ECHA
EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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-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]

CLH-O-0000007139-70-01/F

Adopted

2 June 2022

2 June 2022

CLH-O-0000007139-70-01/F

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

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

Chemical name:

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-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]**

The proposal was submitted by **Germany** and received by RAC on **16 July 2021.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **6 September 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **5 November 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Beata Pęczkowska**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **2 June 2022** by **consensus.**

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	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-5-hydroxy-3,6-bis{[4-(vinylsulfonyl) phenyl]diazenyl}naphthalene-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	-	-	-
RAC opinion		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-5-hydroxy-3,6-bis{[4-(vinylsulfonyl) phenyl]diazenyl}naphthalene-2,7-disulfonate [3]			Resp. Sens. 1A Skin Sens. 1	H334 H317	GHS08 Dgr	H334 H317	-	-	-
Resulting Annex VI entry if agreed by COM		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-			Resp. Sens. 1A Skin Sens. 1	H334 H317	GHS08 Dgr	H334 H317	-	-	-

		aminophenyl)sulfonyl]ethyl hydrogen sulfate, sodium salts; [2] disodium 4-amino-5-hydroxy-3,6-bis{[4-(vinylsulfonyl) phenyl]diazenyl}naphthalene-2,7-disulfonate [3]									

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The substances: 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-5-hydroxy-3,6-bis{[4-(vinylsulfonyl) phenyl]diazenyl}naphthalene-2,7-disulfonate [3] have no current entries in Annex VI to the CLP regulation.

Substance [1] is a mono-constituent substance while substance [2] is a UVCB substance having substance [1] as a constituent in relevant concentrations ranging from 50-80%. Substance no [3] can be formed under basic conditions from substance [1] and is a metabolite of substance [1], representing its activated form.

The CLH report has been created based on 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. It is not possible to unambiguously state which data has been generated on what substance. 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).

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 unambiguously identify any of these commercial products with either substance [1] or [2], it is assumed, that in most cases substance [2] was described.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

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.

The DS evaluated respiratory sensitising potential of Reactive Black 5 (RB5) based on results of three comprehensive health investigations among dyehouse employees in the United Kingdom (UK), Korea and Sweden and occupational case reports summarised in tables below.

Human data

Summary table of human Cross-sectional studies on respiratory sensitisation

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
Cross-sectional 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 (all identical chemical structure) 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 (SPT) on 19 symptomatic workers, RAST (radio allergo sorbent test) 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)
Cross-sectional study (dye-producing industry, Korea) 309 workers of dye-producing factory	RB5 (trade name Rifazol black GR) 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 exposure 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, i.e. 1.6% of all employees (2/5 early reaction, 3/5 dual (early/late reaction) SPT positive: 25/309 (8.1 %) RAST positive : 52/309 (16.8 %) RAST inhibition: Orange 3R showed cross reactivity with RB5, inhibition only effective with HSA-dye conjugate (and not free dye)	(Park et al., 1991b)
Cross-sectional study Textile plants in Sweden 1142 employees, 162 exposed	RB5 (trade name Remazol black B) No information on purity of	1. Interviews in 15 textile plants in western Sweden (1142 employees) 2. Clinical investigations of 162 workers exposed to reactive dyes (RB5 among others)	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	(Nilsson et al., 1993)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
to reactive dyes	the test substance	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)	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	

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) 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 working in a reactive dye producing company	RB5 (trade name Levafix black E-B) No information on purity of the test substance	Patient developed rhinitis and cough and 1 year later eczema on hands and front of the neck after company started to produce reactive dyes (RB5 among others) Tests: SPT, RAST, IgE-level	Positive for RB5, skin allergy developed later than respiratory symptoms 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	(Thorén et al., 1986)
Specific IgE measurements in blood sera of allergic dye house operatives	RB5 (trade name Remazol Black B) 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 (Remazol Black B) Dye powder used as obtained without purification	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 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):	(Estlander, 1988)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
(RB5 among others)	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.	2/5 patients exhibited skin symptoms only (eczema) Tests: patch, scratch chamber and prick test, nasal challenge (only for patients showing respiratory symptoms)	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.	
Occupational case report on 9 patients, all exposed to reactive dyes during their work (dye industry, Korea) Exposure to RB5 and other reactive dyes	RB5 (trade name Rifazol black GR) No information on purity of the test substance	9 patients with asthmatic symptoms Measurements: IgE level, RAST, RAST inhibition (cross-reactivity), Prick skin test, Bronchial challenge with methacholine and reactive dyes Methods of bronchoprovocation: Forced expiratory volume in 1 second (FEV_1) 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 FEV_1 was recorded; pulmonary function test was performed every 9 or 10 hours after challenge	Clearly positive for RB5 - SPT: 9/9 positive to RB5 -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	(Park et al., 1989)
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) 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 and Hong, 1991)
Study on specificity of IgE antibodies	RB5 (trade name Rifazol black GR) 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-human serum albumin (HSA) conjugates and cross-reactivity with orange 3R differed from one patient to another	(Hong and Park, 1992)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
Study on prevalence of specific IgE and IgG in workers of a dye factory and four 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)	RB5 (trade name Rifazol black GR) No information on purity of the test substance	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 %) 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	(Park et al., 1991a)
Study of relevance of SPT and specific IgE measurements in diagnosis of occupation asthma	RB5 (trade name Rifazol black GR) 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 %) → negative predictivity (real negatives (non-allergic and negative tests) among all negative tests): 80/99 (80.8 %)	(Park et al., 2001b)
Study on long-term occupational asthma	RB5 (trade names Remazol black GR (Black SF-GR) and Remazol black B (Black B))	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	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 %)	(Park et al., 2007)

Type of data/report	Test substance	Relevant information about patients/study, tests performed	Observations / Results	Reference
	No information on purity of the test substance	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.	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>Second examination:</u> 0 (negative) – 10/11 (Black B), 8/11 (Black SF-GR) < 0-1 – 1(11) (Black B), 3/11 (Black SF-GR)	

Animal Studies

There are three non-guideline *in vivo* studies performed in guinea pigs and one LLNA in BALB/c mice summarised in tables below.

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)	Test material information: RB5 (Remazol Schwarz B - Pt. 7/88), 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)	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 (Dossier RB5, Key study)

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, duration of exposure	Evaluation parameters	Results	Reference
	8 in test group: 8	Male: 150, 210 mg/m^3 Female: 140, 180 mg/m^3			
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, obtained from Hoechst-Celanese, no information about purity) Induction: subcutaneous 6.7×10^{-3} M, 6.7×10^{-4} M, 6.7×10^{-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, 48 h 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 and Clark, 1992) (Dossier RB5, supporting study)
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, 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 and Clark, 1992)
LLNA (guideline with changes: other strain than	BALB/c mice, n=4	Test substance: RB5 (Remazol Schwarz B, 70 % pure) C = 5,10,	Lymph nodes	Positive, Dose-dependent proliferation of lymphocytes, SI >3 in	(Dearman et al., 2013)

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, duration of exposure	Evaluation parameters	Results	Reference
recommended in the guideline		25 % in dimethyl formamide (DMF) (25 µL), initiation: daily dermal application for 3 days, challenge after 5 days with i.v. 20 µCi of ^{3}H-methyl thymidine		all three tested concentrations	

In Vitro Studies

There are few *in vitro* studies available on RB5, summarised in table below.

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 and Belin, 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 and Clark, 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	Comparison of known respiratory sensitiser to known skin sensitiser in DPRA	RB5: Both strong: 99.5 % lysine and 77.3 % cysteine depletion	(Lalko et al., 2012)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
	55 %) among others		Majority of respiratory sensitisers had a higher reactivity towards lysine.	
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)

Human data

In the three cross-sectional studies in dyehouses the overall frequency (point prevalence) of occupational asthma induced by RB5 among the employees exposed to dye powder seems to be in the range of 1.2-3.3 % (frequency of respiratory symptomatic workers compatible with asthma with confirmed RB5 allergy through a positive RAST or SPT result). Furthermore, in one of the studies 1.6% of employees had a positive specific bronchial provocation test for RB5. 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 a cross-sectional setting it is not possible to assess how many of the immunologically sensitised would have later developed respiratory symptoms or a clinically established asthma or other allergic respiratory disease. 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 necessary. 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.

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.

All three cross-sectional studies were performed 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. 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): 10/11 patients positive at baseline. 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 methacholine 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 all of the patients were on proper asthma medications and had stopped smoking after the diagnosis. Interestingly, skin reactivity examined by SPT disappeared almost completely over time.

Animal data

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 and Clark (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. Since all low molecular weight respiratory sensitisers are also skin sensitisers (ECHA guidance R.7a, 2017), they should thus give positive results in skin sensitising test methods such as LLNA. Nevertheless, it has to be noted that a positive result in a skin sensitising test is not always correlated with respiratory sensitisation potential.

In vitro studies

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.

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".

Several available *in vitro* studies support the assumption that activation of RB5 is required for efficient protein binding:

- a negative peptide binding study by Wass and Belin (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 and Clark (1992) confirming covalent binding to guinea-pig serum albumin when protein and dye are reacting under alkaline conditions.

The respiratory sensitisation potential of RB5 is clearly evident based on the data available for Reactive Black 5. Due to the severity of the symptoms reported in the case studies and a high frequency among occupationally 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 proposed by DS.

Comments received during consultation

One MSCA commented the proposed classification for respiratory sensitisation and supported the DS proposal for classification as Resp. Sens. 1A, however MSCA questioned sub-categorisation and no clear threshold value for considering "high frequency" of occurrence in humans for respiratory sensitisation in CLP guidance but agrees with taking into account consideration criteria for high frequency for skin sensitisation.

One organisation – importer (lead registrant of substance [2]) commented that self-classification – Resp. Sens. 1 is allocated in their registration dossier, however, lead registrant supports the harmonised classification proposed by DS.

Assessment and comparison with the classification 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
b) if there are positive results from an appropriate animal test.

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.

There are no validated animal studies for the identification of respiratory sensitisers, however the experimental animal data available clearly demonstrate that RB5 is a skin sensitiser (Dearman et al., 2013). 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.

According to ECHA guidance on the application of the CLP criteria (2017) "there is currently no clear way of establishing sub-categories for respiratory sensitisation, however if compelling evidence were available such as observations in the workplace, it may be possible to determine a sub-category".

From health investigations among dyehouse workers, frequencies of occurrence of respiratory sensitisation 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 RB5 related asthma around 1.2-3.3 % among dye powder exposed workers. It is also noted that due to the

cross-sectional design, these estimates underestimate the true frequency as they do not include cases that already left the company due to severe symptoms or those already sensitised that would develop asthma and other respiratory symptoms after the cross-sectional survey. 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, use of proper medication and cessation of smoking, there is no recovery in lung function, i.e. effects are irreversible.

The available data are considered as sufficient for sub-categorisation:

- 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. 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. Severity of reaction may also be considered."

The substance shows a high frequency of occurrence of respiratory sensitisation in humans[1]. Furthermore, considering severity of reactions (Park et al. 2007; Estlander, 1988) and the probability of occurrence of a high respiratory sensitisation rate in humans based on animal LLNA study (Dearman et al., 2013), RAC agrees with the DS that sub-categorization is warranted. Therefore, RB5 (substances [1] and [2]) and RB5 bis-vinyl (substance [3]) should be classified **as a Resp. Sens. 1A, H334**, according to CLP Regulation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS evaluated skin sensitising potential of Reactive Black 5 (substances [1] and [2]) and Reactive Black 5 bis-vinyl (substance [3]) based on results of two *in vivo* animal studies and human data on skin sensitisation and supportive *in vitro* protein binding studies.

All available studies are listed in the tables below

[1] since no threshold values are provided in 'ECHA guidance on the application of the CLP criteria (2017)', the thresholds from table 3.2 of CLP guidance were taken into account for considering "high frequency" of occurrence of respiratory sensitisation

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) Non GLP **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 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,** **Stimulation index (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) GLP	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), 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 (Registration Dossier RB5)

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 producing company Exposure to RB5 and other reactive dyes	RB5 (trade name Levafix black E-B) No information on purity of the test substance	Patient developed rhinitis and cough and 1 year later **eczema on hands and front of the neck** after company started to produce reactive dyes Tests: SPT, RAST, IgE-level	**Positive for RB5, skin allergy developed 1 year later than respiratory symptoms** SPT: mild positive with human serum albumin (HSA)-conjugated to RB5, strong positive with non-conjugated RB5 RAST: negative IgE level was elevated Patient's symptoms disappeared after exposure ceased	(Thorén et al., 1986)
Occupational case reports of 5 patients, all worked in dyehouses or textile plants being exposed to dye powders (RB5 among	RB5 (Remazol Black B) TLC analysis of RB5 used indicates impurities. The dye contained 9 µg/g water	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 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:	(Estlander, 1988)

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
others)	soluble chromium.	**2/5 patiens had only skin symptoms (eczema)** Tests: patch, scratch chamber and prick test, nasal challenge (only with patients showing respiratory symptoms)	- scratch- or prick-testing (RB5) - nasal challenge (RB5) 4/5 patients could not continue their work due to severe allergic reactions.	
Case study of a textile artist (46 y, f) developing dermatitis on her hands and arms	RB5	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)
Cross-sectional study (dye industry, Korea) 309 dye exposed workers	RB5 (trade name Rifazol black GR) No information on purity of the test substance dye powder used as obtained without purification	All exposed 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)	**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)
Cross-sectional study Textile plants in Sweden 1142 exployees, 162 exposed to reactive dyes	RB5 (trade name Remazol black B) 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 performed 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 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, 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),**	(Nilsson et al., 1993)

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
			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**	
Case study on patients referred to allergological department who underwent patch testing	RB5 (Remazol Black B Gran, Hoechst©)	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 the hands, wrists and forearms, working as chemical process operator	RB5 among other dyes	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 and McGechaen, 1996)
Case report on 3 patients which became sensitised to reactive dyes through Patch tests	RB5 among others	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 and Wilkinson, 2000)
Case studies with 644 patients suspected to have textile caused contact dermatitis	RB5 (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 (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 day 2 and day 4 (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 and Goossens, 2005)

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
Case report of one patient (f, 54 y) which showed a late reaction to reactive dyes in Patch test	RB5 (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 and Ingber, 2005)
Case report of a patient (f, 27 y) with non-occupational skin allergy against reactive dyes	RB5 (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, submammary folds, flanks, periaxillary, and lumbar areas. She had a history of mild atopic dermatitis, controlled with emollients.	**Positive reaction at D4 (++) for RB5** Symptoms probably caused by textiles, became better after avoidance of dark textiles	(Pérez-Crespo et al., 2009)
Case study of 41 children (< 18 y) with sole dermatitis	RB5 (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)

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) 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) 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) 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 amino acid 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)

Two animal studies on skin sensitisation (Dearman et al., 2013 and Hoechst AG, 1987) were performed most likely with substance [2].

The local lymph node assay (LLNA) was conducted in accordance with OECD TG 429 and principles of GLP (Dearman et al., 2013) and is considered reliable with restriction due to using other strain of mice than usual. 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.

In the guinea pig Maximisation Test conducted with OECD TG 406 considered reliable (Klimisch score 1) in REACH registration dossier, no reaction was reported. However, the negative results have to be questioned due to a blue staining that may have prevented the detection of redness of the skin.

Additional evidence for the skin sensitising potential of RB5 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.

Protein binding studies (Lalko 2012 and 2013a and b) using Direct Peptide Reactivity Assay (DPRA) performed with a range of known respiratory and skin sensitising chemicals were positive for RB5 (most likely with substance [2]) as well. As discussed in detail in the previous chapter, it 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 translation of test results of "Reactive Black 5" (as monoconstituent substance [1] as such or as a constituent of substance [2]) to its metabolite RB5 bis-vinyl [3].

In conclusion, there is sufficient evidence for a skin sensitising potential of "Reactive Black 5" (as monoconstituent substance [1] as such or as a constituent 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.

The DS has proposed to classify substances [1] and [2] (collectively referred to as Reactive Black 5) and Reactive Black 5 bis-vinyl [3] as Skin Sens. 1, H317 (May cause an allergic skin reaction) according to criteria of CLP Regulation. A generic concentration limit of 1.0 % is proposed.

Comments received during consultation

One MSCA commented the proposed classification for skin sensitisation and supported the DS proposal for classification as Skin Sens. 1, H317, without sub-categorisation, based on the positive LLNA and human data.

One organisation – importer (registrant) commented that classification - Skin Sens. 1, proposed by DS, is in agreement with self-classification provided in registration dossier for substance [2].

Assessment and comparison with the classification criteria

Animal data

There are positive results from LLNA test available (Dearman et al., 2013). In this key animal study RB5 [2] showed a simulation index (SI) well above 3 at all tested concentrations (5% - SI=8.6; 10% - SI=6.8; 25%- SI=11.8; concentrations < 5% were not tested). Since LLNA study results do not show linear dose-response relationship extrapolation of data to obtain reliable EC3 value and to exclude classification for skin sensitisation in category 1A is not possible. Based on results of LLNA study (EC3 value < 5%) classification of RB5 [2] as Skin Sens. 1 without sub-categorisation is warranted.

In the second animal study (Hoechst AG, 1987) no reaction was reported 48 h and 72 h after challenge with concentration 100% of RB5. However, the reliability of the study is questioned since blue staining might prevent the detection the effects of skin sensitisation (i.a. redness). Furthermore, the concentrations of pure RB5 in intradermal and dermal induction and dermal challenge doses were significantly lower than reported concentrations of tested material, what can be assumed from composition of Remazol-Schwarz B given in point 4.2.1 of confidential Annex to the CLH report.

Human data

According to the classification criteria of Regulation (EC) 1272/2008 (Annex I section 3.4.2.2.2) human evidence for Sub-categories 1A and 1B, respectively, can include the following type of data:

	Human data
Sub-category 1A	(a) positive responses at ≤ 500 µg/cm^2 (HRIPT, HMT – induction threshold); (b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure; (c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.
Sub-category 1B	(a) positive responses at > 500 µg/cm^2 (HRIPT, HMT – induction threshold); (b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure; (c) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

HRIPT: Human Repeat Insult Patch Test; HMT: Human Maximisation Test

The Guidance on the Application of the CLP Criteria (Section 3.4.2.2.3.1., Table 3.2) further outlines how high or low frequency of occurrence of skin sensitisation shall be assessed):

Human diagnostic patch test data	High frequency	Low/moderate frequency	RB5
General population studies	≥ 0.2 %	< 0.2 %	No studies
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %	1 study on consecutive dermatitis patients 0.1 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %	2 studies 1) 0.3% 2) 5.3%
Workplace studies: 1: all or randomly selected workers 2: selected workers with known exposure or dermatitis	≥ 0.4% ≥ 1.0%	< 0.4% < 1.0%	No studies 2 study: 1) 1.8% positive SPT (3 cases/162-exposed workers) 0.3% positive SPT (3 cases/1142 employees) 2) 0.9%
Number of published cases	≥ 100 cases	< 100 cases	21

There are no studies on general population. In one study on unselected consecutive dermatitis patients (Manzini et al., 1996) the frequency of positive patch test with RB5 was low.

Frequency of positive patch tests to RB5 in selected dermatitis patients was low in the case study by Lazarov (2004) and high in the case study by Darling et al (2012). 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 the cross-sectional study by Park at al. (1991b) frequency of skin symptoms in selected workers was low to moderate. In the second study in selected workers with dermatitis by Nilsson et al. (1993) no positive patch test was observed however high frequency of positive skin prick test (SPT) was high amongst workers exposed to reactive dyes.

The number of published patch-test-positive cases is below the limit for high frequency.

In the study by Sommer and Wilkinson (2000) 99 patients suspected of having a clothing dermatitis to 8 reactive dyes, 2 patients of whom became sensitized as a result of the patch test with 5% Reactive Black 5 in petrolatum. Therefore high frequency (>2%) can be considered but in relation to high exposure (based only on tested concentration, >1%).

Only results of the Lazarov (2004) study in selected dermatitis patients may support category 1B (0.3% positive patch test response to 5% RB5 (in petrolatum)) taking into account low frequency in relation to high exposure.

However, there are no comprehensive exposure data available in any of the available studies to estimate the exposure index according to Table 3.3 of the 'Guidance on the Application of the CLP Criteria' and to assess the level of exposure required to decide on a subcategory 1A or 1B.

There is evidence in humans that "Reactive Black 5" (as monoconstituent substance [1] as such or as a constituent 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.

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

In conclusion, **there is sufficient evidence for the skin sensitising potential of Reactive Black 5** (as monoconstituent substance [1] as such or as a constituent of substance [2] and RB5 bis-vinyl [3]). 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, H317** is supported by RAC, according to CLP Regulation.

ANNEXES:

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

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