Committee for Risk Assessment

RAC

Annex 1
Background document
to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 September 2021

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

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

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

Index Number: **n.a.**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 9-[2-(ETHOXYCARBONYL)PHENYL]-3,6-BIS(ETHYLAMINO) -2,7-DIMETHYLXANTHENIUM CHLORIDE; BASIC RED 1

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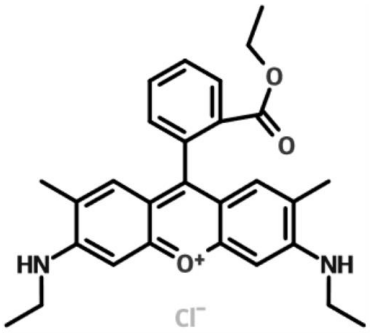
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*9-[2-(Ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthenium chloride*
Other names (usual name, trade name, abbreviation)	9-[2-(Ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride; Basic Red 1; Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, chloride (1:1) [CAS name]; Rhodamin 6G
ISO common name (if available and appropriate)	n.a.
EC number (if available and appropriate)	213-584-9
EC name (if available and appropriate)	9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride
CAS number (if available)	989-38-8
Other identity code (if available)	C.I. Basic Red 1
Molecular formula	C28H31ClN2O3
Structural formula	
SMILES notation (if available)	[Cl-].CCNc1cc2[o+]c3cc(NCC)c(C)cc3c(c2cc1C)c4ccccc4C(=O)OCC
Molecular weight or molecular weight range	479.01 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the	*mono-constituent substance; purity not relevant*

entry in Annex VI)	

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
9-[2-(Ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride (CAS No. 989-38-8)	**≤ 100 % w/w**	Skin Sens. 1B, H317	Acute Tox. 3, H301 Eye Dam. 1, H318 Aquatic Acute 1, H400 Aquatic Chronic 1, H410

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
-				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
-					

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

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

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	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. 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	

Table 7: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no current harmonized classification for Basic Red 1 (BR 1; 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride) but it is listed by ECHA as Annex III substance (substances predicted as likely to meet criteria for category 1A or 1B carcinogenicity, mutagenicity, or reproductive toxicity).

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).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Reason for a need for action at Community level:

Differences in self-classification

Further detail on need of action at Community level

As reported on the ECHA dissemination site, there are in total a number of 1310 notifications in the C&L inventory (as of 07.08.2020).

Disagreement by DS with current self-classification

Notified classification and labelling are inconsistent and contradictory as seen below:
Acute Tox. 4 = 541/1310
Acute Tox. 3 = 683/1310
Skin Sens. 1B = 2 / 1310
Eye Dam. 1= 1034/1310
Skin Irrit. 2 = 2 /1310
Eye Irrit. 2 = 19 /1310
Muta. 1B = 116 /1310
Repr. 1B = 116/1310
Muta. 2 = 47/1310
Repr. 2 = 47/1310
STOT SE 3 = 1 /1310
Aquatic Acute 1 /Chronic 1 = 679/1310
Aquatic Chronic 1 = 58/1310
Aquatic Chronic 2 = 207/1310
No classification for aquatic environment = 323/1310.

A number of 21/1310 notifications did not classify Basic Red 1 at all.
This outcome justifies a proposal for harmonised classification.

5 IDENTIFIED USES

This substance is manufactured and/or imported in the European Economic Area in 1 - 10 tonnes per year. It is used in the laboratory settings, as well as industrially and professionally. It might be found in products like inks and toners but also as binding agent in paints and coatings or adhesives[1].

6 DATA SOURCES

In addition to the information that is available on the website of ECHA (as of December 2019[1]) and in the IUCLID registration dossier for substance 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride (Basic Red 1), a literature search was conducted in several relevant online resources (e.g. PubMed, SCOPUS, Web of Science, Wiley, Toxnet, Science Direct).

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid red powder	REACH registration data	experimental result (visible inspection)
Melting/freezing point	229.4 °C (under decomposition; decomposition starting at approx. 220 °C at atmospheric pressure (1007 hPa))	REACH registration data	experimental result [EU Method A.1 (Melting / Freezing Temperature); OECD Guideline no. 102 "Melting Point/Melting Range"; differential scanning calorimetry]
Boiling point	decomposes before boiling	REACH registration data	experimental result [EU Method A.2 (Boiling Temperature); OECD Guideline 103 (Boiling Point); dynamic method]
Relative density	D^{20}_4= 1.297	REACH registration data	experimental result [EU Method A.3 (Relative Density); OECD Guideline 109 (Density of Liquids and Solids); EPA OPPTS 830.7300 (Density / Relative Density / Bulk Density); pycnometer method]
Vapour pressure	results of the Effusion method: T = 128°C: 4.70 x 10^{-4} Pa T = 138°C: 1.55 x 10^{-3} Pa T = 143°C: 3.18 x 10^{-3} Pa T = 148°C: 5.08 x 10^{-3} Pa	REACH registration data	calculated by extrapolation of the measured vapour pressure curve [EU Method A.4 (Vapour Pressure); OECD Guideline 104 (Vapour Pressure Curve);

[1] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25449

Property	Value	Reference	Comment (e.g. measured or estimated)
	T = 153°C: 7.73 x 10^{-3} Pa calculated: T = 20°C: 8.0 x 10^{-12} Pa T = 25°C: 2.4 x 10^{-11} Pa T = 50°C: 3.9 x 10^{-9} Pa		OECD Test Guideline 113 (1981) Thermal Stability; effusion method: vapour pressure balance]
Surface tension	Not applicable (based on structure, surface activity is not expected).	REACH registration data	-
Water solubility	77.9 g/L at 20 °C and pH 2.8	REACH registration data	experimental result [OECD Guideline 105 (Water Solubility); EU Method A.6 (Water Solubility); flask method]
Partition coefficient n-octanol/water	log Pow = 0.1 ± < 0.1 at 24 °C (pH 3.9 - 4.4)	REACH registration data	experimental result [OECD Guideline 107 (Partition Coefficient (n-octanol / water), Shake Flask Method); EU Method A.8 (Partition Coefficient - Shake Flask Method); shake-flask-method]
Granulometry	D10 = 2.3 µm D50 = 9.6 µm D90 = 23.8 µm (The results are average values: two test series of three measurements each were performed)	REACH registration data	[OECD Guideline 110; ISO 13320; EPA OPPTS 830.7520; Laser scattering/diffraction

The information in this table marked with „REACH registration data“ is based on information taken from the REACH registration dossier and ECHA’s public registration information as accessed on 11-07-2019.

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed for this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

There is no data available to the DS. Induction of skin sensitisation takes place locally in the skin at the site of contact; therefore, systemic availability of the hapten is not relevant. Proof of sensitisation after dermal contact also proves that a sufficient amount of hapten has been taken up.

10 EVALUATION OF HEALTH HAZARDS

For the substances 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride (Basic Red 1) and 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride (Basic Red 1:1) read-across is used in the registration dossier. The registrant provides a read-across justification document in their dossier using the analogue approach.

The only structural difference between target and source substance is an methyl instead of a ethyl group at a carboxyl group on a benzene ring. The following studies are available which show similar toxicological profiles of the two substances.

Table 9: Summary table of data for substances 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride (Basic Red 1) and 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)-phenyl]-2,7-dimethylxanthylium chloride (Basic Red 1:1)

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

The current self-classifications show a similar pattern supporting the similarities in the toxicological profile.

Table 10: Summary table of self-classifications for human health endpoints

Current self-classification	Basic Red 1 (Target substance)	Basic Red 1:1 (Source substance)
	Acute Tox. 3 (oral) Eye Dam. 1	Acute Tox. 4 (oral) Acute Tox. 2 (inhalation) Eye Damage 1, Skin Sens. 1B

Furthermore, the registrant provides data from the QSAR toolbox showing that the target and source substances are very similar.

There are studies available using 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride (Basic Red 1) for acute oral toxicity and eye damage which are used for classification. For the endpoint skin sensitisation the study available in the registration dossier of 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride (Basic Red 1) was performed with 3,6-bis(ethyl-amino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride (Basic Red 1:1). This study is used for classification based on the read-across considerations of the registrant as summarised above.

Acute toxicity

There is one study available for acute toxicity oral route; there is no data on other routes.

10.1 Acute toxicity - oral route

Table 11: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity similar to OECD 401, no GLP, Standard acute method	Rat, male/female, strain not specified N=5	9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride Vehicle: carboxymethyl cellulose	200, 250, 400, 800, 1250, 1600, 3200 mg/kg bw All animals in the 4 highest dose groups died after 14 days	250 mg/kg bw	(BASF, 1973)[2] REACH Registration dossier for Basic Red 1

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

One study is available which was performed in rats similar to OECD TG 401. All animals in the four highest dose groups (400, 800, 1250, 1600 and 3200 mg/kg bw) died after 14 days. A LD_{50} value of 250 mg/kg bw was determined for 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride.

10.1.2 Comparison with the CLP criteria

As described above, the lowest available LD_{50} value, taken from a study performed similar to OECD guideline 401, is 250 mg/kg bw for rats.

According to the criteria shown in the Table 3.1.1 of Annex I, Part 3 of CLP, substances can be allocated to one of four toxicity categories based on acute toxicity by the oral route. In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. Acute toxicity values are expressed as approximate LD_{50} values (oral) or as acute toxicity estimates (ATE):

Acute oral toxicity - Category 3: $50 < ATE \leq 300$ mg/kg bw.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the results shown above, it is proposed to classify 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethyl-amino)-2,7-dimethylxanthylium chloride as:

Acute Tox. 3 after oral exposure (H301 – Toxic if swallowed).

An ATE value of 250 mg/kg bw is proposed based on the LD_{50} value from the study described.

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

[2] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25449/7/3/2

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[3], 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[4], 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.

[3] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25449/7/3/2

[4] D.J.Finney (1952) Probit Analysis (2nd Ed), Journal of the Institute of Actuaries, 78 (3): 388-390.

10.2 Acute toxicity - dermal route

There is no study available for acute toxicity dermal route.

10.3 Acute toxicity - inhalation route

There is no study available for acute toxicity inhalation route.

10.4 Skin corrosion/irritation

Not assessed in this dossier.

10.5 Serious eye damage/eye irritation

A study from 1973 is available in the REACH registration dossier. The study is similar to OECD guideline 405. In the opinion of the DS, the study allows classification of the test substance due to occurrence of severe eye damage.

Table 12: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance,</th><th>Dose levels duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr>
<td>Study performed

Non-GLP; but equivalent to OECD guideline 405 with deviations:

→observation period ended after 8 days but it was sufficient to determine irreversibility of the eye damage

→no test substance removal after 1h of exposure; according to OECD TG 405: “If the solid test substance has not been removed from the eye of the test animal by physiological mechanisms at the first observation time point of 1 hour after treatment, the eye may be rinsed with saline or distilled water.”

→not measured:</td>
<td>Vienna White rabbits

n=2/ exp.</td>
<td>9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride

Solid, no vehicle</td>
<td>50 mg

Observation period:

10 min, 1 h, 2 h, 24 h, 48 h, 96 h, 7d and 8 days</td>
<td>Irritation parameters (see Annex I for details)

<u>Iris score</u>

n.m.= not measured

<u>Conjunctivae score</u>

n.m.= not measured

<u>Chemosis score</u>
<table>
<tr><td>No.</td><td>Time</td><td>Score</td><td>Obs.</td></tr>
<tr><td>♯1</td><td>24h</td><td>3-4</td><td>NFR8D</td></tr>
<tr><td>♯1&2</td><td>48h</td><td>-</td><td>n.m.</td></tr>
<tr><td>♯1&2</td><td>72h</td><td>-</td><td>n.m.</td></tr>
<tr><td>♯1</td><td>8days</td><td>3-4</td><td>Corr., Ulcer.</td></tr>
<tr><td>♯2</td><td>8days</td><td>3-4</td><td>Corr., Ulcer.</td></tr>
</table>
<u>Cornea opacity</u>
<table>
<tr><td>No.</td><td>Time</td><td>Score</td><td>Obs.</td></tr>
<tr><td>♯1</td><td>24h</td><td>3</td><td>NFR8D</td></tr>
<tr><td>♯1&2</td><td>48h</td><td>-</td><td>n.m.</td></tr>
<tr><td>♯1</td><td>8days</td><td>4</td><td>Corr., Ulcer.</td></tr>
<tr><td>♯2</td><td>24h</td><td>3</td><td>NFR8D</td></tr>
<tr><td>♯2</td><td>8days</td><td>4</td><td>Corr., Ulcer.</td></tr>
</table>
NFR8D = not fully reversible within: 8 days

Corr.= corrosion

Ulcer.= ulceration

n.m. = not measured

Eye dam. Cat. 1</td>
<td>(Badische Anilin- & Soda-Fabrik AG, 1973)[5]

REACH Registration dossier for Basic Red 1</td>
</tr>
</table>

[5] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25449/7/4/3

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results - Observations and time point of onset - Mean scores/animal - Reversibility**	**Reference**
Iris and Conjunctivae score Test reliability 2: reliable with restriction					

9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride was applied to the Vienna White rabbit's eye. This study has been carried out similar to OECD TG 405 – Acute Eye Irritation Study, non-GLP, with some deviations as described above. Iris score and conjunctivae score were not measured, but chemosis score and cornea opacity showed scores of ≥ 3 which were irreversible after 8 days. After treatment and during the observation period several signs of severe eye damage were documented while concerns for animal pain and discomfort were obvious. Conform 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, in these experimental conditions.

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

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 test substance causes serious eye damage.

10.5.2 Comparison with the CLP criteria

According to the CLP criteria, classification as Eye Dam 1 needs to be assigned if:

Study	Criteria according to CLP regulation	Relevant result	Resulting Classification
Study similar with the OECD guideline 405 (Badische Anilin- & Soda-Fabrik AG, 1973)	Category 1 A substance produces effects (1) at least in one animal of 3 in cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; (2) and/or a substance produces a positive response in 2 of 3 tested animals of (i) corneal opacity ≥ 3 and/or (ii) iritis ≥ 1.5 calculated as the mean scores following grading at 24, 48 and 72 hours after application of the test material.	(2) Chemosis score = 3-4 Cornea opacity = 3 or 4 In two test animals Not reversible after 8 days Severe damage to the eye of the rabbits that are not expected to be reversible after 21 days	Eye Dam. 1

The results of the available study meet the criteria described above as the corneal opacity score is ≥ 3 for all measured time points and for all test animals. A single dose of tested substance induced severe damage to the eye of the rabbits that are not expected to be reversible after 21 days, thus the observation period of 8 days showed to be sufficient to evaluate the irreversibility of the effects.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

The test substance 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride causes severe damage to rabbit eyes and therefore is proposed to be classified according with CLP as **Eye Dam. 1, H318 (Causes serious eye damage)**.

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.

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'**.

10.6 Respiratory sensitisation

During the literature search, the DS did not identify studies demonstrating a potential of 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride to cause respiratory sensitisation.

10.7 Skin sensitisation

In the REACH registration dossier an in vivo LLNA skin sensitisation test is available. The study was performed in 2017 according with the OECD Guideline 429 Local Lymph Node Assay (vs. 2010), GLP, using the read-across substance 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethyl-xanthylium chloride (Basic Red 1:1, CAS 3068-39-1, EC 221-326-1).

Table 13: 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
Mouse local lymph node assay (LLNA) GLP reliability of the study 2: reliable with restrictions	Mouse; CBA:J; female n=5	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride (Basic Red 1:1, CAS 3068-39-1, EC 221-326-1) **(read-across substance)** Positive control substance: hexyl cinnamic aldehyde (CAS No 101-86-0)	Concentrations 0, 10, 25 and 50 % in acetone/olive oil (4:1 v/v)	Mean DPM/animal values: vehicle control = 572 DPM 10 % = 1709 DPM 25 % = 3238 DPM 50 % = 2081 DPM Calculated SI values: 10 % = 3.0 ± 0.8 25 % = 5.7 ± 1.6 50 % = 3.6 ± 1.2 See details in Annex I. *EC3 value of 10 % was calculated; when SI =3.* **Skin Sens. 1B**	(Charles River Laboratories Den Bosch B.V., 2017)[6] Same experimental study in REACH Registration dossier for Basic Red 1 and Basic Red 1:1

[6] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25449/7/5/2

For this study, 10 weeks old female mice with weight ranging 18.7 to 24.5 g ± 20 % were used with an allowed acclimation period of at least 5 days. A pre-screen test was performed with two concentrations (25 %, 50 %) in two animals per concentration. Mice were treated with one concentration on three consecutive days. Ear thickness measurements were done prior to dosing on Days 1, 3, and 6 and animals were sacrificed after the final observation. No erythema was observed while the variations in ear thickness was less than 25 %. Piloerection and diarrhoea was 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) and excision of the nodes was done on day 6 followed by the tissue processing for radioactivity. 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 were 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 EC=10 %.

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

The outcome of the available LLNA study of - 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride (read-across substance) 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.

9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride is considered to react the same way as 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride. An extra methyl group should not influence the skin sensitising potential of the substance.

10.7.2 Comparison with the CLP criteria

The results from the LLNA study available in the registration dossier under REACH were compared with the CLP criteria for skin sensitisers in the Table below.

Table 14: Comparison of results confirming the skin sensitisation potential of 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride and 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride with the respective CLP criteria from CLP regulation.

Assay and Reference	Criteria according to CLP regulation[7]	Relevant result	Resulting Classification
Local lymph node assay (LLNA) (Charles River Laboratories Den Bosch B.V., 2017)	**Skin Sens. 1A:** EC3 value ≤ 2 % **Skin Sens. 1B:** EC3 value > 2 %	EC3 = 10 % (when SI =3)	**Skin Sens. 1B**

In the LLNA study described using 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride an EC3 value > 2 % was determined leading to a classification as Skin Sens. 1B. It should be noted that lower concentrations than 10 % were not tested which means that Skin Sens. 1A cannot be completely excluded. However, it is reasonable to assume that concentrations < 2 %, which would allow classification as Skin Sens. 1A, would be considered as not sensitising in this case as the lowest tested concentration of 10 % showed a SI=3 and the EC3 was calculated as 10 %. The proposed classification should be applied for the target substance 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride which is in line with the current self-classification of the registrant.

The skin sensitisation potency of the tested substance was derived from the LLNA study according to the Guidance to CLP Criteria Table 3.6 [8]).

EC3-value (% w/v)	Potency	Resulting sub-category
≤ 0.2	Extreme	1A
> 0.2 - ≤ 2	Strong	1A
> 2	**Moderate**	**1B**

According to these criteria the skin sensitising potency of 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride based on the data from read-across substance 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride is considered as moderate given that the resulting EC3 value > 2 %. Therefore the generic concentration limit (GCL) of 1 % (w/v) for moderate potency should be applied for 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride (as laid down in the Guidance to CLP Criteria Table 3.9).

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the results from a LLNA study according to OECD guideline 429 performed with the read-across substance 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride should be classified in

[7] Guidance on the Application of the CLP Criteria, Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures
https://echa.europa.eu/documents/10162/13643/clpriteria_v5_part3_caracal_en.pdf/c1b2f195-acdb-995c-f430-7675ca96ca9f

[8] https://chesar.echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5

Category 1B (indication of skin sensitising potential) based on GHS criteria (**Skin Sens. Category 1B, H317 – May cause an allergic skin reaction**). No Specific Concentration Limit (SCL) is proposed.

RAC evaluation of skin sensitisation

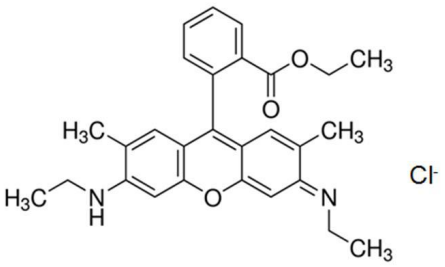
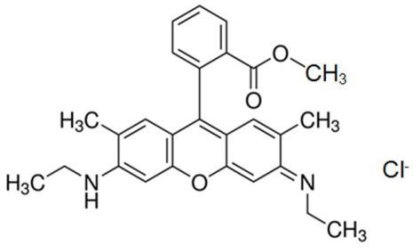
Summary of the Dossier Submitter's proposal

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.

10.8 Germ cell mutagenicity

Not assessed in this dossier.

10.9 Carcinogenicity

Not assessed in this dossier.

10.10 Reproductive toxicity

Not assessed in this dossier.

10.11 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

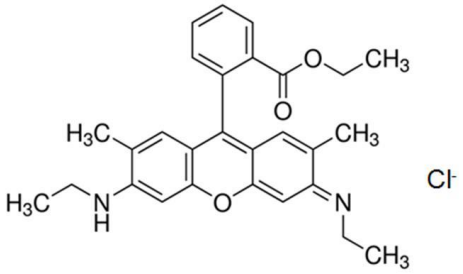
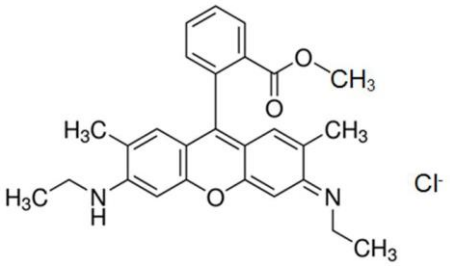
Not assessed in this dossier.

10.13 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

For the substance 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride no experimental data for degradability, fish and algae toxicity are available. In the REACH registration dossier (Basic Red 1) for some endpoints a read-across to the structurally similar substance 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride (Basic Red 1:1) is used. The same analogue approach has been chosen for classification purpose to assess the above-mentioned endpoints (see further details in the following chapters of the corresponding endpoints). The target substance 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride (CAS 989-38-8) and the source substance 3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride (CAS 3068-39-1) have the same structure and differ only in the chain length at the carboxyl group (methyl vs. ethyl residue).

	Target substance	**Source substance**
Substance name	9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthylium chloride; Basic Red 1	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)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	13.49 – 18.9 g/L at 20 – 28 °C and pH 3 – 3.1
Partition coefficient n-octanol/water	log Pow = 0.1 ± < 0.1 at 24 °C (pH 3.9 – 4.4)	log Pow = 1.7 at 20 °C (pH 7) log Pow = 1.21 at 25 °C
Surface tension	Not applicable (based on structure, surface activity is not expected).	65.4 mN/m @ 1 g/L and 20 °C
Ready biodegradability	Not readily biodegradable (EPI Suite v.411 BIOWIN v4.10)	Not readily biodegradable (OECD TG 301B)
Short-term toxicity to aquatic invertebrates	EC_{50}= 0.16 mg/L (similar OECD TG 202)	EC_{50}= 1 mg/L
Toxicity to algae		E_rC_{50}= 0.023 mg/L

11.1 Rapid degradability of organic substances

Table 15: Summary of relevant information on rapid degradability

Method	Test material	Results	Remarks	Reference
OECD 301B	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride / Basic Red 1:1 / CAS 3068-39-1 / EC 221-326-1	2 % and 5 % degradation (CO_2 evolution) after 28 days (duplicates)	Read-across Reliability: 1 GLP	Registration dossier for Basic Red 1 (Charles River Laboratories Den Bosch BV, 2017b)
OECD 301D	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride / Basic Red 1:1 / CAS 3068-39-1 / EC 221-326-1	31.45 % degradation (O2 consumption) after 28 days	Read-across Reliability: 3 (reliability in registration dossier: 1) No GLP	Registration dossier for Basic Red 1:1 (CAS 3068-39-1) (Sustainability Support Services, 2018)
BIOWIN (v4.10)	9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthenium chloride / Basic Red 1 / CAS 989-38-8 / EC 213-584-9	Not readily biodegradable (BIOWIN 3: slower than weeks, BIOWIN 5: < 0.5) BIOWIN 1: biodegrades fast (0.8803); BIOWIN 2: biodegrades fast (0.9574); BIOWIN 3: ultimate biodegradation within months (2.1478); BIOWIN 4: primary biodegradation within days or weeks (3.3942); BIOWIN 5: not readily degradable (0.0698) (own calculation: 0.2914); BIOWIN 6: not readily degradable (0.0079) (own calculation: 0.0454);	Reliability: 2	Registration dossier for Basic Red 1 (EPI Suite v.411 BIOWIN v4.10.)
BIOWIN (v4.10)	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride / Basic Red 1:1 / CAS 3068-39-1 / EC 221-326-1	Not readily biodegradable (BIOWIN 3: slower than weeks, BIOWIN 5: < 0.5) BIOWIN 1: biodegrades fast (0.6532); BIOWIN 2: biodegrades fast (0.8028); BIOWIN 3: ultimate biodegradation within months (2.0439); BIOWIN 4: primary biodegradation within days or weeks (3.3060); BIOWIN 5: not readily degradable (0.1607); BIOWIN 6: not readily degradable (0.0127)	Read-across Reliability: 2	(EPI Suite v.411 BIOWIN v4.10.)
OECD	3,6-bis(ethylamino)-9-[2-	Half-lives:	Read-across	Registration

Method	Test material	Results	Remarks	Reference
111	(methoxycarbonyl)phenyl]-2,7-dimethylxanthylium chloride / Basic Red 1:1 / CAS 3068-39-1 / EC 221-326-1	pH 4 > 1 year (25°C) pH 7 = 266 days (20°C) pH 7 = 187 days (25°C) pH 9 = 324 hours (20°C) pH 9 = 168 hours (25°C) no transformation products analysed	Reliability: 1 GLP	dossier for Basic Red 1:1 (CAS 3068-39-1) (Charles River Laboratories Den Bosch BV, 2017c)

11.1.1 Ready biodegradability

For the substance itself (Basic Red 1) no ready biodegradability test is available.

Data from BIOWIN shows similar results for Basic Red 1 and the structurally similar substance Basic Red 1:1 (see Table 13). Therefore, experimental data from Basic Red 1:1 is used to assess the ready biodegradability of Basic Red 1.

Nevertheless, two ready biodegradability studies with Basic Red 1:1 according to OECD Guideline 301B (GLP compliant) and OECD Guideline 301D (not GLP compliant) were applied. In the test according to OECD Guideline 301B an initial concentration of 17 mg/L (test material), corresponding to 12 mg TOC/L, was used (Charles River Laboratories Den Bosch BV, 2017b). 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 pH range of 7.6 – 8.0. In the registration dossier it is stated that "S*ince the test material was not sufficiently soluble to allow preparation of an aqueous solution at a concentration of 1 g/L, weighed amounts were added to the 2-litre test bottles containing medium with microbial organisms and mineral components. To this end, 10 mL of Milli-RO water was added to each weighing bottle containing the test material. After vigorous mixing (vortex) the resulting suspension was added quantitatively to the test medium. The test solutions were continuously stirred during the test to ensure optimal contact between the test material and test medium".* After 28 days 2 and 5 % $ThCO_2$ were measured in the test bottles with test material (duplicates), respectively. In the toxicity control 27 % $ThCO_2$ was measured after 14 days. Hence, the test material was assumed not to inhibit microbial activity. The reference substance (sodium acetate) showed 60 % degradation within 14 days.

In the study according to OECD Guideline 301D polyseed was used as inoculum (Sustainability Support Services, 2018). One polyseed capsule were added in 500 ml distilled water and then stirred for one hour for proper mixing and functioning of inoculum. This gave the bacterial count as 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 degraded with 58.43 % after 14 days. Hence, the validity criterion is not fulfilled (≥ 60 % after 14 days).

In conclusion, Basic Red 1 is predicted to be not readily biodegradable based on the data of the structurally similar substance Basic Red 1:1.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

For the substance itself no data is available. Nevertheless, a hydrolysis study with Basic Red 1:1 according to OECD Guideline 111 (GLP compliant) is available and documented in the registration dossier of this substance (Charles River Laboratories Den Bosch BV, 2017c). 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 time at 25 °C and pH 4 is greater than 1 year. In the main study half-lives of 187 days (25 °C) and 226 days

(20 °C) were observed for pH 7 and 168 hours (25 °C) and 324 hours (20 °C) for pH 9. No hydrolysis products were analysed in this study. Hence, it cannot be demonstrated whether the hydrolysis products do not fulfil the criteria for classification as hazardous for the aquatic environment. Consequently, the study should not be used for classification.

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not relevant.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

No data available.

11.1.4.4 Photochemical degradation

No data available.

11.2 Environmental fate and other relevant information

No data available.

11.3 Bioaccumulation

Table 16: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD 107	Log Kow = 0.1 ± < 0.1	Reliability: 1	Registration dossier (Consilab mbH, 2018)

11.3.1 Estimated bioaccumulation

No data available.

11.3.2 Measured partition coefficient and bioaccumulation test data

A log Kow of 0.1 was determined by the shake-flask-method according to the OECD Test Guideline 107 (GLP compliant) at 24 °C and pH 3.9 - 4.4.

11.4 Acute aquatic hazard

Table 17: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[9]	Remarks	Reference
OECD TG 203	*Leuciscus idus*	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthenium chloride / Basic Red 1:1 / CAS 3068-39-1 / EC 221-326-1	96h-LC_{50} was 6.85 mg/L (nominal)	Read-across Reliability: 2 (only 4 conc., no analytical monitoring)	Registration dossier for Basic Red 1:1 (BASF AG, 1990a)
OECD TG 202	*Daphnia magna*	9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethylxanthenium chloride / Basic Red 1 / CAS 989-38-8 / EC 213-584-9	48h-EC_{50}= 0.16 mg/L (nominal)	Reliability: 2 (no analytical monitoring)	Registration dossier for Basic Red 1 (BASF AG, 1990b)
OECD TG 202	*Daphnia magna*	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthenium chloride / Basic Red 1:1 / CAS 3068-39-1 / EC 221-326-1	48h-EC_{50}= 1 mg/L (nominal)	Read-across Reliability 1 (registrant)	Registration dossier for Basic Red 1:1 (Charles River Laboratories Den Bosch B.V., 2017d)
OECD TG 201 GLP	*Pseudokirchneriella subcapitata*	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthenium chloride / Basic Red 1:1 / CAS 3068-39-1 / EC 221-326-1	72h-E_rC_{50}= 0.023 mg/L (meas. TWA)	Read-across Reliability: 1	Registration dossier for Basic Red 1 (Charles River Laboratories Den Bosch B.V., 2017e)

As there is only an acute toxicity test on aquatic invertebrates available for Basic Red 1, the acute aquatic toxicity data for the read-across substance Basic Red 1:1 is included in the dossier (acute toxicity to fish, to aquatic invertebrates and toxicity to algae). The results of the available acute toxicity tests on *Daphnia magna* for Basic Red 1 and Basic Red 1:1 show 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 is the most sensitive aquatic test species. The EC_{50} from the algae test (with Basic Red 1:1) is lower than the one from the *Daphnia* study with Basic Red 1. Thus, the data with algae and Basic Red 1:1 should be 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.

11.4.1 Acute (short-term) toxicity to fish

Not available for Basic Red 1.

A short-term toxicity test to fish (*Leuciscus idus*) with the read-across substance Basic Red 1:1 is described here for comparison reason. The test was conducted according to OECD TG 203 with the deviation that only four dose levels were examined. No analytical monitoring was conducted and no vehicle was used. A static test type was used. The test conditions were: hardness was 2.5 mmol/L, test temperature was 19 to 20 °C, the pH value was about 8.0 and the dissolved oxygen was 7.8 to 8.7 mg/L. The nominal test concentrations

[9] E_rC_{50} … endpoint: growth

were: 1.0, 2.15, 4.64 and 10 mg/L. Glass aquaria with a fill volume of 10 L were used. 10 fish per vessel were used per concentration. The biomass loading rate was 6.0 g fish/L test water. The resulting 96h-LC_{50} was 6.85 mg/L (nominal).

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

One short-term toxicity test to aquatic invertebrates is available. The test is conducted according to OECD TG 202 with *Daphnia magna* in a static test type. No analytical monitoring and no vehicle were used. 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. The test concentrations were: 0, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039 and 0.019 mg/L. The test conditions were: hardness was 2.89 mmol/L, test temperature was 18.85 to 20.85 °C, the pH value was 8.1 and the conductivity was 600 to 700 µS/cm. Test vessels with 10 mL test volume were used with 5 organisms per vessel and 4 replicates per concentration/control. The resulting 48h-EC_{50} was 0.16 mg/L (95 % CI: 0.12-0.2 mg/L).

A short-term toxicity test to aquatic invertebrates (*Daphnia magna*) with the read-across substance is described here for comparison reason. The test is conducted according to OECD TG 202 in a static test type with analytical monitoring. No vehicle was used. The nominal test concentrations were: 0, 0.10, 0.22, 0.46, 1.0 and 2.2 mg/L (measured concentrations: 92 to 105 % of nominal). The test conditions were: hardness 180 mg $CaCO_3$/L, 20 °C, pH 7.9 to 8.1 and 8.9 to 9.1 mg O_2/L. The 100 mL-test vessels were filled with 80 mL test solution. 5 organisms per vessel and 4 replicates per concentration/ control were used. The resulting 48h-EC_{50} was 1 mg/L (95 % CI: 0.12-0.2 mg/L).

11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

The registration dossier contains for this endpoint a read-across study for the source substance Basic Red 1:1 (CAS 3068-39-1). It was conducted according to OECD TG 201 (GLP) with analytical monitoring and without the use of a vehicle. The test was conducted with *Pseudorkirchneriella 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). 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} is 0.023 mg/L (meas. TWA).

11.4.4 Acute (short-term) toxicity to other aquatic organisms

Not available.

11.5 Long-term aquatic hazard

Table 18: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[10]	Remarks	Reference
OECD TG 201 GLP	*Pseudokirchneriella subcapitata*	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)phenyl]-2,7-dimethylxanthenium chloride / 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	Registration dossier for Basic Red 1:1 (Charles River Laboratories Den Bosch B.V., 2017e)

[10] E_rC_{50} … endpoint: growth rate

11.5.1 Chronic toxicity to fish

Not available.

11.5.2 Chronic toxicity to aquatic invertebrates

Not available.

11.5.3 Chronic toxicity to algae or other aquatic plants

The registration dossier contains for this endpoint a read-across study to the source substance Basic Red 1:1 (CAS 3068-39-1). It was conducted according to OECD TG 201 (GLP) with analytical monitoring and without the use of a vehicle. The test was conducted with *Pseudorkirchneriella 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). 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_{10} is 0.014 mg/L (meas. TWA).

11.5.4 Chronic toxicity to other aquatic organisms

Not available.

11.6 Comparison with the CLP criteria

11.7 Acute aquatic hazard

Table 19: Comparison with criteria for acute aquatic hazards

	Criteria for acute environmental hazards	9-[2-(Ethoxycarbonyl)phenyl]-3,6-bis(ethyl-amino)-2,7-dimethylxanthylium chloride	Conclusion
Acute Aquatic Toxicity	Cat. 1: $LC_{50}/EC_{50}/ErC_{50} \leq 1$ mg/L	Fish: 96h-LC_{50}= 6.85 mg/L (n) (*Leuciscus idus*) (read-across) Invertebrates: 48h-EC_{50}= 0.16 mg/L (n) (*Daphnia magna*) Algae: 72h-ErC_{50}= 0.023 mg/L (m) (*Pseudokirchneriella subcapitata*) (read-across)	**Aquatic Acute 1, M= 10** *(based on 72h-E_rC_{50}= 0.023 mg/L)*

11.7.1 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 20: Comparison with criteria for long-term aquatic hazards

	Criteria for environmental hazards	9-[2-(Ethoxycarbonyl)-phenyl]-3,6-bis(ethyl-amino)-2,7-dimethyl-xanthylium chloride	Conclusion
Rapid Degradation	Half-life hydrolysis < 16 days Readily biodegradable in a 28-day test for ready biodegradability (> 70 % DOC removal or > 60 % theoretical oxygen demand, theoretical carbon dioxide)	No data available 2-5 % biodegradation after 28 days (read-across substance) => not readily biodegradable	**Not rapidly degradable**
Bioaccumulation	BCF ≥ 500 or if not available log Kow ≥ 4	BCF not available log Kow = 0.1	**Not bioaccumulative (low potential for bioconcentration in the aquatic environment)**
Aquatic Toxicity	Not rapidly degradable substances: Cat. 1: NOEC ≤ 0.1 mg/L Cat. 2: NOEC ≤ 1 mg/L <u>Surrogate approach in absence of appropriate chronic toxicity reference data:</u> Not rapidly degradable substances and/or bioaccumulative substances: Cat. 1: $E/LC_{50} \leq 1$ mg/L Cat. 2: $E/LC_{50} \leq 10$ mg/L	Fish: No appropriate long-term toxicity tests are available. Invertebrates: No appropriate long-term toxicity tests are available. Algae: 72h-E_rC_{10}= 0.014 mg/L (m) (*Pseudokirchneriella subcapitata*) (read-across) Fish: - Invertebrates: 48h-EC_{50}= 0.16 mg/L (n) (*Daphnia magna*)	**Aquatic Chronic 1, M= 1** *(based on 72h-E_rC_{10}= 0.014 mg/L (m) with Pseudokirchneriella subcapitata as well as an acute 48h-EC_{50}= 0.16 mg/L for Daphnia magna)*

	Cat. 3: E/LC_{50} ≤ 100 mg/L		

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Basic Red 1 is not rapidly degradable and has a low potential for bioaccumulation. The most sensitive valid short-term toxicity E/LC_{50}-value is 0.023 mg/L (based on the read-across substance). This results in a classification of Basic Red 1 as Aquatic Acute 1 (M-factor of 10) and a labelling with H400.

The most sensitive valid long-term toxicity no effect concentration is 0.014 mg/L (read-across substance; measured concentrations). This results in a classification of Basic Red 1 as Aquatic Chronic 1 (M-factor of 1) and a labelling with H410 based on the criteria given in Table 4.1.0(b)(i) of the CLP Regulation.

Basing the chronic classification on the available acute toxicity studies with Basic Red 1 itself, the most sensitive acute E/LC_{50}-value is 0.16 mg/L (nominal concentration, Daphnia magna). With the surrogate approach, it results as well in a classification of Basic Red 1 as Aquatic Chronic 1 and a labelling with H410 based on the criteria indicated by Table 4.1.0(b)(iii) of the CLP Regulation. The resulting M-factor is 1.

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).

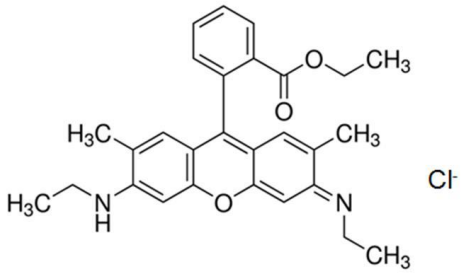
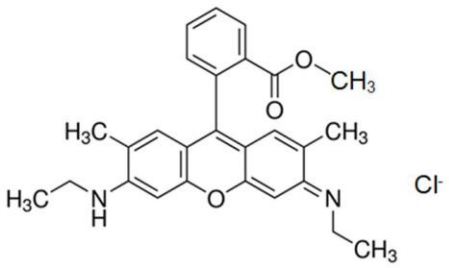
Read-across

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(ethylamino)-2,7-dimethylxanthylium chloride; **Basic Red 1**	3,6-bis(ethylamino)-9-[2-(methoxycarbonyl)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	LC_{50} = 6.85 mg/L (OECD TG 203), no analytical monitoring)
Acute toxicity to aquatic inverte brates	EC_{50}= 0.16 mg/L (similar OECD TG 202, not GLP, no analytical monitoring)	EC_{50}= 1 mg/L (nominal based on analytical monitoring, GLP, OECD TG 202)
Toxicity to algae	No data - Read-across	E_rC_{50}= 0.023 mg/L E_rC_{50}= 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 $\mu E/m^2/s$ 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, $0.01<EC_{10}\leq 0.1$ 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.**

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