Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

9-[2-(Ethoxycarbonyl)phenyl]-3,6-bis(ethylamino) -2,7-dimethylxanthenium chloride; Basic Red 1

EC Number: 213-584-9
CAS Number: 989-38-8

CLH-O-0000007031-88-01/F

Adopted

16 September 2021

16 September 2021

CLH-O-0000007031-88-01/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: **9-[2-(Ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthenium chloride; Basic Red 1**

EC Number: **213-584-9**

CAS Number: **989-38-8**

The proposal was submitted by **Germany** and received by RAC on **4 September 2020.**

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 **5 October 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 December 2020**.

ADOPTION OF THE OPINION OF RAC

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

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

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 **16 September 2021** by **consensus**.

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

	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	tbd	9-[2-(ethoxycarbonyl) phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride; Basic Red 1	213-584-9	989-38-8	-						
Dossier submitters proposal					Acute Tox. 3 Eye Dam. 1 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H301 H318 H317 H400 H410	GHS05 GHS06 GHS09 Dgr	H301 H318 H317 H410	-	oral ATE = 250 mg/kg bw M=10 M=1	
Resulting Annex VI entry if agreed by RAC and COM					Acute Tox. 3 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H301 H318 H317 H400 H410	GHS05 GHS06 GHS09 Dgr	H301 H318 H317 H410	-	oral ATE = 280 mg/kg bw M=10 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Basic Red 1, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino) -2,7-dimethylxanthylium chloride currently has no entry in Annex VI to the CLP regulation.

The CLH report is based on data submitted by the lead registrant in the REACH registration dossier for Basic Red 1 and available on the website of ECHA. A literature search was conducted in several relevant online resources (e.g. PubMed, SCOPUS, Web of Science, Wiley, Toxnet, Science Direct).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity - oral route

One study (BASF, 1973) is available. This study was reported as being similar to OECD Testing Guideline 401, but was not GLP compliant, performed in male and female rats (5/sex/dose) with Basic Red 1 (unspecified purity) administrated orally using a 0.1-30% aqueous suspension in carboxymethyl cellulose as a vehicle. There were no deaths reported at a dose of 200 mg/kg bw and all animals in the four highest dose groups (of the seven tested groups at 200, 250, 400, 800, 1250, 1600 and 3200 mg/kg bw) died during the 14-day observation period. A LD_{50} value of 250 mg/kg bw was determined for the substance.

Based on the results, the Dossier Submitter (DS) proposed to classify Basic Red 1 as Acute Tox. 3 after oral exposure (H301: Toxic if swallowed), ATE value of 250 mg/kg bw.

There are no studies available for acute toxicity dermal and inhalation route of exposure.

Comments received during consultation

One MSCA commented the proposed classification for acute oral toxicity and supported the DS proposal Acute Tox. 3 via oral route (the MSCA erroneously referring in its comment to H302 instead of H301; ATE=250mg/kg bw).

Assessment and comparison with the classification criteria

One study (BASF, 1973), reported as reliable with restrictions and conducted with Basic Red 1, has been included in the assessment of acute oral toxicity.

Based on the report summary provided[1], in an acute oral toxicity study (BASF, 1973), groups of 5 rats/sex/dose were given a single oral dose of Basic Red 1 and observed for 14 days. The mortality incidences are summarized in the following Table:

Administered dose (mg/kg bw)	Mortality (number of deaths / number of tested animals per dose group) during the 14-day observation period		
	males	females	Males and females (combined)
200	0/5	0/5	0/10
250	2/5	2/5	4/10
400	3/5	5/5	8/10
800	5/5	5/5	10/10
1250	5/5	5/5	10/10
1600	5/5	5/5	10/10
3200	5/5	5/5	10/10

The oral LD_{50} of 250mg/kg bw for males and females was claimed by the author of the study, but it is not substantiated by the data above. From classification point of view it is important to note that LD_{50} value is between 250 and 400 mg/kg bw, therefore it meets criteria for category Acute Tox. 3; H301. Using probit statistical analysis[2], the calculated LD_{50} is 279 mg/kg bw (95% confidence interval 227-342mg/kg bw) in male and female rats. Taking these data into account RAC considers that Basic Red 1 warrants classification as **Acute Toxicity Category 3 with hazard statement H301 'Toxic if swallowed'**, because the LD_{50} value is in a range of 50-300 mg/kg bw (table 3.1.1 of Annex I, Part 3 of CLP Regulation). Based on the calculated LD_{50} of 279 mg/kg bw. RAC proposes an **ATE of 280 mg/kg bw** (rounded value to 2 significant figures) instead of the ATE value of 250 mg/kg bw proposed by DS.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

One study (BASF AG, 1973) with Basic Red 1 is available. This study was reported as similar to the OECD Testing Guideline 405 but was not GLP compliant and had deviations (observation period ended after 8 days, no test substance removal after 1h of exposure). A quantity of 50 mg of the neat test substance was applied to the eyes of two Vienna White rabbits.

Iris and conjunctivae scores were not measured, but chemosis and corneal opacity showed scores of ≥ 3 (read only 24h after exposure) which were irreversible after 8 days. After treatment and during the observation period, several signs of severe eye damage were documented. In conformity with OECD TG 405, experiments with animals that have achieved post treatment severe eye lesions (in this case grade 4 corneal opacity) should be terminated early since these lesions are generally not reversible, therefore the 8 days observation period is sufficient to establish the magnitude and the irreversibility of the eye damage.

[1] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25449/7/3/2
[2] D.J.Finney (1952) Probit Analysis (2nd Ed), Journal of the Institute of Actuaries, 78 (3): 388-390.

The results of the available study demonstrate that the application of the solid test substance to the eyes of the rabbits caused irreversible damage. Eight days after exposure the eyes of both animals showed severe edema, while corrosion and ulceration were observed, effects that are not expected to be reversible. Thus, the DS proposed to classify Basic Red 1 as Eye Dam. 1 (H318 - causes serious eye damage).

Comments received during consultation

One MSCA commented the proposed classification for eye hazard and supported the DS proposal Eye Dam.1, H318.

Assessment and comparison with the classification criteria

One non GLP compliant study (BASF AG, 1973) with Basic Red 1, reported as reliable with restrictions, is available for the evaluation of the serious eye damage/irritation in rabbits. Iris and conjunctivae scores were not reported in the study report as well as cornea opacity scores at 48 and 72 hours after installation of the test material. However, grade 4 cornea lesions with corrosion and ulceration at day 8 after exposure were observed in both test animals.

RAC agrees with the argumentation presented by the DS that the observation period of 8 days is sufficient to evaluate irreversibility of the eye lesions and that the results of the available study meet the irreversibility criterion given in the CLP Regulation, for classification of Basic Red 1 as **Eye Dam. 1, H318 'Causes serious eye damage'**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

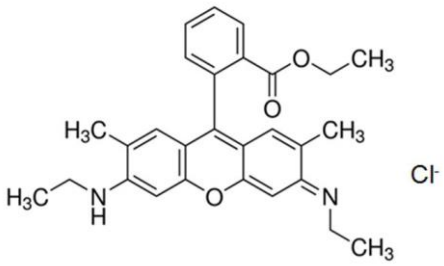
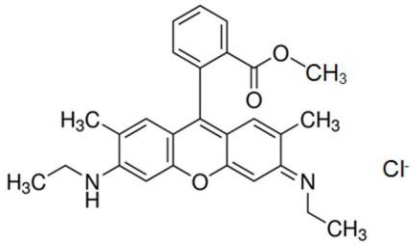
There is no specific human or animal data on skin sensitisation available for Basic Red 1 itself. Therefore, the proposed harmonised classification was based on read across using the source substance Basic Red 1:1 (3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl] -2,7-dimethylxanthylium chloride; CAS 3068-39-1, EC 221-326-1).

Read across

The DS proposed read across from the source substance Basic Red 1:1 using the analogue approach.

Table: *Identity of the target (Basic Red 1) and source (Basic Red 1:1) substances*

	Basic Red 1 (Target substance)	**Basic Red 1:1 (Source substance)**
EC name	9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl) phenyl]-2,7-dimethylxanthylium chloride
CAS no	989-38-8	3068-39-1
EC no	213-584-9	221-326-1
Molecular weight	479.01 g/mol	464.98 g/mol
Molecular formula	C28H31N2O3.Cl	C27H29N2O3.Cl

Structural formula		

The only structural difference between the target and source substance is a methyl instead of an ethyl substituent on a carboxyl group on one benzene ring.

The following studies are available which show similar toxicological profiles of the two substances.

***Table**: Summary table of data for substances Basic Red 1 and Basic Red 1:1 (based on REACH registration dossiers)*

Endpoint	Basic Red 1 (Target substance)	Basic Red 1:1 (Source substance)
Acute toxicity oral	LD50 250 mg/kg bw	LD50 449 mg/kg bw
Acute toxicity inhalation	No data	LC50 within range of 0.05 to 0.5 mg/l
Skin irritation	Not irritating	Not irritating
Eye irritation	Eye damaging	Eye damaging
Skin sensitisation	No data	Sensitising
Genetic toxicity	Negative in Ames test	Negative in Ames test

Furthermore, the registrant provides data from the QSAR toolbox showing that the target and source substance have very similar structure (similarity value of 92.54%).

Summary of skin sensitisation test presented in the CLH report

In the REACH registration dossier of Basic Red 1, an in vivo LLNA skin sensitisation test (2017) is available. The study was performed according to the OECD Testing Guideline 429 Local Lymph Node Assay (2010), GLP, using the read-across source substance Basic Red 1:1 (purity not reported). Three groups of five female mice (CBA:J) were treated with one test material concentration per group.

To determine the highest non-irritant and technically applicable test item concentration, a pre-test was performed in two mice with two concentrations: 25 and 50% (w/v). No erythema was observed while the variations in ear thickness was less than 25%. Piloerection and diarrhoea were noted for all animals. The highest test material concentration (50%) was selected for the main study.

In the main study the induction occurred consecutively in the first 3 days. The dorsal surface of both ears was topically treated (25 µL/ear) at concentrations of 10, 25 and 50% in vehicle (acetone/olive oil (4:1 v/v)). An excision of the nodes was done on day 6 followed by the tissue processing for radioactivity measurements. Each animal was injected via the tail vein with 0.25 mL of sterile phosphate buffered saline (PBS) containing 20 µCi of 3H-methyl thymidine. All animals were killed after five hours, the draining (auricular) lymph node of each ear was excised, and the nodes were pooled for each animal. Precipitates were recovered by centrifugation, re-suspended in 1 mL TCA and transferred to scintillation fluid. Radioactivity measurements were performed using a Packard scintillation counter (2800TR). The scintillation counter was programmed to automatically subtract background and convert Counts Per Minute (CPM) to

Disintegrations Per Minute (DPM). Positive control was alpha-hexylcinnamaldehyde in concentrations of 5, 10 and 25% in Acetone/Olive oil (4:1 v/v; AcOO).

No erythema was observed in the main study, no mortalities occurred and no clinical signs of systemic toxicity were observed. Body weight loss was found in some animals but independent of dosing. Pink discolouration of skin, urine and faeces was observed, probably due to the colour of the test material. The SI values for 10%, 25% and 50% test substance were calculated as 3.0, 5.7 and 3.6, respectively. The estimated concentration that will give an SI=3 was given as EC3=10%.

The outcome of the LLNA study demonstrates the skin sensitising potential of the tested substance. All concentrations tested (10%, 25% and 50 %) showed SI values ≥3 which according to the CLP regulation is considered as significant skin sensitising effect. Lower concentrations were not tested, but an EC3 value of 10% was calculated which coincides with the lowest concentration tested.

Target substance Basic Red 1 is considered to react the same way as the source substance Basic Red 1:1. An extra methyl group should not influence the skin sensitising potential of the substance.

The DS concluded that these results warrant Skin Sens. sub-category 1B, according to the criteria given in Table 3.4.4 of the CLP regulation (LLNA: EC3 value >2%).

Comments received during consultation

Two MSCA commented the proposed classification for skin sensitisation hazard and one supported the DS proposal for the read-across analogue approach and classification as Skin Sens. 1B, H317.

The other MSCA disagreed with the proposal and recommends classification as Skin Sens. 1 without sub-categorisation. The reason for the disagreement is lack of results of LLNA study at concentrations lower than 10%. Furthermore, dose response relationship has not been analysed or discussed in the CLH proposal. An MSCA noted that the LLNA study was scored as reliable with restrictions without consideration of its limitations, and how the choice of vehicle other than acetone/olive oil (4:1 v/v) affect the solubility of the test substance and the outcome of the study. The study has been allocated reliability 1 (reliable without restriction) in the REACH registration dossier disseminated on ECHA webpage.

In their response, the DS concluded that the available data on skin sensitisation lacks information on choice of vehicle and dose selection. Therefore, based on the dose selection, lack of information on a dose-response at lower doses and on solubility of the substance in the vehicle chosen, category 1A (although unlikely) cannot be formally excluded.

However, according to the LLNA study report available on ECHA website, it was reported that 'the vehicle was selected on the basis of maximising the solubility of tested substance'.

Assessment and comparison with the classification criteria

Read across from Basic Red 1:1 to Basic Red 1

The read across is based on similar toxicological profiles of both substances and high structural similarity between Basic Red 1:1 and Basic Red 1.

Experimental toxicological data indicate similar acute oral toxicity, lack of skin irritation properties, lack of mutagenicity in Ames test, and eye damaging property in both substances. The target substance Basic Red 1 (CAS 989-38-8) and the source substance Basic Red 1:1 (CAS 3068-39-1)

have the same structure and only differ in the type of substituent (methyl or ethyl) in one of the benzene rings.

The comparison of the QSAR Toolbox profiling schemes for the target and the source substances shows that they are very similar. Therefore, QSAR clearly supports the applied read-across approach.

RAC agrees with the justification for an analogue approach using read across from the source substance Basic Red 1:1 to the target substance Basic Red 1.

Comparison with the criteria

RAC considers that for regulatory purposes, LLNA skin sensitisation test (2017) performed according with the OECD Testing Guideline 429 (2010), conducted under GLP, using the read across source substance Basic Red 1:1 provides enough information on study methodology and results, despite its limitations and dose-response relationship. The study reports lack information on justification for the choice of vehicle, justification for dose selection, and why lower concentrations than 10% was not tested.

RAC agrees that the substance has skin sensitising potential since SI ≥ 3 were observed at all tested concentrations (10%, 25 and 50%), and an EC3 value of 10% was calculated. According to the criteria given in table 3.4.4 of Annex I, Part 3 of the CLP Regulation, an EC3 > 2% indicates that a classification in category 1B is warranted. However, since lower concentrations than 2% were not tested, classification in category 1A could not formally be excluded. Taking into account the lack of linear dose response relationship (SI values of 3.0 ± 0.8, 5.7 ± 1.6 and 3.6 ± 1.2 at concentrations of 10, 25 and 50%, correlation coefficient r=0.07, very weak or no correlation), extrapolation of results to lower concentrations is not appropriate. ECHA CLP Guidance indicates that, when Category 1A cannot be excluded, Category 1 (as a default) should be applied instead of Category 1B, particularly when results at lower doses are absent or in the absence of adequate dose-response information.

Based on section 3.4.2.2.1.1 of Annex I, Part 3 of the CLP Regulation), skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation. Therefore, classification as **Skin Sens. 1, H317 'May cause an allergic skin reaction'**, without sub-categorisation is proposed by RAC for Basic Red 1. No Specific Concentration Limit (SCL) is proposed.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

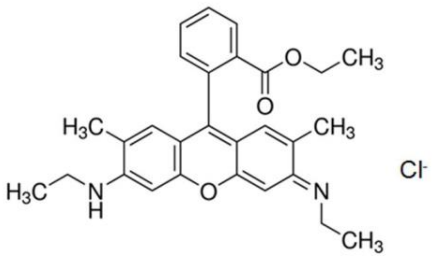
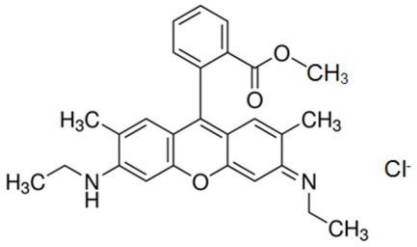
The substance is not currently listed in Annex VI Table 3.1 of the CLP Regulation. The Dossier Submitter (DS) proposed to classify Basic Red 1) using read-across from Basic Red 1:1. The basis for proposing Aquatic Acute 1, M=10 was an E_rC_{50} of 0.023 mg/L for algae (data on Basic Red 1:1). The proposal for Aquatic Chronic 1, M=1 was based on the substance being not rapidly degradable (data on Basic Red 1:1) and on an E_rC_{10} value of 0.014 mg/L for algae (data on Basic Red 1:1).

Read-across

There was no experimental data available for Basic Red 1 on degradability or toxicity to fish and algae. The DS used read-across to a structurally similar substance 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride (Basic Red 1:1) to assess these endpoints. The target substance and the source substance have the same structure and differ only in the chain length at the carboxyl group (methyl vs. ethyl residue). Based on the QSAR Toolbox (version 4.2) similarity options, both substances share a very high degree of similarity with a similarity value of 92.54%.

Information relevant for classification presented in the CLH Report on both substances is presented in the following table. The information was available in the REACH Registration Dossiers last modified 21.2.2019 (Basic Red 1) and 28.5.2018 (Basic Red 1:1).

Table: *Information relevant for classification on Basic Red 1 and Basic Red 1:1*

	Target substance	**Source substance**
Substance	9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethyl amino)-2,7-dimethylxanthylium chloride; Basic Red 1	3,6-bis(ethylamino)-9-[2-(methoxycarbo-nyl)phenyl]-2,7-dimethylxanthylium chloride; Basic Red 1:1
CAS number	989-38-8	3068-39-1
Structural formula		
Water solubility	77.9 g/L at 20 °C and pH 2.8 (GLP, OECD TG 105)	18.9 g/L at 20 °C and pH 3 – 3.1 (GLP, OECD TG 105) 13.49 g/L at 28 °C (OECD TG 105 and 101)
Partition coefficient n-octanol/water	log Pow = 0.1 ± < 0.1 at 24 °C (pH 3.9 – 4.4) (GLP, OECD TG 107)	log Pow = 1.7 at 20 °C (pH 7) (GLP, OECD TG 107) log Pow = 1.21 at 25 °C (OECD TG 117)
Surface tension	Not applicable (based on structure, surface activity is not expected).	65.4 mN/m @ 1 g/L and 20 °C (GLP, OECD) – not surface active
Hydrolysis	No data - Read-across	Stable at pH 4 and 7; DT50 <16 days at pH 9; transformation products not analysed (GLP, OECD TG 111)
Ready biodegr.	Not readily biodegradable (EPI Suite v.411 BIOWIN v4.10)	2% and 5% degradation, not readily biodegradable (GLP, OECD TG 301B)
Acute toxicity to fish	No data – Read-across	LC50 = 6.85 mg/L (OECD TG 203), no analytical monitoring)
Acute toxicity to aquatic invertebrates	EC50= 0.16 mg/L (similar OECD TG 202, not GLP, no analytical monitoring)	EC50= 1 mg/L (nominal based on analytical monitoring, GLP, OECD TG 202)
Toxicity to algae	No data - Read-across	ErC50= 0.023 mg/L ErC50= 0.014 mg/L (measured, TWA, GLP, OECD TG 201, analytical monitoring)

Degradation

There was no data available on the hydrolysis of Basic Red 1. However, a hydrolysis study (OECD TG 111, GLP) was available for Basic Red 1:1 in the REACH registration dossier. At the preliminary study ≥ 10% hydrolysis was observed at pH 7 and pH 9 after 5 days. For pH 4 < 10% hydrolysis was observed. Hence, the half-life at 25 °C and pH 4 was greater than 1 year. Half-lives of 187 days and 226 days were observed at pH 7 and half-lives of 7 days and 13.5 days at pH 9 at 25 °C and 20 °C, respectively. No hydrolysis products were analysed in this study. Hence, it could not be demonstrated whether the hydrolysis products do fulfil the criteria for classification as hazardous for the aquatic environment.

No ready biodegradability test was available for Basic Red 1. Data from BIOWIN showed similar results for Basic Red 1 and the structurally similar substance Basic Red 1:1. Therefore, the DS used experimental data from Basic Red 1:1 to assess the ready biodegradability of Basic Red 1.

Two ready biodegradability studies were available with Basic Red 1:1; OECD TG 301B (GLP) and OECD TG 301D (not GLP). In the test according to OECD TG 301B an initial concentration of 17 mg/L (test material), corresponding to 12 mg TOC/L, was used. Activated sludge from a municipal wastewater treatment plant (predominantly domestic, 4.9 g/L suspended solid) was used as inoculum. The inoculum was rated to be not adapted to the test substance. The study was conducted at 21.8 – 22.9°C and at pH range of 7.6 – 8.0. After 28 days, 2 and 5% biodegradation of the test material in duplicate bottles was observed based on $ThCO_2$. In the toxicity control 27% biodegradation was measured after 14 days. Hence, the test material was assumed not to inhibit microbial activity. The reference substance (sodium acetate) showed 60% biodegradation within 14 days.

In the study according to OECD TG 301D polyseed was used as inoculum. One polyseed capsule was added in 500 ml distilled water and then stirred for one hour for proper mixing and functioning of the inoculum. This gave a bacterial count of 10^7 to 10^8 CFU/ml. The concentration of the test substance and the reference substance (sodium benzoate) was 4 mg/L, while that of inoculum was 32 ml/L. The study was performed at 20 °C and a pH range of 6.1 – 7.0. After 28 days 31.45% O_2 consumption was observed. The reference substance sodium benzoate degraded with 58.43% after 14 days. Hence, the validity criterion for the reference substance to reach the pass level by day 14 was not fulfilled (≥ 60% after 14 days).

The DS concluded that Basic Red 1 was not readily biodegradable based on the data of the structurally similar substance Basic Red 1:1.

Altogether the DS considered Basic Red 1 as not rapidly degradable.

Bioaccumulation

There were no experimental bioconcentration data available. The log Pow (OECD TG 107) was 0.1 ± <0.1 at 24 °C and pH 3.9 – 4.4. The DS concluded that Basic Red 1 has low potential for bioaccumulation.

Acute aquatic toxicity

***Table**: Relevant information on acute aquatic toxicity from the REACH Registration Dossiers*

Method	Species	Test material	Results	Remarks
OECD TG 203, static	*Leuciscus idus*	Basic Red 1:1	96h-LC_{50} was 6.85 mg/L (nominal)	Read-across Reliability: 2 (only 4 conc., no analytical monitoring)
OECD TG 202, static, not GLP	*Daphnia magna*	Basic Red 1	48h-EC_{50}= 0.16 mg/L (nominal [1])	Reliability: 2 (no analytical monitoring)
OECD TG 202, static, GLP	*Daphnia magna*	Basic Red 1:1	48h-EC_{50}= 1 mg/L (nominal)[2]	Read-across Reliability 1 (registrant) (analytical monitoring)
OECD TG 201, static, GLP	*Pseudokirchneriella subcapitata*	Basic Red 1:1	72h-E_rC_{50}= 0.023 mg/L (meas. TWA)	Read-across Reliability: 1 (analytical monitoring)

[1] DS: As the test substance has a good water solubility and is not readily biodegradable, a disappearance of the substance from the test system is not expected.
[2] measured concentrations 92-105% of nominal

As there was only one acute toxicity test (*Daphnia*) available for Basic Red 1, the acute aquatic toxicity data (fish, *Daphnia*, algae) for the read-across substance Basic Red 1:1 was included in the dossier. The results of the acute toxicity tests on *Daphnia magna* for Basic Red 1 and Basic Red 1:1 showed that Basic Red 1 seems to be more toxic than Basic Red 1:1. The results of the three acute aquatic toxicity tests with Basic Red 1:1 reveal that algae was the most sensitive aquatic test species. The EC_{50} from the algae test was lower than the one from the *Daphnia* study with Basic Red 1. Thus, the data with algae and Basic Red 1:1 were used for classification keeping in mind that the real toxicity of Basic Red 1 to algae might be even higher than anticipated by the data for Basic Red 1:1.

The study with the source substance Basic Red 1:1 was conducted according to OECD TG 201 (GLP) with analytical monitoring and without the use of a vehicle. The test was conducted with *Pseudokirchneriella subcapitata* with an initial cell density of $1x10^4$ cells/mL. The test temperature was 22 to 24 °C, the light intensity was 60 to 120 µE/m^2/s and effective wavelength ranges of 400 to 700 nm (continuous photoperiod). The hardness was 24 mg $CaCO_3$/L, the pH was 8.0 to 8.2. The test concentrations were: 0.046, 0.10, 0.22, 0.46 and 1.0 mg/L (nominal concentrations) and 0.0046, 0.014, 0.041, 0.14 and 0.42 mg/L (time weighted average concentrations, TWA). The test vessel volume was 250 mL containing 30 mL of test solution. Three replicates per test concentration were used. For the control 5 replicates were used (instead of 6 replicates because one of the replicates fell from the shaking table and possibly part of the solution was lost). The validity criteria were all fulfilled. The resulting 72h-E_rC_{50} was 0.023 mg/L (measured TWA).

The DS concluded that the lowest acute toxicity value to be used for classification was a 72h-E_rC_{50} of 0.023 mg/L for algae. The DS proposed to classify Basic Red 1 as Aquatic Acute 1, H400 with an M-factor of 10 ($0.01<E_rC_{50} \leq 0.1$ mg).

Chronic aquatic toxicity

***Table**: Relevant information on chronic toxicity from REACH Registration Dossiers*

Method	Species	Test material	Results	Remarks
OECD TG 201 GLP	*Pseudokirchneriella subcapitata*	Basic Red 1:1 / CAS 3068-39-1 / EC 221-326-1	72h-E_rC_{10}= 0.014 mg/L (meas. TWA)	Read-across Reliability: 1

There were no chronic toxicity data available on Basic Red 1. The registration dossier contained a read-across algae study to the source substance Basic Red 1:1. The study details are described under Acute aquatic toxicity. The resulting 72h-E_rC_{10} was 0.014 mg/L (measured TWA).

The DS concluded that the lowest chronic toxicity value to be used for classification was a 72h-E_rC_{10} of 0.014 mg/L for algae. Since data on chronic toxicity to fish and invertebrates is missing, the surrogate approach based on a 96h-LC_{50} of 6.85 mg/L for fish and on a 48h-EC_{50} of 0.16 mg/L for Daphnia was also considered. Consequently, the DS proposed to classify Basic Red 1 with Aquatic Chronic 1, H410 and an M-factor of 1 (not rapidly degradable substance, $0.01<EC_{10}\leq0.1$ mg/L) based on the chronic data for algae. Using the surrogate system for Daphnia data would lead to the same classification whereas using surrogate system for fish data would lead to Aquatic Chronic 2 classification.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the Dossier Submitter (DS) approach to use read-across to a structurally similar substance Basic Red 1:1 to assess degradability and fish and algae toxicity of Basic Red 1. The target substance and the source substance have the same structure and differ only in the chain length at the carboxyl group (methyl vs. ethyl residue). Based on the QSAR Toolbox (version 4.2) similarity options, both substances share a very high degree of similarity with a similarity value of 92.54%.

Degradation

RAC agrees with the DS to conclude that Basic Red 1 is not rapidly degradable based on read-across to Basic Red 1:1. There were no data available on hydrolysis or ready biodegradability of Basic Red 1. Data for Basic Red 1:1 showed that:

- Basic Red 1:1 is not readily biodegradable (2 and 5% biodegradation after 28 days in OECD TG 301B test)
- Hydrolysis half-life for Basic Red 1:1 is < 16 days at pH 9, hydrolysis products were not analysed in the study → rapid degradation was not shown (DT_{50}: at pH 4 > 1 year, 187 and 226 days at pH 7 and 7 and 13.5 days at pH 9)

Bioaccumulation

RAC agrees with the DS to consider Basic Red 1 as having a low potential for bioaccumulation. There were no experimental bioconcentration data available. The experimental log P_{ow} was 0.1 ± <0.1, which does not fulfil the classification criteria log Kow ≥ 4.

Aquatic toxicity

RAC agrees with the DS to use toxicity data on Basic Red 1:1 for aquatic toxicity classification when data on Basic Red 1 is not available.

Acute toxicity

There were data available on three trophic levels. For fish the 96h-LC_{50} was 6.85 mg/L (Basic Red 1:1) and for *Daphnia* 48h-EC_{50} values were 0.16 mg/L (Basic Red 1) and 1 mg/L (Basic Red 1:1). Algae was the most sensitive trophic level with the 72h-E_rC_{50}= 0.023 mg/L (Basic Red 1:1).

RAC agrees with the DS that although nominal concentration without analytical monitoring were used in the fish (Basic Red 1:1) and Daphnia test (Basic Red 1), disappearance of the substance from the test substance is not expected but cannot be excluded. Analytical monitoring was used in the Basic Red 1:1 Daphnia test and in the algae test were results were expressed as measured time-weighted-average (TWA) concentration.

RAC agrees with the DS to classify Basic Red 1 to Aquatic Acute Category 1, H400, M-factor of 10 based on the 72h-E_rC_{50}= 0.023 mg/L for algae, which is lower than the cut-off ≤ 1 mg/L for Acute Category 1. M-factor of 10 is warranted because the 0.01 mg/L < E_rC_{50} ≤ 0.1 mg/L.

Chronic toxicity

There were no chronic toxicity data available on Basic Red 1. Only an algae study was available on Basic Red 1:1 resulting to a 72h-E_rC_{10} of 0.014 mg/L as measured TWA. Both acute and chronic algae toxicity values originate from the same test.

In the absence of chronic toxicity data on fish and *Daphnia*, the surrogate system was used. For fish the use of the surrogate system for 96h-LC_{50} of 6.85 mg/L with a not rapidly degradable substance leads to Aquatic Chronic 2 classification. The *Daphnia* 48h-EC_{50} of 0.16 mg/L for Basic Red 1:1 warrants the same classification (Aquatic Chronic 1) as concluded with the chronic algae data as does the 48h-EC_{50} of 1 mg/L for *Daphnia* with Basic Red 1.

The chronic test result for algae, 48h-EC_{50} of 0.16 mg/L, warrants Aquatic Chronic 1, H410, M-factor of 1 classification the E_rC_{10} value for a not rapidly degradable substance being smaller than 0.1 mg/L cut-off for Category 1. M-factor of 1 is warranted because the 0.01 mg/L < E_rC_{10} ≤ 0.1 mg/L.

Consequently, RAC agrees to the Dossier Submitter's proposal to classify Basic Red 1 as **Aquatic Acute 1, H400, M=10 and Aquatic Chronic 1, H410, M=1.**

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).