to the textile.

In good agreement with this, only the activated bis-vinyl form triggers profiler alerts in the OECD QSAR Toolbox (version 4.4) for: a) protein binding by OECD and by OASIS through Michael addition of polarised alkenes forming polarised alkenesulfones and b) protein binding potency by GSH “highly reactive”.

During the dyeing process, the activated form is generated by basic treatment of RB5. Several available *in vitro* studies (Table 18) support the assumption that activation of RB5 is required for efficient protein binding:

- a negative peptide binding study by Wass andBelin (1990), where incubation of RB5 dye and peptide was performed at neutral pH and 37 °C (incubation time 10 min),
- another study by the same authors (Wass et al., 1990) with the aim of improving sensitivity of the RAST assay through optimisation of HSA-RB5 conjugation efficiency. Best results were obtained by incubating HSA and RB5 at pH 8.8 and 20 °C for 1 h.
- protein binding study by Sarlo andClark (1992) confirming covalent binding to guinea-pig serum albumin when protein and dye are reacting under alkaline conditions.

It has to be noted that in abiotic degradation studies (Lead Dossier substance [2]) not only under alkaline condition, but also at neutral pH and elevated temperature, Reactive Black 5 [1] is hydrolysed. The absence of peptide binding at neutral pH can be explained by low temperature in combination with short reaction time.

There is no validated test model for respiratory sensitisation in animals. The few available animal studies do not significantly contribute to the overall evidence of respiratory sensitisation potential of RB5 and its activated bis-vinyl derivative. Sarlo andClark (1992) reported positive effects in an active cutaneous anaphylaxis test and dose-dependent IgG antibody production in the inhalation model. Neither in inhalation, nor in dermal induction experiments with guinea pigs an impairment of lung function was observed. However, the evaluated lung function parameters are limited and not validated to estimate allergic responses of the respiratory tract.

In addition, in a modified LLNA reported by (Dearman et al., 2013) the outcome was clearly positive and dose-dependent proliferation of lymphocytes was observed. The LLNA is a validated and accepted test method to determine skin sensitisation potential of chemicals (OECD Test No. 429). However, to current knowledge, all low molecular weight respiratory sensitisers are also skin sensitisers (ECHA, 2017). Thus they should give positive results in skin sensitising test methods such as LLNA. Nevertheless, it has to be noted that a positive result is not always correlated with a respiratory sensitisation potential.

Based on human data, the registrants self-classified the UVCB substance [2] as skin and respiratory sensitiser, category 1. Overall, the respiratory sensitisation potential of RB5 is clearly evident. Due to the severity of the symptoms reported in the case studies and a high frequency among ocupationally exposed populations as well as occurrence in (occupational) exposure groups with rare contact to dye powders, a high potency is assumed and classification into subcategory 1A is considered justified.

10.4.5 Comparison with the CLP criteria

According to CLP (Annex I, Table 3.4.1), "*Substances shall be classified as respiratory sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:*

(a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity; and /or

There is evidence in humans that RB5 induces asthma. Furthermore, it is apparent that also RB5 bis-vinyl induces asthma as this substance has been identified as a metabolite of RB5 and as RB5 bis-vinyl represents the activated form of RB5. Activation of RB5 is the prerequisite for hapten formation, one of the key events leading to respiratory sensitisation.

(b) if there are positive results from an appropriate animal test.

No adequate animal studies performed with RB5 are available to conclude on respiratory sensitisation. But as stated on note ([1]) of Annex I, Table 3.4.1: "([1]) *At present, recognised and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circum stances, data from animal studies may provide valuable information in a weight of evidence assessment."*

The experimental animal data available clearly demonstrate that RB5 is a skin sensitiser. As all known respiratory sensitisers are skin sensitisers as well, these data may thus be indicative of the potential of RB5 to cause respiratory sensitisation in humans.

Are data sufficient for subcategorisation?

- *Subcategory 1A: "Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests ([1]). Severity of reaction may also be considered.*

- *Subcategory 1B: "Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests ([1]). Severity of reaction may also be considered."*

From health investigations among dyehouse workers, frequencies of occurrence can only be derived for occupationally exposed populations. Studies by Docker et al. (1987), Park et al. (1991b), Nilsson et al. (1993) indicate a high frequency of around 1.4 – 3.3 % among dye powder exposed workers. In addition, severity of the symptoms reported in the case studies is high, which in many cases impedes continuation of the current

work and requires relocation of employees to other workplaces. In addition, the long-term study of Park et al. (2007) demonstrated that in cases with severe occupational asthma, even after long-time avoidance of the causative dyes, there is no recovery in lung function, i.e. effects are irreversible. Taken together, classification into subcategory 1A is justified.

10.4.6 Conclusion on classification and labelling for respiratory sensitisation

Reactive Black 5 (substances [1] and [2]) and Reactive Black 5 bis-vinyl (substance [3]) should be classified as Resp. Sens. Cat. 1A, H334 according to the CLP Regulation. The generic concentration limit of ≥ 0.1 % is proposed.

10.5 Skin sensitisation

All available studies are listed in the tables below.

Table 19: Summary table of animal studies on skin sensitisation.

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results**	**Reference**
LLNA (OECD TG 429 with changes: other strain than usual) **KEY-study, Reliabilty 2**	BALB/c strain mice, n=4	RB5, 70 % pure (Ecological and Toxicological Association of Dye and Organic Pigments Manufacturers)	C = 5,10, 25 % in dimethyl formamide (DMF) (25 µL), Initiation: daily dermal application for 3 days, after 5 days i.v. 20 µCi of ^{3}H-methyl thymidine	**Positive, Dose-dependent proliferation of lymphocytes,** **SI >3 in all three tested concentrations:** (5 %, SI 8.6; 10 %, SI 6.8; 25 %, SI 11.8)	Dearman et al. (2013)
Guinea pig maximisation test, OECD TG 406 Reliability 1 (dossier)	Guinea pig, female, Determination of primary not irritating concentration: 6 Determination of intradermal tolerability: 3 Sentinel group: 5 Control group: 5 Treatment group: 10	RB5 (Remazol-Schwarz B, [2]), Constituent (liquid), for detailed information about purity see confidential annex	Induction: intradermal 5 % and epicutaneous 100 % Challenge: epicutaneous (occlusive) 100 % First reading 48 h after challenge, second reading 72 h after challenge	**Negative** No animals with reactions: 0/10 after 48 h and 72 h Clinical observation: blue staining of skin	Hoechst AG (1987)

Table 20: Summary table of human data on skin sensitisation

Type of data/report	**Test substance**	**Relevant information about patients/study, tests performed**	**Observations / Results** **(bold: summary of results, bold/blue: exposure group and frequency according to CLP guidance Table 3.2)**	**Reference**
Occupational case report of 1 patient working in a reactive dye	RB5 (trade name Levafix black E-B) (most likely substance [2]) No information on	Patient developed rhinitis and cough and 1 year later **eczema on hands and front of the neck** after company	**Positive for RB5, skin allergy developed 1 year later than respiratory symptoms** SPT: mild positive with human serum albumin (HSA)-conjugated to RB5,	Thorén et al. (1986)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results (bold: summary of results, bold/blue: exposure group and frequency according to CLP guidance Table 3.2)	Reference
producing company Exposure to RB5 and other reactive dyes	purity of the test substance	started to produce reactive dyes Tests: SPT, RAST, IgE-level	strong positive with non-conjugated RB5 RAST: negative IgE level was elevated Patient's symptoms disappeared after exposure ceased	
Occupational case reports of 5 patients, all worked in dyehouses or textile plants being exposed to dye powders (RB5 among others)	RB5 (Remazol Black B) (most likely substance [2]) TLC analysis of RB5 used indicates impurities. The dye contained 9 µg/g water soluble chromium.	5 cases of occupational **eczema**, **urticarial** and respiratory disease 3/5 patiens had respiratory symptoms (2/5 asthma), all three **additionally** had **skin symptoms** (eczema and/or urticaria) **2/5 patiens had only skin symptoms (eczema)** Tests: patch, scratch chamber and prick test, nasal challenge (only with patients showing respiratory symptoms)	**2 patients demonstrated clear allergic reactions to RB5** Patch test: 2/5 patients positive (**one with only skin symptoms**, **1 with both skin and respiratory symptoms**) The patient with respiratory symptoms was also positive after: - scratch- or prick-testing (RB5) - nasaly challenge (RB5) 4/5 patients could not continue their work due to severe allergic reactions.	Estlander (1988)
Case study of a textile artist (46 y, f) developing dermatitis on her hands and arms	RB5 (most likely substance [2])	Symptoms started 6 years after the patients started to work with reactive dyes Test: Patch tests	**Strongly positive patch test results for RB5** (and Reactive Blue 21) No further details given on methods/results.	Estlander et al. (1990)
Case-control study (dye industry, Korea) 309 dye exposed workers	RB5 (trade name Rifazol black GR) (most likely substance [2]) No information on purity of the test substance dye powder used as obtained without purification	All exposed employees (309): 1. Questionaire 2. Clinical Test: SPT, RAST, RAST inhibition (cross-reactivity of different dyes), specific IgE level, bronchoprovocation Co-exposure of workers to other reactive dyes also produced within the company: Rifacion orange HE 2G (O-20), Rifacion red HE 313, Rifacion navy blue HER, Rifafix yellow 3 RN, Rifafix red BBN,	**Allergic reactions to RB5 confirmed correlated with occupational exposue to reactive dye powder** 1. Questionnaire: 78 (25.2 %) of the exposed workers had work-related lower respiratory tract symptoms; **3 of them with additional skin symptoms (0.9 %)** SPT positive: 25/309 (8.1 %) RAST positive : 53/309 (16.8 %) **Selected workers with known exposure (non-quantified), Low-moderate frequency (0.9 %)**	Park et al. (1991b)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results (bold: summary of results, bold/blue: exposure group and frequency according to CLP guidance Table 3.2)	Reference
		Rifazol brilliant orange 3R (O-16)		
Case-control study Textile plants in Sweden 1142 exployees, 162 exposed to reactive dyes	RB5 (trade name Remazol black B) (most likely substance [2]) No information on purity of the test substance	1. Interviews in 15 textile plants in western Sweden (1142 employees) 2. clinical investigations of 162 workers exposed to reactive dyes (RB5 among others) Tests: spirometry, metacholine challenge test, IgE levels, RAST, RAST inhibition, SPT and patch test RAST, SPT and patch test perfomed with 9 suspected commercial reactive dye powders (brought in by the patients)	**RB5 causative agent for occupational respiratory allergy which is sometimes accompanied by skin symptoms** 1. Interviews: 162/1142 workers exployed in dyehouse and laboratory departments exposed to dye powder 2. Clinical investigations Workers with respiratory symptoms: -10 (6 %) of exposed workers had work-related respiratory or nasal symptoms: 8 rhinitis, 6 asthma, 7 bronchitis, SPT positive (RB5): 5 RAST positive (RB5): 4 Patch test: none positive IgE level slightly elevated in 5 patients **5/10 had additional skin symptoms (dermatitis, urticaria), 3/10 (1.8 % of all exposed workers, 0.3 % among all workers) positive SPT against RB5** -5 workers only skin symptoms, no positive Patch test/ SPT to reactive dyes, no increased IgE levels **All workers: low/moderate frequency** **Exposed workers: high frequency**	Nilsson et al. (1993)
Case study on patients referred to allergological department who underwent patch testing	RB5 (Remazol Black B Gran, Hoechst©) (most likely substance [2])	Patch tests with GIRDCA (Italian Research Group on Contact and Environmental Dermatitis) standard series and 12 reactive dyes in 1813 patients (non-occupational exposed)	18/1813 positive to reactive dyes, among them **2 positive with RB5** (< 0.1 %) **Consecutive dermatitis patients** **Low frequency**	Manzini et al. (1996)
Case report of a 32-year old man with work-related dermatitis on the dorsa of	RB5 among other dyes (most likely substance [2])	Patch test with standard textile series and samples of suspected dyes	The patient gave positive results for RB5 and Reactive Blue 225 in the patch test on day 4.	Wilkinson andMcGechaen (1996)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results (bold: summary of results, bold/blue: exposure group and frequency according to CLP guidance Table 3.2)	Reference
the hands, wrists and forearms, working as chemical process operator				
Case report on 3 patients which became sensitised to reactive dyes through Patch tests	RB5 among others (most likely substance [2])	Patch test with standard, medicaments and textiles series, textile series included a 5 % pet. dilution of RB5	2/3 patients became sensitised to RB5 through initial negative patch test.	Sommer andWilkinson (2000)
Case studies with 644 patients suspected to have textile caused contact dermatitis	RB5 (most likely substance [2]) (5 % pet.), as part of textile colour finish series	All patients were Patch tested with: standard series (TRUE Tests), textile colour and finish series (TCFS) and additional series, as well as clothing extracts in 21 cases	**2 patients (0.3 %) positive patch test response to RB5** **Selected dermatitis patients** **Low fequency**	Lazarov (2004)
Case report on 1 patient with axillary and neck dermatitis which had been present for about 1 year	RB5 (most likely substance [2]) (5 % pet.), as part of textile series (Chemotechnique)	The patient had a history of intolerance to perfumes, nickel and to dark cotton T-shirts he had to wear at workplace Patch testing was performed with the European standard series and the Chemotechnique textile series (Malmö, Sweden)	**Positive patch test (2+ on D2 and D4)** Additionally, the patient database revealed another patient with textile dermatitis (eczema from dark sport pants) and a positive patch test for RB5 (1+, D4)	Moreau andGoossens (2005)
Case report of one patient (f, 54 y) which showed a late reaction to reactive dyes in Patch test	RB5 (most likely substance [2]) (incl. in textile series)	Patient with recurrent events of acute dermatitis Patch testing was performed with the European standard series and textile series	**Initial patch test negative, symptoms occurred at patch test area after 2 weeks;** **Retesting gave positive result for RB5 at D17 (+1 reaction)**	Slodownik andIngber (2005)
Case report of a patient (f, 27 y) with non-occupational skin allergy against	RB5 (most likely substance [2]) (1 % pet) (as part of textile series)	A 27-year-old woman, working in bakery delivery, presented with a 7-month history of pruriginous eczematous lesions affecting the medial aspect of her arms, palms,	**Positive reaction at D4 (++) for RB5** Symptoms probably caused by textiles, became better after avoidance of dark textiles	Pérez-Crespo et al. (2009)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results (bold: summary of results, bold/blue: exposure group and frequency according to CLP guidance Table 3.2)	Reference
reactive dyes		submammary folds, flanks, periaxillary, and lumbar areas. She had a history of mild atopic dermatitis, controlled with emollients.		
Case study of 41 children (<18y) with sole dermatitis	RB5 (most likely substance [2]) (5 % pet.) as part of textile series for patch test	Retrospective analysis of patch test data from 1997-2009 of Edinburgh Department of Dermatology	**19 children tested with textile series, thereof one patient with positive patch test for RB5 (and other reactive dyes)** **Selected dermatitis patients (involving the soles)** **High frequency**	Darling et al. (2012)

Table 21: Summary table of other studies relevant for skin sensitisation.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD 442C - in chemico skin sensititisation	RB5 (Black GR) (most likely substance [2]) purity 55 %	Comparison of known respiratory sensitiser with known skin sensitiser in DPRA	RB5: Both strong: 99.5 % Lysine (Lys) and 77.3 % Cysteine (Cys) depletion Majority of respiratory sensitisers had a higher reactivity towards Lys.	Lalko et al. (2012)
Modified DPRA	RB5 (Black GR) (most likely substance [2]) purity 55 %	Peroxidase Peptide Reactivity Assay (PPRA) as a refinement to the DPRA	RB5 was also positive here, but selectivity to Lys was lost for all respiratory sensitisers	Lalko et al. (2013a)
DPRA	RB5 (Black GR) (most likely substance [2]) purity 55 %	DPRA with known respiratory sensitisers on lysine-, cysteine-, histidine-, arginine- and tyrosine-bearing peptides; Competitive DPRA with different Lys:Cys ratios	Ratio for RB5 with slight preference for Lys over Cys was confirmed (85 % Lys depletion, 82 % Cys depletion), RB5 has no reactivity towards other aminoacid residues investigated Majority of respiratory sensitisers had a higher reactivity towards Lys, with the exception of isocyanates which show preference for cysteine.	Lalko et al. (2013b)

10.5.1 Short summary and overall relevance of the provided information on skin sensitisation

To the current knowledge it is assumed that all low molecular weight respiratory sensitisers exhibit skin sensitising properties due to the common key event of hapten formation in the underlying mechanism of the induction (ECHA, 2017).

Supporting this assumption for RB5, protein binding studies (Lalko 2012 and 2013) and a mouse LLNA (Dearman et al., 2013) performed most likely with substance [2] were positive. As discussed in detail in the previous chapter, it is is assumed that activation of the sulphoxyethylsulfonylphenyl group of substance [1] yielding the vinylsulphonylphenyl group is a prerequisite for the protein binding ability and thus justifies to

translate test results of “Reactive Black 5” (as monoconstitute substance [1] as such or as a constitutent of substance [2]) to its metabolite RB5 bis-vinyl [3].

In the LLNA study, a dose-dependent proliferation of lymphocytes after dermal exposure to RB5 was observed. The study deviated from OECD TG 429 in the choice of animal strain: BALB/c mice were used instead of CBA/Ca mice as recommended in the guideline. However, the authors performed further tests on these two mouse strains using two other reference chemicals and a broad concentration range in order to demonstrate that the BALB/C strain is of sufficient sensitivity for characterizing responses in the LLNA assay. In all concentrations used, the tested substance yielded stimulation indexes (SI) clearly above 3. It is expected that concentration below 5 % will result in a stimulation index at or above 3. However a concentration below 5 % has not been tested, and the dose-response relationship also does not allow to extrapolate the data to obtain a reliable EC3 value. Thus the available data do not allow to draw a conclusion on the potency of “Reactive Black 5” for skin sensitisation.

In contrast, the lead dossier for the UVC substance [2] contains a report on a negative guinea pig maximization test performed occording to OECD TG 406 with most likely the UVCB substance [2] and the dossier submitter therefore assigned a reliability of Klimisch score 1. Even though no reaction was reported, is has to be noted that a blue staining of the animals skin was observed in all animals. This staining might have prevented the detection of redness of the skin, which is the primary readout of the test, so that the negative results have to be questioned. Considering the clearly positive result of the LLNA and supporting evidence from human cases, no weight is given to this test.

Additional evidence for the skin sensitising potential of “Reactive Black 5” is obtained from human case reports. In these reports, dermal eczema and urticaria frequently accompanied respiratory symptoms in occupationally exposed workers (Estlander, 1988; Nilsson et al., 1993; Park et al., 1991b; Thorén et al., 1986). In some cases, the development of skin symptoms only is reported, where the allergic reaction could be specifically attributed to RB5 by SPT or patch tests (Estlander, 1988; Estlander et al., 1990; Moreau and Goossens, 2005; Wilkinson and McGechaen, 1996).

In addition, it was reported that in some patients sensitisation to “Reactive Black 5” was induced through the patch testing (Slodownik and Ingber, 2005; Sommer and Wilkinson, 2000). Here, initially negative patch tests became positive upon a secondary testing strongly indicating skin sensitising potential of “Reactive Black 5”. Few cases are reported in which a textile caused contact dermatitis which could subsequently be attributed to an allergy against “Reactive Black 5” (Lazarov, 2004; Moreau, 2005; Pérez-Crespo, 2009; Darling 2012).

The overall frequency of allergic skin reactions derived from work place studies or studies on dermatitis patients is low to moderate. Only from the study by Darling et al (2012) a high frequency can be derived upon selected dermatitis patients (children with affected sole). However, the overall number of patients in this study is only 19 and the frequency derived from other studies with higher numbers of patients/test persons is thus more reliable. In neither of the available studies exact exposure concentrations have been measured or reported.

In conclusion, there is sufficient evidence for a skin sensitising potential of “Reactive Black 5” (as monoconstitute substance [1] as such or as a constitutent of substance [2]). The overall frequency for allergic skin reactions is low to moderate, but as neither human exposure data are available nor derivation of the potency from animal studies is possible, sub-categorisation based on the available data is not possible. Thus, classification as Skin Sens. 1 is proposed.

10.5.2 Comparison with the CLP criteria

According to CLP (Annex I, Table 3.4.2), “*Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:*

(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

There is evidence in humans that “Reactive Black 5” (as monoconstitute substance [1] as such or as a constitutent of substance [2]) induces contact allergy. Furthermore, it is apparent that also RB5 bis-vinyl [3] induces contact allergy as this substance has been identified as a metabolite of substance [1] and as RB bis-vinyl [3] represents the activated form of substance [1]. Activation of substance [1] is the prerequisite for

hapten formation, one of the key events leading to skin sensitisation.

(b) if there are positive results from an appropriate animal test."

According to the CLP guidance, the sensitising potential of a substance is identified if a significant effect has been observed in an acceptable in vivo test such as the LLNA or the GPMTand the Buehler assay. A significant response in an LLNA assay is given when a Stimulation Index (SI) ≥ 3 is obtained. The LLNA performed most likely with substance [2] (Dearman et al., 2013) yields a SI ≥ 3 for all three tested concentrations (5, 10, 25 %) and is thus clearly positive.

Data are sufficient for subcategorisation, if the following is observed (according to CLP Annex I, Table 3.4.2):

"Subcategory 1A: high frequency of occurrence in humans and/or a high potency in animals
Subcategory 1B: a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals"

Based on these criteria, the available data for Reactive Black 5 is not sufficient for sub-categorisation.

From the human case report, an overall low to moderate frequency of occurrence can be derived, while the studies do not provide exposure data. Subcategorisation from LLNA results requires an EC3 value below or above 2 %. However, the available data does not allow for the determination of an EC3 value (as a concentration below 5 % was not tested and extrapolation is not possible) and consequently no potency can be derived.

10.5.3 Conclusion on classification and labelling for skin sensitisation

It is poposed to classify substances [1] and [2] (collectively refered to as "Reactive Black 5") and Reactive Black 5 bis-vinyl [3] as Skin Sens. 1, H317 according to CLP Regulation. A generic concentration limit of ≥ 1.0 % is proposed.

10.6 Germ cell mutagenicity

Not assessed.

10.7 Carcinogenicity

Not assessed in this dossier

10.8 Reproductive toxicity

Not assessed in this dossier

10.9 Specific target organ toxicity-single exposure

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

13 ADDITIONAL LABELLING

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15 ANNEXES

Confidential Annex to the CLH report