Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

disodium 4-amino-6-((4-((4-(2,4-diaminophenyl)azo)phenylsulfamoyl)phenyl)azo) -5-hydroxy-3-((4-nitrophenyl)azo)naphthalene - 2,7-disulfonate

EC Number: 421-880-6
CAS Number: 201792-73-6

CLH-O-0000001412-86-165/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 public 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

22 September 2017

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),

Annex VI, Part 2

Substance Name: Acid Black 210 Na

disodium 4-amino-6-((4-((4-(2,4-diaminophenyl)azo)phenylsulfamoyl)phenyl)azo)-5-hydroxy-3-((4-nitrophenyl)azo)naphthalene-2,7-disulfonate

EC Number: **421-880-6**

CAS Number: **201792-73-6**

Index Number: **611-159-00-6**

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Dossier prepared by Industry in accordance with Article 37(6) of CLP Regulation,

Version number: 2 **Date: 10/10/2016**

CONTENTS

Part A.

Part A.

1. PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1. Substance

Table 1: Substance identity

Substance name:	Acid Black 210 Na
EC number:	421-880-6
CAS number:	201792-73-6
Annex VI Index number:	611-159-00-6
Degree of purity:	>= 60.0 — < 100.0 % (w/w)
Impurities:	See table 6

1.2. Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Eye Damage 1 (Hazard statement: H318: Causes serious eye damage.) Aquatic Chronic 3 (Hazard statement: H412: Harmful to aquatic life with long lasting effects.)
Current proposal for consideration by RAC	No classification
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	No classification

1.3. Proposed harmonised classification and labelling based on CLP Regulation

The proposal is to remove the current classification and the entry from Annex VI of CLP Regulation, based on the fact that new information has been provided in the framework of REACH Regulation on the concerned end points, which overcome the current evaluations.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	none		none	conclusive but not sufficient for classification
2.2.	Flammable gases	none		none	conclusive but not sufficient for classification
2.3.	Flammable aerosols	none		none	conclusive but not sufficient for classification
2.4.	Oxidising gases	none		none	conclusive but not sufficient for classification
2.5.	Gases under pressure	none		none	conclusive but not sufficient for classification
2.6.	Flammable liquids	none		none	conclusive but not sufficient for classification
2.7.	Flammable solids	none		none	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	none		none	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	none		none	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	none		none	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	none		none	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	none		none	conclusive but not sufficient for classification
2.13.	Oxidising liquids	none		none	conclusive but not sufficient for classification

2.14.	Oxidising solids	none		none	conclusive but not sufficient for classification
2.15.	Organic peroxides	none		none	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	none		Data lacking	Data lacking
3.1.	Acute toxicity - oral	none		none	conclusive but not sufficient for classification
	Acute toxicity - dermal	none		none	conclusive but not sufficient for classification
	Acute toxicity - inhalation	none		none	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	none		none	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	**No classification**		**Eye Damage 1**	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	none		Data lacking	Data lacking
3.4.	Skin sensitisation	none		none	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	none		none	conclusive but not sufficient for classification
3.6.	Carcinogenicity	none		Data lacking	Data lacking
3.7.	Reproductive toxicity	none		none	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	none		none	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	none		none	conclusive but not sufficient for classification
3.10.	Aspiration hazard	none		Data lacking	Data lacking

4.1.	Hazardous to the aquatic environment	**No classification**		**Aquatic Chronic 3**	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	none		none	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: No signal word

Hazard statements: No Hazard statements

Precautionary statements:No Precautionary statement

Proposed notes assigned to an entry: no notes

2. BACKGROUND TO THE CLH PROPOSAL

2.1.History of the previous classification and labelling

The substance has been classified in the framework of NONS, and the classification was inserted in Annex VI of CLP ATP 01.

In the **20th Meeting** summary record of the CMR group **on Classification and Labelling of New Notified Substances (Ispra, 14th November 2005) it is reported:**

421-880-6 (96-01-0395-00) Proposed classification: R52-53

" labelling: R: 52/53, S: 61

written comments: UK: The UK believe that R41 is warranted based on the irreversible colouration of the cornea observed in one animal. R41 would also require the addition of S26-39.

F: Xi R41 should be added, but substance not marketed anymore (february 2005), Propose R52-53. ErC50 = 13.7. **ES**: we need some clarifications about the values of the EC_{50}'s of the algae tests. **D:** Discuss R41 (irrev. coloration of the eyes). (**M21: R52-53 agreed).**

Agreed classification: Xi; R41, R52-53

" labelling: Xi, R: 41-52/53, S: (2-)26-39-61

For the purpose of this CLH proposal all registration dossiers available in REACH-IT in July 2014 have been considered by the Italy CA.

2.2. Short summary of the scientific justification for the CLH proposal

Currently, Acid Black 210 sodium salt has a harmonised classification (Regulation (EC) 1272/2008, Annex VI) for the following toxicological endpoints: Eye Damage 1 (Hazard statement: H318: Causes serious eye damage.) and Aquatic Chronic 3 (Hazard statement: H412: Harmful to aquatic life with long lasting effects.).

However, new studies are available to be considered to update the current classification.

The substance Acid Black 210 sodium salt (EC 421-880-6 – ABl210-Na) has been evaluated taking into consideration the structural analogue potassium salt (EC: 286-384-2 – ABl210-K). The read across approach has been used for two purposes:

- in order to support and confirm the outcomes from the existing studies on the ABl210-Na

- in order to predict and assess endpoint information for the target substance ABl210-Na, avoiding unnecessary studies.

The Read Across justification is detailed in the document attached in the IUCLID dossier, section 13.

Eye irritation/corrosion:

The current classification for corrosion was based on a study presented in the framework of DSD Notification Of New Substances, reporting eye damage not reversible within 28 days (Stahl Europe B.V., 1996a).

It can be assumed that the tested substance is the sodium salt of Acid Black 210, but the full composition of the substance is unknown (main component content and impurity profile), as well as several further details on the study like the physical form of the applied substance and if the eye has been rinsed or not.

Several studies have been performed independently on the different salts of the substance by different producers (BASF SE 1984, 67 % litium/potassium salt and J. Zapatero 1997, 65 % potassium salt) and no eye irritation has been observed following CLP criteria. Those two studies are considered fully reliable.

An *in vitro* test according to OECD TG 437, on the Acid Black 210 sodium salt is available (S. Cinelli, 2014). The test resulted in a medium IVIS of 25.5 due to alterations of the mean cornea opacity. However, since the test item is coloured, the mean opacity value can be affected by the substance remaining on the corneal surfaces. No relevant increases in corneas permeability were recorded after treatment with the test item when compared to those of negative control. The result according to the new update of OECD TG 437 of the 26 July 2013 cannot lead to a clear conclusion about the classification regarding irritating properties, but clearly exclude serious eye damage.

Aquatic toxicity

Classification for aquatic toxicity has been based on a study on Algae Growth Inhibition presented in the framework of DSD Notification Of New Substances, reporting EC_{50} at 72 hours based on Grow rate of 13.7 mg/l. (Stahl Europe B.V., (i)).

It can be assumed that the tested substance is the sodium salt of Acid Black 210, but it was not possible to recover the original report of the study, the full composition of the substance is unknown, as well as many details on the study like nominal or measured concentrations.

A second test (Dirk Scheerbaum, 2011) was performed on Acid Black 210 potassium salt (read across) according to OECD series on testing and assessment Number 23 (2000): "Guidance document on aquatic toxicity testing of difficult substances and mixtures", paragraph 3.8, Coloured substances

The NOEC is > 1 mg/l and EC_{50} is between 10 and 100 mg/l (nominal).

The observed algae toxicity is reasonably due to the shadowing effect of the substance in the tested medium. Several studies on algae conducted on dark dyes, including those with a modified test system for coloured substances, showed that the growth inhibition is not due to a toxic effect of the dye, but to the light absorption of the stained water. Modified test system is usually conducted putting the dye above the algae testing solution, in a different vessel and not into contact with the alga. This method has some limitation because it focuses on the shadowing effect but gives no information on the real potential toxicity for algae or aquatic plants of the tested substance.

At present new information is available on aquatic toxicity. A Lemna growth inhibition test has been conducted following OECD TG 221 on Acid Black 210 potassium salt (for read across consideration see above) (Alexa Caduff, 2012) which resulted in EC_{50} > 2000 mg/l.

Lemna is an aquatic plant that develops his leaves on the surface of the water, while nourishing substances are taken from the water solution. With this test the observed effect is only related to the potential toxicity of the substance and not to the potential shading effect of a classical Alga study.
Acute toxicity on Fish and Daphnia has been performed both on the substance and on the potassium salt and they don't reveal any toxicity at high levels of dosing.

Furthermore, a short summary is available of a study performed and submitted in the framework of the notification of the substance S124668 (Acid Black 210, sodium salt) under DSD in 1997 (JH Moore, 1997). The summary indicates that a long term reproductive study on Daphnia has been performed on the substance. The result is indicating that the substance has a NOEC long term > 1 mg/l, therefore based on Regulation 286/2011, amending Regulation 1272/2008 (CLP) the study confirms that no classification for the environment is warranted.

2.3. Current harmonised classification and labelling

Classification		Labelling		
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram Signal Word Code(s)	Hazard Statement Code(s)	Suppl. Hazard statement code(s)
Eye Dam. 1 Aquatic Chronic 3	H318 H412	GHS05 Dgr	H318 H412	

Specific Concentration Limits and M Factors: none
Pictogram(s)
 Corrosion

2.4. Current self-classification and labelling

No classification

RAC general comment

Background: current classification and read-across

Acid Black 210 sodium salt (also referred to below as ABl210-Na) has an existing harmonised classification (Regulation (EC) 1272/2008, Annex VI) for Eye Damage 1 (H318: Causes serious eye damage) and Aquatic Chronic 3 (H412: Harmful to aquatic life with long lasting effects). Since new information has become available after this classification had been decided under the Dangerous Substances Directive (DSD), the Dossier Submitter (DS) proposed removal of the classifications for both hazard classes based on the CLP criteria. For this proposal, the DS considered all the registration dossiers available in REACH-IT up to July 2014.

The proposed revision relies on read-across from the potassium salt. The Acid Black 210 potassium salt (ABl210-K) has no entry in the Annex VI of CLP.

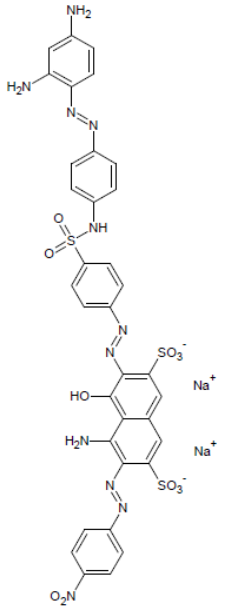
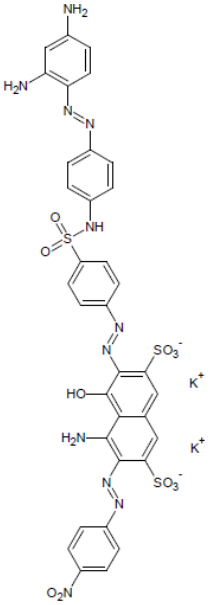
Justification for the Read Across from Acid Black 210 Potassium salt

The DS proposed a one-to-one read across to Acid Black 210 Sodium salt as the target chemical from Acid Black 210 Potassium salt as the source chemical. This approach is consistent with Article 13 of REACH and makes use of the recommendations included in the *ECHA Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals*. In particular, the analysis takes into account: a) the structural similarity, b) common precursors, c) typical compositions and d) physico-chemical properties. In addition, the toxicological profile was assessed (see below)

a) Structural similarity

Acid Black Na and its potassium salt counterpart have the same molecular and structural formula except for the alkali metal as shown by the DS in the following table:

Table: Molecular and structural formulas of the target and source chemicals.

Common name	**ABl210-Na**	**ABl210-K**
Molecular formula	$C_{34}H_{25}N_{11}Na_2O_{11}S_3$	$C_{34}H_{25}K_2N_{11}O_{11}S_3$
Structure		

Both sodium and potassium salts dissociate in water to form the identical base structure and their respective counter-ions. The entire chromophore structure may be seen as an identical functional group having the same properties in the dissociated form. On the other hand, it is noted that, due to its greater molecular size, potassium is slightly less electronegative and its salt is potentially less soluble; consequently, potassium can form monoacid sulphated salts more readily than sodium and thus may influence the impurity profile.

b) *Common precursors*

Both the source and the target chemicals are reported to have the same precursors (See Background Document).

c) Typical composition

Both Acid Black 210 sodium salt and the source compound used in the read-across assessment are multicomponent products *sensu stricto*; they are industrial products of variable composition. It is expected that the commercial batches should present very similar characteristics and the composition profile as presented by the DS is shown in the following table:

Table: The composition profile of the target and source chemicals.

	ABl210-Na	**ABl210-K**
Main component	> 60%	65-77%
Sodium chloride	0-15%	0-15%
Water	0-20%	0-15%
Organic impurities	0-8% *	n.a.
Sodium sulphate	0-3%	-
Potassium chloride	-	0-10%

*This includes isomers of the main component (0-4%) and other organic impurities (0-3.9%); n.a. not applicable

The relatively low concentration of the main component and therefore a notable percent of impurities in the substance raises the question of whether the product should be considered a mono-constituent substance or a mixture. The following arguments had been brought by the DS in favour of the first option:

- Both the Acid Black sodium salt and the potassium salt are produced, used and characterised as one product;
- The ABl210-Na was presented as a mono-constituent substance in the framework of Dangerous Preparation Directive (DPD, 99/45/EC) and ECHA recognised the substance presented by the Lead Registrant in 2011 as the same substance.

Given the argumentation, RAC agrees with the DS that, for the purpose of the read-across, the approach regarding both substances should be the same as that for mono-constituent substances.

d) Physico-chemical properties

A brief comparison of the selected properties is given in the following table:

Table: Selected physical-chemical properties of the target and source chemicals.

Indicator	**ABl210-Na**	**ABl210-K**
Melting point	> 330 °C	Decomposition starting from 200 °C
Relative density	1.43 at 20 °C	1.29 at 20 °C
Water solubility	270 g/L at 20 °C and pH ca. 8.7	183 g/L at 20 °C and pH ca. 9
Partition coefficient n-octanol/water (Log Kow)	-3.1 at 25°C (pH not reported)	-1.73 at 20° and pH 8.64

As noted under the heading *Structure similarity* above, there is a slight difference in water solubility. It is expected that the bioavailability would be highly comparable.

e) Toxicological profile

The inorganic salts deriving from the production process have not been shown to influence the toxicological and eco-toxicological properties, that is to potentiate or diminish the biological effects of the main component;

The studies considered for toxicological characterisation were performed with the industrial chemical; consequently, the results reflect the behaviour of the substance as it is, including the isomers and impurities;

Additional key elements

Common precursors

Both the source and the target chemicals are reported to have the same precursors as follows: water (EC: 231-791-2; CAS: 7732-18-5), 4-amino-N-(4-aminophenyl)benzenesulfonamide (EC: 240-834-4; CAS: 16803-97-7), 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid (EC: 201-975-7; CAS: 90-20-0. Other name: H Acid), p-nitroaniline (EC: 202-810-1; CAS: 100-01-6), m-phenylendiamine (EC: 203-584-7; CAS: 108-45-2), sodium nitrite (EC: 231-555-9; CAS: 7632-00-0) and hydrochloric acid (EC: 231-791-2; CAS: 7732-18-5). The precursors undergo a reaction process in six identical steps to produce the same organic moiety. The only difference in the manufacturing process is the last step, in which the neutralisation is made with KOH to obtain the source chemical (ABl210-K) and NaOH for the target ABl210-Na.

Conclusion

Given the argumentation above, RAC agrees that, based on their similar physico-chemical properties, the read-across between the sodium and a potassium salt of the same organic moiety is viable since comparable activity is anticipated *in vivo*. RAC also agrees that the read-across approach to evaluate environmental fate and aquatic toxicity can be considered reliable.

3. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A change in an existing entry (Eye Damage 1 and Aquatic Chronic 3) is considered justified due to new data becoming available after the harmonised classification was agreed.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

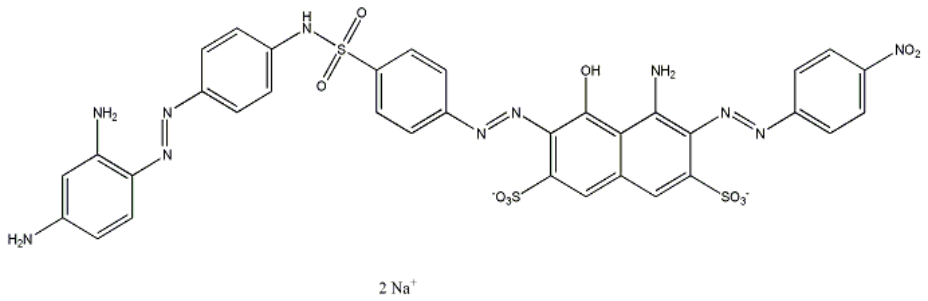
4. IDENTITY OF THE SUBSTANCE

4.1. Name and other identifiers of the substance

Table 4: Substance identity

EC number:	421-880-6
EC name:	disodium 4-amino-6-((4-((4-(2,4-diaminophenyl)azo)phenylsulfamoyl)phenyl)azo)-5-hydroxy-3-((4-nitrophenyl)azo)naphthalene-2,7-disulfonate
CAS number (EC inventory):	201792-73-6
CAS number:	201792-73-6
CAS name:	2,7-Naphthalenedisulfonic acid, 4-amino-6-[2-[4-[[[4-[2-(2,4-diaminophenyl)diazenyl]phenyl]amino]sulfonyl]phenyl]diazenyl]-5-hydroxy-3-[2-(4-nitrophenyl)diazenyl]-, sodium salt (1:2)
IUPAC name:	disodium 4-amino-6-[[4-(N-(4-((E)-(2,4-diaminophenyl)diazenyl)phenyl)sulfamoyl)phenyl)diazenyl)-5-hydroxy-3-((E)-(4-nitrophenyl)diazenyl)naphthalene-2,7-disulfonate
CLP Annex VI Index number:	611-159-00-6
Molecular formula:	C34H25N11Na2O11S3
Molecular weight range:	ca. 905.8

Structural formula:



4.2. Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Acid Black 210 Na EC no.: 421-880-6	ca. 66.4 % (w/w)	>= 60.0 — < 100.0 % (w/w)	

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Impurity_1	ca. 0.17 % (w/w)	>= 0.0 — < 0.5 % (w/w)	
Impurity_2/Acid_Black_210Na	ca. 0.5 % (w/w)	>= 0.0 — < 1.0 % (w/w)	for specific concentration on the sample refer to the analytical certificate
Impurity_3	ca. 0.4 % (w/w)	>= 0.0 — < 0.5 % (w/w)	for specific concentration on the sample refer to the analytical certificate
Impurity_4	ca. 0.04 % (w/w)	>= 0.0 — < 0.1 % (w/w)	for specific concentration on the sample refer to the analytical certificate
Impurity_5/oligomeric impurity	ca. 0.99 % (w/w)	>= 0.0 — <= 1.5 % (w/w)	for specific concentration on the sample refer to the analytical certificate
Impurity_6	ca. 2.71 % (w/w)	>= 0.0 — < 4.0 % (w/w)	for specific concentration on the sample refer to the analytical certificate
Impurity_8	ca. 0.2 % (w/w)	> 0.0 — < 0.3 % (w/w)	

Impurity	Typical concentration	Concentration range	Remarks
sodium chloride EC no.: 231-598-3	ca. 7.4 % (w/w)	>= 0.0 — < 15.0 % (w/w)	for specific concentration on the sample refer to the analytical certificate
sodium sulfate EC no.: 231-820-9	ca. 1.86 % (w/w)	>= 0.0 — < 3.0 % (w/w)	for specific concentration on the sample refer to the analytical certificate
water EC no.: 231-791-2	ca. 16.0 % (w/w)	>= 0.0 — < 20.0 % (w/w)	

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

4.3. Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference
Physical state at 20°C and 1013 hPa Acid Black 210 sodium salt Purity: Unknown	Solid: black odourless powder	Qualitative assessment
Melting / freezing point Acid Black 210 sodium salt Purity: Unknown EU Method A.1: Kofler hot bar	Decomposition > 200 °C	Stahl Europe B.V. (a) Only the visual appearing of a dividing line between solid and liquid can be assessed by this method, irrespectively from the fact that decomposition or actual melting has happened.
Melting/freezing point Acid Black 210 potassium salt Purity: 67 % EU Method A.1: Differential Scanning Calorimetry	Decomposition > 200 °C	Michal Bartos (2011a) The onset of a thermal decomposition was observed at 200°C. No peak corresponding to melting point was observed.
Boiling point	Decomposition > 200 °C	Michal Bartos (2011b)

Property	Value	Reference
Acid Black 210 potassium salt Purity: 67 % EU Method A.1: Differential Scanning Calorimetry		The onset of a thermal decomposition was observed at 200°C. No peak corresponding to melting/boiling point was observed.
Relative density Acid Black 210 sodium salt Purity: unknown EU Method A.3	1.43 at 20 °C	Stahl Europe B.V. (b)
Relative density Acid Black 210 potassium salt Purity: 67 % EU Method A.3: pycnometer	1.286 at 20 °C	Michal Bartos (2011c)
Vapour pressure Acid Black 210 acid form	10E-45 Pa at 25 °C	Estimated with SPARC v4.6
Surface tension Acid Black 210 sodium salt Purity: unknown EU Method A.5:	71.8 mN/m at 26°C at 1 g/L	Stahl Europe B.V. (c) Concentration of the solution: 1000 mg/l
Water solubility Acid Black 210 sodium salt Purity: unknown EU Method A.6: flask method	270 g/l at pH 8.7 and 25 °C	Stahl Europe B.V. (d)
Water solubility Acid Black 210 potassium salt	183 g/L at pH 9 and 20 °C	Michal Bartos (2011d)

Property	Value	Reference
Purity: 67 % EU Method A.6: flask method		
Partition coefficient n-octanol/water (log value) Acid Black 210 sodium salt Purity: unknown EU Method A.8: shake flask method	-3.1 at 25 °C	Stahl Europe B.V. (e)
Partition coefficient n-octanol/water (log value) Acid Black 210 potassium salt Purity: 67 % EU Method A.8: shake flask method	-1.73 at 20 °C	Michal Bartos (2011e)
Flash point	Not applicable	Flash point is a property of liquid or solids with a very high vapour pressure. The substance is a solid with very low vapour pressure
Flammability Acid Black 210 sodium salt Purity: unknown EU Method A.10, A.12, A.13	Non flammable	Stahl Europe B.V. (f)
Explosive properties Acid Black 210 sodium salt Purity: unknown EU Method A.14	Not explosive	Stahl Europe B.V. (g)
Self-ignition temperature Acid Black 210 sodium salt Purity: unknown EU Method A.16	215°C at 1013 hPa	Stahl Europe B.V. (h)

Property	Value	Reference
Oxidising properties Acid Black 210 potassium salt Purity: 67 % EU Method A.17	Not oxidising properties	Michal Bartos (2011f)
Granulometry Acid Black 210 potassium salt Purity: 67 % EU Method ISO 13320	D50=52.51 µm	Michal Bartos (2011g) Since particle size is more depending from the production process than from the individual chemical characteristics of the substance, it is realistic to assume that also sodium salt, produced with the same process in the same plant will have the same characteristics. The value is considered within the inhalable fraction, but not in the thoracic or respirable fraction. Since the usual form present on the market is liquid and the solid form is always treated with anti-dusting materials, this value is too much conservative in respect to the real form present in the market, therefore it is not indicative of the need to test the respiratory toxicity as preferred exposure route.
Dissociation constant Acid Black 210 potassium salt Purity: 67 % Method: OECD TG 112	7.62	Michal Bartos (2011h) The pKa of the sulfonic groups was not determined being the tested substance already in the salt form. The experimental value obtained is due to the protonation of the amino groups of the molecule. The data is therefore irrilevant
Viscosity	not scientifically feasible, the substance is a solid	

Discussion of physico-chemical properties

The substance is a black solid powder that decomposes for T >200 °C, with high solubility and negative partition coefficient. It does not show flammable, oxidising or explosive properties and has particle size distribution D_{50} ca 52 µm. The substance is in its salt form, completely dissociated in water and does not show any surface active properties.

Many studies are available on the substance received from ECHA following the inquiry, presented in the NONs submission by Stahl Industrial Colorants s.a.s. (France), but no enough details and no information on composition of the tested substance is provided. Some other studies are available on a similar substance with complete GLP reports and good identification of the substance: the potassium salt of the same dye. Physicochemical properties reported for the two substances have been here compared in order to evaluate the similarity of the two substances and the possibility to use the well assessed results on the similar substance to assess the endpoint instead than the old very poor study presented by Stahl Industrial Colorants s.a.s. and received from ECHA.

Table 9: comparison of properties

Property	**Acid Black 210 Na salt**	**Acid Black 210 K salt**
Physical state at 20°C and 1013 hPa	Black odourless powder	Black odourless powder
Melting / freezing point	Decomposition > 200°C	Decomposition > 200°C
Boiling point	Decomposition > 200°C	Decomposition > 200°C
Relative density	1.43	1.29
Water solubility	Very soluble (270 g/l at 25°C and pH 8.7)	Very soluble (183 g/l at 25°C and pH 9)
Partition coefficient n-octanol/water (log value)	Negative (log Kow at 25°C, pH not reported = -3.1)	Negative (log Kow at 20°C and pH 8.64 = -1.73)
Oxidising properties	Not oxidiser	Not oxidiser

5. MANUFACTURE AND USES

5.1. Manufacture

The substance is imported; therefore no production method is reported

5.2. Identified uses

The substance is used in water-based formulations mainly for industrial leather dyeing, either in wet-end, than for finishing applications. Secondary uses can be with similar processes in textile and paper formulation

6. CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not relevant for the purpose of this CLH report

7. HUMAN HEALTH HAZARD ASSESSMENT

7.1. Toxicokinetics (absorption, metabolism, distribution and elimination)

Not relevant for the purpose of this CLH report.

7.2. Acute toxicity

Not relevant for the purpose of this CLH report.

7.3. Specific target organ toxicity – single exposure (STOT SE)

Not relevant for the purpose of this CLH report.

7.4. Irritation

7.4.1. Skin irritation

Not relevant for the purpose of this CLH report.

7.4.2. Eye irritation

7.4.2.1. Non-human information

The results of experimental studies on eye irritation are summarised in the following table:

Table 10. Overview of experimental studies on eye irritation

Method	Results	Remarks	Reference
rabbit (New Zealand White): 3 animals Vehicle: water Amount: 0.1 ml EU Method B.5 (Acute Toxicity: Eye Irritation / Corrosion)	not irritating Cornea score: 0 of max. 4 (animal #1) (Time point: mean of 24, 48 and 72 h) 0 of max. 4 (animal #2) (Time point: mean of 24, 48 and 72 h) 0.33 of max. 4 (animal #3) (Time point: mean of 24, 48 and 72 h) (fully reversible within: 48 h) Iris score: 0 of max. 4 (animal #1) (Time point: mean of 24, 48 and 72 h)	1 (reliable without restrictions) key study read-across from supporting substance (structural analogue or surrogate) **Test material (Common name): Acid Black 210 potassium salt** **Purity: ca. 65 %** Impurities: sodium sulfate, sodium chloride, potassium chloride	J. Zapatero (1997)

Method	Results	Remarks	Reference
	0 of max. 4 (animal #2) (Time point: mean of 24, 48 and 72 h) 0 of max. 4 (animal #3) (Time point: mean of 24, 48 and 72 h) Conjunctivae score: 0.78 of max. 4 (animal #1) (Time point: mean of 24, 48 and 72h) (fully reversible within: 72 h) 0.78 of max. 4 (animal #2) (Time point: mean of 24, 48 and 72 h) (fully reversible within: 72 h) 1 of max. 4 (animal #3) (Time point: mean of 24, 48 and 72 h) (fully reversible within: 6 d) 0 of max. 4 (animal #1) (Time point: mean of 24, 48 and 72 h) 0.33 of max. 4 (animal #2) (Time point: mean of 24, 48 and 72 h) (fully reversible within: 48 h) 0 of max. 4 (animal #3) (Time point: mean of 24,48 and 72 h) For one animal, one hour after administration, 2 % of aqueous sodium fluorescein solution has been applied, followed by washing the area with a 0.9 % physiological saline solution. The corneal alterations were observed with the aid of a transilluminator with a cobalt blue filter In the course of the first hour after instillation, the test substance induced in one animal slight swelling (grade 1) of the eyelids and nictating membrane and slightly congestive iris (grade 1). Similarly, all the treated animals presented slight lacrimation. Evaluation of hyperaemia in the conjunctivae was not possible due to the colouring caused by the product in them. 24 hours after treatment, all the animals presented some blood vessels definitely hyperaemic (grade 1) in the conjunctivae, accompanied by slight swelling (grade 1) of the eyelids and nictating membrane in one animal and diffuse areas of opacity in the cornea (grade 1), affecting at least a quarter of the corneal area in another animal. In the reading made 48 hours after administration, the observed lesions had remitted and by the end of 72 hours after		

Method	Results	Remarks	Reference
	treatment only one animal continued to present some blood vessels definitely hyperaemic (grade 1) in the conjunctivae. This lesion had completely disappeared by the additional reading made 6 days after administration		
rabbit (Vienna White): 3 animals, 1 male, 2 female Vehicle: none equivalent or similar to OECD TG 405 (Acute Eye Irritation / Corrosion)	not irritating Cornea score: ca. 0 of max. 4 (mean (of the 3 tested animals) over the 3 time point: 24, 48 and 72 hour Iris score: ca. 0 of max. 2 (mean (of the 3 tested animals)) over the 3 time point: 24, 48 and 72 hour Conjunctivae score: ca. 0 of max. 3 (mean (of the 3 tested animals)) over the 3 time point: 24, 48 and 72 hour Chemosis score: ca. 0 of max. 4 (mean (mean of the 3 tested animals)) over the 3 time point: 24, 48 and 72 hour Irritation index could not be read because of the staining due to the colour of the test substance just after 1 hour instillation for all three animals	1 (reliable without restrictions) supporting study read-across from supporting substance (structural analogue or surrogate) **Test material (Common name): Acid Black 210 litium/potassium salt** **Purity: ca. 67 %** Form: solution	BASF SE (1984)
in vitro study Bovine eyes Vehicle: physiol. saline OECD TG 437 (Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants)	The test item could not be clearly classified : ca. 25.5 of max. 100 (mean IVIS = mean opacity score + (15 x mean permeability OD 490 score))	1 (reliable without restriction) supporting study experimental result **Test material (Common name): Acid Black 210 sodium salt** **Purity: ca. 66.4 %** Impurities: sodium sulfate, sodium chloride	S. Cinelli (2014)
rabbit (New Zealand White): 3 animals	not classified Cornea score: 0 (animal #1) (Time point: mean at 24, 48 and 72h)	4 (non assignable) disregarded study experimental result	Stahl Europe B.V. (1996a)

Method	Results	Remarks	Reference
EU Method B.5 (Acute Toxicity: Eye Irritation / Corrosion)	0 (animal #2) (Time point: mean at 24, 48 and 72h) 0.3 (animal #3) (Time point: mean at 24, 48 and 72h) Iris score: 0 (animal #1) (Time point: mean at 24, 48 and 72h) 0 (animal #2) (Time point: mean at 24, 48 and 72h) 0.3 (animal #3) (Time point: mean at 24, 48 and 72h) Conjunctivae score: (Max. duration: 28h.Max. value at the end of observation period:. (related to al animals)) Chemosis score: 0 (animal #1) (Time point: mean at 24, 48 and 72h) 0 (animal #2) (Time point: mean at 24, 48 and 72h) 0.3 (animal #3) (Time point: mean at 24, 48 and 72h) Other effects: The application in the eye caused mild pain initially in animals (Class 2 on a scale of 0 to 5). However, the ophthalmologic examination revealed no abnormalities. Additional observations include staining of the third eyelid (this lasted until the 28th day in only one animal). Black coloration of the third eyelid reversible day 14 in 2 animals and not reversible at day 28 in 1 animal. This coloring prevented the appreciation of the conjunctival erythema until day 14.	**Test material (Common name): Acid Black 210 sodium salt** Form: powder **Purity: unknown** Impurities: unknown	

7.4.2.2. Human information

No data available

7.4.2.3. Summary and discussion of eye irritation

Classification for corrosion was based on a study presented in the framework of DSD notification of new substances, reporting eye damage not reversible within 28 days (Stahl Europe B.V. (1996a)).

Few information is available on the study and basic information useful for a correct classification are missing (i.e. purity of the tested substance, identities of impurities, if the eyes have been rinsed or not and if the substance has been added directly in powder form) and several attempts to recover the original reports have been made, without success, either by the owner of the notification or the responsible Member State (France, presumably).

The study has therefore been disregarded in the framework of this CLH proposal because of its deficiencies

Studies on analogous salts hare taken into consideration since the counter ion has little influence on the outcome of the eye irritation study and the main analogue substance (potassium salt) can be taken as a conservative representative

Either dyes (the sodium and the potassium salt) in fact are highly soluble and they dissolve into the lachrymal fluid, that is mainly composed of water and salts. In this respect it can be evaluated if sodium or potassium as such, or better, as counterions of other molecules well studies have ever demonstrated a different behaviour regarding eye irritation, maybe due to potential differences in permeability properties. Compairing the behaviour of different salt based on the same anionic part and having the results on either the sodium and the potassium analogue it can be noticed that the potassium analogues are generally more irritant then the sodium one.

Futhermore the specific impurities for the analogues substances (lithium sulphate, Lithium chloride, Potassium sulphate) have eye irritant properties by themselves, therefore again the analogue substances are conservative also considering their impurities

The study J. Zapatero (1997) is a complete study, well conducted following GLP and performed according to OECD TG 405. The substance has been identified with a purity of about 65% and impurities indicated as the following salts: sodium sulphate, sodium chloride and potassium chloride. All the effects have been properly assessed and described in detail. In the course of the first hour after instillation, the test substance induced in one animal slight swelling (grade 1) of the eyelids and nictating membrane and slightly congestive iris (grade 1). Similarly, all the treated animals presented slight lacrimation. Evaluation of hyperaemia in teh conjunctivae was not possible due to the colouring caused by the product in them.

24hours after treatment, all the animals presented some blood vessels definitely hyperaemic (grade 1) in the conjunctivae, accompanied by slight swelling (grade 1) of the eyelids and nictating membrane in one animal and diffuse areas of opacity in the cornea (grade 1), affecting at least a quarter of the corneal area in another animal.

In the reading made 48 hours after administration, the observed lesions had remitted and by the end of 72 hours after treatment only one animal continued to present some blood vessels definitely hyperaemic (grade 1) in the conjunctivae. This lesion had completely disappeared by the addtional reading made 6 days after administration.

The second key study is BASF SE (1984). This study has been rated Klimisch 1 even if it not performed according to GLP because the study is complete and performed according the OECD TG 405 without deviations. The substance is well identified and a certificate has been provided for exact composition: the tested substance consists of a 67 % of the dye, considered as the mixture of potassium and lithium salt, 30 % of water and a remaining 3 % of inorganic salts (lithium chloride).

All the effects have been properly assessed and described in detail. No effect has been reported for any end point after 72 hours in any animal. Irritation index could not be read because of the staining due to the colour of the test substance just after 1 hour instillation.

A BCOP *in vitro* test according to OECD TG 437 (S. Cinelli, 2014), has been conducted on the Acid Black 210 sodium which composition consists of 66.5 % of the dye, 16 % of water, about 10 % of inorganic salts (sodium sulphate and sodium chloride) and a remaining 3 % of identified impurities.

The BCOP test method is recommended as an initial step within a tiered-testing strategy to identify chemicals inducing serious eye damage, i.e. chemicals to be classified as UN GHS Category 1, without further testing. A chemical that is not predicted as causing serious eye damage or as not classified for eye irritation/serious eye damage with the BCOP test method would require additional testing (in vitro and/or in vivo) to establish a definitive classification

According to the updated OECD TG 437 of 17 September 2012, the IVIS cut-off values for identifying test chemicals as inducing serious eye damage (UN GHS Category 1) and test chemicals not requiring classification for eye irritation or serious eye damage (UN GHS No Category) are given hereafter:

IVIS	**UN GHS**
< 3	No Category
>3; < 55	No prediction can be made
> 55	Category 1

The test resulted in a medium IVIS of 25.5 and alterations of the mean cornea opacity were observed. However, since the test item was coloured, the mean opacity value is affected by the substance remaining on the corneal surfaces. No relevant increases in corneas permeability were recorded after treatment with the test item when compared to those of negative control.

Conclusions

Two Klimisch 1 studies (J. Zapatero, 1997 and BASF SE, 1984) have been performed respectively on the potassium salt and on a mixture of litium/potassium salts of the dye showing no effect that can trigger a classification. They are well performed, the substance is well identified and their results are consistent. An in vitro study, according to OECD TG 437 has been also performed on ABl 210-Na. The result according to the new update of OECD TG 437 of the 17 September 2012 cannot lead to a conclusion about the classification; the study performed on sodium salt (Stahl Europe B.V. (1996a)) has been disregarded as not adequate, reliable and scientifically valid for the purpose of the classification due to the poor reporting

7.4.2.4. Comparison with criteria

The positive responses in both J.Zapatero (1997) and in BASF SE (1984) did not meet the criteria for classification, since all scores were <1 and effects were reversible within maximum 6 days

7.4.2.5. Conclusions on classification and labelling

Based on the study results the no classification for eye irritation/corrosion is proposed under Regulation 1272/2008

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS provided three *in vivo* and one *in vitro* studies.

Zapatero – 1997 (Key study)

This report was rated as Klimisch 1 and was considered by the DS to be the key study. The test protocol followed the *EU Method B.5 – Acute Toxicity: Eye irritation/Corrosion*. The Acid Black 210 potassium salt of ca. 65% purity was instilled in a single application of 0.1 mL using water as vehicle; no details regarding the sample preparation were given. The grading of the ocular lesions is shown in the following table:

Table: Grading of the ocular lesions in the Zapatero study.

Score*	Cornea (min.0-max.4)	Iris (min.0-max.2)	Conjunctivae (min.0-max.3)	Chemosis (min.0-max.4)
Animal #1	0	0	0.78 (fully reversible within 48 h)	0
Animal #2	0	0	0.78 (fully reversible within 48 h)	0
Animal #3	0.33 (fully reversible within 48 h)	0	1 (fully reversible within 48 h)	0

*All values are reported at the time point representing the mean of the 24, 48 and 72 h observations.

Regarding the time of observation, the DS gave the following details:

- 1 hour after treatment; the test substance induced in one animal a slight (grade 1) swelling of the eyelids and nictitating membrane and grade 1 congestive iris. All animals showed slight lacrimation. The evaluation of hyperaemia in the conjunctivae was not possible due to the coloration caused by the substance;
- 24 hours after treatment; all the animals presented grade 1 hyperaemic blood vessels in the conjunctivae. One animal presented additional grade 1 swelling of the eyelids and nictitating membrane; another animal presented grade 1 diffuse areas of opacity in the cornea affecting at least one quarter of the corneal area;
- 48 hours after treatment; the observed lesions had remitted;
- 72 hours after treatment; one animal had grade 1 hyperaemic blood vessels in the conjunctivae that completely disappeared after 6 days from instillation.

BASF SE – 1984 (supportive study)

This study was performed on 3 rabbits (1 male and 2 female) according to a protocol stated as being "equivalent or similar" to *OECD TG 405 method – Acute Toxicity: Eye irritation/Corrosion*. The test material was well identified and a certificate was provided with the exact composition: Acid Black 210 lithium/potassium salt ca. 67%, 3% inorganic salt

(lithium chloride) and 30% water. No ratio of the lithium vs. potassium salts of the organic moiety is given. The material (0.1 mL) was instilled without further preparation since the test material was a water solution.

The study was rated as Klimisch 1 and considered as supporting data. The grading of the ocular lesions is given in the following table:

Table: Grading of the ocular lesions in the BASF study.

Score	Cornea (min.0-max.4)	Iris (min.0-max.2)	Conjunctivae (min.0-max.3)	Chemosis (min.0-max.4)
Mean of the 3 tested animals over the time points of 24, 48 and 72 h observations	0	0	0	0

After the instillation the eyes of the animals were not washed out. The observation period was 8 days, but no effect in any animal was reported after 72 hours.

Stahl Europe B.V. – 1996 (study disregarded by the DS)

This study was performed on 3 rabbits according to *EU Method B.5 – Acute Toxicity: Eye irritation/Corrosion*. The test material was Acid Black 210 sodium salt; the purity of the main component as well as the impurities are unknown. No further details were given regarding the application of the test material. However, since the physical state was described as a powder, the amount was stated as 100 mg and no preparation of the sample was described, it was presumably applied as a solid. The study was rated with a Klimisch score of 4 (not assignable) due to poor reporting and was disregarded by the DS. However, it provided the grading of the ocular lesions as follows:

Table: Grading of the ocular lesions in the Stahl Europe B.V. study.

Score*	Cornea (min.0-max.4)	Iris (min.0-max.2)	Conjunctivae** (min.0-max.3)	Chemosis (min.0-max.4)
Animal #1	0	0	-	0
Animal #2	0	0	-	0
Animal #3	0.3	0.3	-	0.3

* All values are reported at the time point representing the mean of the 24, 48 and 72 h observations.
** The values are not reported

It is also noted that the application in the eye initially caused a mild pain rated as 2 on a scale of 0 to 5. Still, the ophthalmologic examination did not reveal abnormalities. In addition, it is noted that the coloration of the third eyelid (the nictitating membrane) was reversible at day 14 in two animals and was not reversible by day 28 in the third animal. The staining of the conjunctiva prevented the evaluation of the conjunctival erythema until day 14.

In vitro study

The Bovine Corneal Opacity and Permeability (BCOP) study performed by Cinelli (2014) was cited by the DS as a supporting study and rated as Klimisch 1. The test followed the protocol of OECD TG 437; the test material was Acid Black 210 sodium salt of 66.4% purity. Following the Public Consultation, specific concentrations of the impurities were added as follows:

sodium sulphate 1.86%, sodium chloride 7.4% and water 16%. The test material was applied at a concentration of 20% w/v using physiological saline as the vehicle. The IVIS score computed according to the method protocol was of 25.5. It is noted that the mean opacity value was affected by the coloration of the corneal surfaces. In addition, when compared to the negative control, no relevant increase in corneal permeability was recorded following the application of the test substance.

Comments received during public consultation

Three Member States Competent Authorities (MSCA) commented on the general issues and classification for the eye irritation/damage hazard class. One MSCA supported the removal of the actual classification without any further comment. Another MSCA disagreed and recommended that the classification should be maintained. The third MSCA stated that the limited information reported makes the interpretation of the data very difficult but no clear position was taken. The concerns raised by the last two MSCA referred to the identity/composition of the substance and the reliability of the presented studies. Furthermore, the decision of the DS to disregard the study based on which the substance has been originally classified was questioned.

Assessment and comparison with the classification criteria

There are two issues that have to be detailed: the staining properties of the test material and the information provided in the original study report.

The staining properties

The tissue staining capacity of the test substance is not unusual since the substance is a dye; furthermore, the black colour hinders the optical evaluation of the tissues. The effect is reported in all the studies but appears to be of low persistence and severity; the coloration shortly disappeared and following the evaluation, the scores revealed low and transitory toxicity. The only exception is the Stahl Europe BV (1996) study in which the coloration of the nictitating membrane lasted until day 14 in two animals and day 28 in one animal.

There are slight differences between the studies regarding the actual concentration of the test materials due to the intrinsic composition and the sample preparation; consequently slight differences regarding the intensity and duration of the coloration are expected. Again, the Stahl study appears to be an exception since the test material was probably applied as a solid. Because the effect is coloration, the rinsing might have influenced the results, as stated by the DS. Regardless, the persistent coloration of the nictitating membrane in one animal is noted but no additional signs of toxicity were revealed.

In contrast to the well characterised diagnostic dyes used in ophthalmology (https://www.reviewofophthalmology.com/article/the-dye-namics-of-dry-eye-diagnosis) the mode of action for the Acid Black dye is unknown; therefore it cannot be determined whether the dye diffuses into the intercellular spaces or is also able to penetrate the cell surface and localise in the cellular nuclei and/or in other organelles - thus manifesting intrinsic cellular toxicity. It is only known that the Acid Black dye induces some effects both *in vivo* and *in vitro*. In the *in vivo* tests, the coloration proved transitory and no remaining signs of irritancy/damage were revealed. Consequently, although conjunctival membrane coloration is certainly an *effect,* its toxicity is questionable. The only consideration is that it hinders the visual observation of the conjunctiva for a limited time. Taking into account the reported *in*

vitro remnant coloration of the cornea, RAC notes that the method is not validated for the evaluation of this effect and the finding is not present *in vivo*; moreover, no further corneal damage was registered either *in vitro* or *in vivo*.

The coloration of the conjunctivae and nictitating membrane cannot be considered as tissue damage; with respect to the definition of irritation, it might be seen as a *change,* but one of equivocal toxicological significance. It cannot be determined if the transient conjunctival hyperaemia is caused by coloration.

Therefore, RAC cannot find any consistent evidence to consider the conjunctival membrane coloration as a toxic effect in the evaluation of the eye irritation/corrosion under the CLP criteria (See Annex *I: 3.3.1.1 of the CLP Guidance).*

The information provided in the original study

The classification under the DSD was based on the study provided by Stahl Europe B.V. (1996) on ABl210-Na. This study was summarised by the DS but was disregarded due to poor reporting. The overall reliability was questioned due to the lack of information regarding the purity of the substance and the identity of the impurities, if the eyes had been rinsed and if the substance had been added directly in powder form. However, the study was performed according to a protocol suitable for classification purposes and the report still contains useful information.

The missing conjunctival scores are considered by RAC as an important drawback. Also, RAC recognises that the presence/absence of rinsing may have influenced the results. With respect to the purity/impurity profile, RAC notes that, given the expected similarity of the industrial batches, the composition should follow the values given in the paragraph above under the heading "*Typical composition".*

RAC notes that the report lists the corresponding scores for cornea, iris and conjunctival oedema (chemosis); all the values are below the thresholds for classification and this is in line with all the studies considered for evaluation. With respect to the conjunctival membrane, difficulties regarding the evaluation were reported due to coloration. This is also in line with the other studies. Since the scores in the study report and methodological difficulties were similar to the rest of the studies, RAC considers that the Stahl report should not be disregarded and the valid information should be used in the context of the weight of evidence.

The Stahl study reports the persistent coloration of the third eyelid after day 21 in one animal out of three. During the public consultation, it was suggested that the classification of ABl210-Na as eye damage should be maintained, in particular due to this finding. Taking note of this, RAC firstly considers that coloration of the conjunctival membrane is difficult to consider as a toxic effect under the CLP Regulation because it is not referred to and nor can it be directly compared with the classification criteria. Secondly, the location of the coloration is inappropriate for consideration. The nictitating membrane is a part of the rabbit's conjunctiva that does not occur in humans. The equivalent anatomical structure in humans (*plica semilunaris* or semilunar fold*)* is only a small fold of conjunctivae representing a vestigial remnant of the nictitating membrane. Due to the anatomical differences between the animal and human conjunctival membrane, the coloration of the nictitating membrane cannot be extrapolated to humans and its relevance in the assessment is therefore questionable.

Given the above argumentation, RAC considers that the study does not support the classification as eye damage category 1 under the CLP Regulation.

The new studies

The key study of Zapatero (1997), in which the potassium salt was used, provides properly assessed information. Following the instillation, signs of irritation appeared gradually but thereafter reduced and disappeared by day six. The second *in vivo* study (BASF SE, 1984) which used the ABl210-Li and -K salt provided conclusions in line with Zapatero (1997): the corresponding scores for cornea, iris, conjunctivae and chemosis were zero.

The BCOP test provides an IVIS score of 25.5; according to test method OECD TG 437 this value falls between 3 and 55 and "no prediction can be made".

Conclusion

RAC considers that findings from a study using ABl210-Na and read-across from ABl210-K is appropriate for the evaluation of eye irritation/eye damage of ABl210-Na. The substances do not have any known physico-chemical properties that would support classification. No human data were available for the assessment. The animal data showed that both substances are mild eye irritants but the severity and reversibility indices had numerical values below the threshold for classification. Both substances and the equivalent lithium salt exhibited staining capacity of the conjunctival membrane; however this finding cannot be used for classification.

In conclusion, RAC agrees that Acid Black 210 sodium salt **does not meet the classification criteria for eye irritation/corrosion** under the CLP Regulation. Consequently, the present classification should be removed.

7.4.3. Respiratory tract irritation

Not relevant for the purpose of this CLH report.

7.5. Corrosivity

Not classified for corrosion

7.6. Sensitisation

Not relevant for the purpose of this CLH report.

7.7. Repeated dose toxicity

Not relevant for the purpose of this CLH report.

7.8. Specific target organ toxicity – repeated exposure (STOT RE)

Not relevant for the purpose of this CLH report

7.9. Germ cell mutagenicity

Not relevant for the purpose of this CLH report.

7.10. Carcinogenicity

Not relevant for the purpose of this CLH report.

7.11. Toxicity for reproduction

Not relevant for the purpose of this CLH report

7.12. Other effects

Not relevant for the purpose of this CLH report.

8. ENVIRONMENTAL HAZARD ASSESSMENT

8.1. Degradation

8.1.1. Stability - Abiotic degradation

8.1.1.1. Hydrolysis

The studies on hydrolysis are summarised in the following table:

Table 11. Overview of studies on hydrolysis

Method	Results	Remarks	Reference
EU Method C.7 (Degradation: Abiotic Degradation: Hydrolysis as a Function of pH) Screening study – HPLC method with UV detector	Half-life (DT50): t1/2 (pH 4.08): > 1 yr at 25 °C t1/2 (pH 6.91): > 1 yr at 25 °C t1/2 (pH 8.8): > 1 yr at 25 °C Recovery (in %): pH 4.08: ca. 97 at 50 °C after ca. 5 d pH 6.91: ca. 96.15 at 50 °C after ca. 5 d pH 8.8: ca. 92.35 at 50 °C after ca. 5 d Transformation products: no	1 (reliable without restrictions) key study read-across from supporting substance (structural analogue or surrogate) **Test material (Common name): Acid Black 210 potassium salt** **Purity: ca. 65 %** Impurities: sodium sulfate, sodium chloride, potassium chloride	Jana Netusilova (2010)

A single screening study (Jana Netusilova, 2010) was performed on Acid Black 210 potassium salt at different pH showing less than 10 % of the test substance hydrolysing within 5 days period. The potassium counter ion has no significant influence on the hydrolysis rate of the substance. The result is then applicable to sodium salt also.

The following information is taken into account for any hazard / risk / persistency assessment:

Not hydrolysable

8.1.1.2. Phototransformation/photolysis

No data

8.1.1.3. Phototransformation in air

The substance will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 0.6 hours.

Half-life in air: 0.636 h

Degradation rate constant with OH radicals: 0.000000002 m³ molecule-1 s-1

8.1.1.4. Phototransformation in water

No data

8.1.1.5. Phototransformation in soil

No data

8.1.2. Biodegradation

8.1.2.1. Biodegradation estimation

No data

8.1.2.2. Screening tests

The test results are summarised in the following table:

Table 12. Overview of screening tests for biodegradation in water

Method	Results	Remarks	Reference
Test type: inherent biodegradability Aerobic, sewage, industrial, non-adapted OECD TG 302 B (Inherent biodegradability: Zahn-Wellens/EMPA Test)	Inherently biodegradable % Degradation of test substance: 38 after 28 d (TOC removal) BOD5*100/COD = 1.3 %	1 (reliable without restrictions) key study read-across from supporting substance (structural analogue or surrogate) **Test material (Common name): Acid Black 210 potassium salt** **Purity: ca. 65 %** Impurities: sodium sulfate, sodium chloride, potassium chloride	Prof. FJ Carrion (1997a)

8.1.2.3. Simulation tests

8.1.3. Summary and discussion of degradation

Abiotic degradation

The substance is considered not hydrolizable in water compartment.

Biotic degradation

The substance is considered not rapidly degradable based on BOD 5 /COD is < 0,5

8.2. Environmental distribution

8.2.1. Adsorption/Desorption

The studies on adsorption/desorption are summarised in the following table:

Table 13. Overview of studies on adsorption/desorption

Method	Results	Remarks	Reference
Study type: adsorption (soil/sewage sludge) HPLC estimation method EU Method C.19 (Estimation of the Adsorption Coefficient (KOC) on Soil and Sewage Sludge Using High Performance Liquid Chromatography (HPLC))	Adsorption coefficient: log Koc (soil, pH=5.6): ca. 2.42 at 25 °C log Koc (sludge, pH=5.6): ca. 2.39 at 25 °C log Koc (soil, pH=7.4): ca. -0.07 at 25 °C log Koc (sludge, pH=7.4): ca. -0.52 at 25 °C	1 (reliable without restrictions) key study read-across from supporting substance (structural analogue or surrogate) **Test material (Common name): Acid Black 210 potassium salt** **Purity: ca. 65 %** Impurities: sodium sulfate, sodium chloride, potassium chloride	Karel Cizek (2011)

Discussion

A study (Karel Cizek, 2011) was performed to estimate the adsorption coefficient Koc of Acid Black 210 potassium salt on soil and sludge by means of HPLC method, following EU Method C.19. log Koc for both soil and sludge are less than 3 for pH 5.6 at 25°C and negative at pH 7.4, showing very low potential of adsorption of the tested substance. No significant influence of the potassium counterion can be estimated on this result

8.2.2. Volatilisation

The following information is taken into account for any environmental exposure assessment:

A value of $9*10^{-9}$ Pa m^3/mol at 25 °C has been calculated on the acid form with KOCWIN v2.00.

8.2.3. Distribution modelling

No data

8.3. Aquatic Bioaccumulation

8.3.1. Aquatic bioaccumulation

No studies available

8.3.1.1. Bioaccumulation estimation

Based on a measured logKow of -3.1 (Stahl Europe B.V. (e)) no bioaccumulation is foreseen.

8.3.1.2. Measured bioaccumulation data

No data

8.3.2. Summary and discussion of aquatic bioaccumulation

The substance is not biodegradable and not hydrolysable, but it is very soluble with logKow of -3.1, therefore no bioaccumulation is foreseen. A quick photolitic degradation both in water and in the air has to be considered. Long-term toxicity to marine and freshwater organisms is expected to be >= 0.01 mg/l, based on the high values observed in acute tests at all three trophic levels and Long term test on Daphnia

8.4. Aquatic toxicity

8.4.1. Fish

8.4.1.1. Short-term toxicity to fish

The results are summarised in the following table:

Table 14. Overview of short-term effects on fish

Method	Results	Remarks	Reference
Poecilia reticulata freshwater static	LC_{50} (48 h): > 2000 mg/l test mat. (nominal) LC_{50} (96 h): ca. 1890 mg/l test mat. (estimated)	1 (reliable without restrictions) key study	Ludmila Dolezalova (1996)

Method	Results	Remarks	Reference
ISO 7346-1 (Determination of the Acute Lethal Toxicity of Substances to a Freshwater Fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 1: Static Method)		read-across from supporting substance (structural analogue or surrogate) **Test material (Common name): Acid Black 210 potassium salt** **Purity: ca. 65 %** Impurities: sodium sulfate, sodium chloride, potassium chloride	
Rainbow trout (Oncorhynchus mykiss) semi-static EU Method C.1 (Acute Toxicity for Fish)	LC_{50} (24 h): > 120 mg/l LC_{50} (48 h): > 120 mg/l LC_{50} (72 h): > 120 mg/l LC_{50} (96 h): > 120 mg/l	2 (reliable with restrictions) supporting study experimental result **Test material: (Common name): Acid Black 210 sodium salt** **Purity: unknown** Impurities: unknown	Stahl Europe B. V. (1996b)

Discussion

A study (Ludmila Dolezalova, 1996) was performed on Acid Black 210 potassium salt testing different concentrations that allowed the extrapolation of a value for LC_{50}, equal to 1890 mg/l.

A limit test was performed on Acid Black 210 sodium salt showing a NOEC > 120 mg/l. The purity of the substance and the identity of the impurities are not known, as well as the measured concentration before and after the test. The result has not been taken into account for classification purposes.

The following information is taken into account for acute fish toxicity :

LC_{50} (96 h) = 1890 mg/l

8.4.1.2. Long-term toxicity to fish

No data available

8.4.2. Aquatic invertebrates

8.4.2.1. Short-term toxicity to aquatic invertebrates

The results are summarised in the following table:

Table 15. Overview of short-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna EU Method C.2 (Acute Toxicity for Daphnia)	EC_{50} (48 h): > 150 mg/l Based on measured concentration	2 (reliable with restrictions) key study experimental result **Test material: (Common name): Acid Black 210 sodium salt** **Purity: unknown** Impurities: unknown	Stahl Europe B. V (1996c)

Discussion

A limit test on *Daphnia Magna* has been performed, showing no mortality at the nominal concentration of 180 mg/l. Since the sample concentration is 83 % of the nominal concentration, the EC_{50} has been set at 150 mg/l

8.4.2.2. Long-term toxicity to aquatic invertebrates

The results are summarised in the following table:

Table 16. Long-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater OECD TG 211 (Daphnia magna Reproduction Test)	NOEC (21 d): ca. 2.5 mg/l (meas. (not specified)) based on: reproduction NOEC (21 d): ca. 2.5 mg/l (meas. (not specified)) based on: growth LOEC (21 d): ca. 8 mg/l (meas. (not specified)) based on: growth	2 (reliable with restrictions) weight of evidence experimental result **Test material: (Common name): Acid Black 210 sodium salt**	JH Moore, MHI Comber (1997)

Method	Results	Remarks	Reference
	LOEC (21 d): ca. 8 mg/l (meas. (not specified)) based on: growth	**Purity: Unknown** Impurities: unknown	

Discussion

The result is indicating that the substance Acid Black 210 sodium salt has a NOEC long term > 1 mg/l, no further information on the purity of the substance or on the impurities has been provided

8.4.3. Algae and aquatic plants

The results are summarised in the following table:

Table 17. Overview of effects on algae and aquatic plants

Method	Results	Remarks	Reference
Lemna minor (aquatic plants) freshwater static OECD TG 221 (Lemna sp. Growth Inhibition Test)	EC_{50} (7 d): > 2000 mg/l act. ingr. (nominal) based on: growth rate Corresponding to EC_{50} (7 d): > 3080 mg/l test material	1 (reliable without restrictions) key study read-across from supporting substance (structural analogue or surrogate) **Test material (Common name): Acid Black 210 potassium salt** **Purity: ca. 65 %** Impurities: sodium sulfate, sodium chloride, potassium chloride	Alexa Caduff (2012)
Desmodesmus subspicatus (algae) freshwater static OECD TG 201 (Alga, Growth Inhibition Test)	EC_{50} (72 h): > 10 — < 100 mg/l test mat. (meas. (not specified)) based on: growth rate EC_{10} (72 h): ca. 10.8 mg/l test mat. (meas. (initial)) based on: growth rate	2 (reliable with restrictions) disregarded study read-across from supporting substance (structural analogue or surrogate) **Test material (Common name): Acid Black 210 potassium salt** **Purity: ca. 65 %**	Dirk Scheerbaum (2011)

Method	Results	Remarks	Reference
		Impurities: sodium sulfate, sodium chloride, potassium chloride	
Scenedesmus subspicatus (new name: Desmodesmus subspicatus) (algae) EU Method C.3 (Algal Inhibition test)	EC_{50} (72 h): 3.8 mg/l based on: biomass EC_{50} (72 h): 13.7 mg/l based on: growth rate NOEC (72 h): < 1.9 mg/l based on: growth rate NOEC (72 h): < 1.9 mg/l based on: biomass	4 (not assignable) disregarded study experimental result **Test material: (Common name): Acid Black 210 sodium salt** **Purity: unknown** Impurities: unknown	Stahl Europe B.V. (i)

8.4.4. Other aquatic organisms (including sediment)

No data

8.4.5. Discussion on classification and labelling for environmental hazards (sections 8.4.1 – 8.4.4)

Classification for aquatic toxicity has been based on a study of Algae Growth Inhibition presented in the framework of DSD Notification Of New Substances, reporting EC_{50} at 72 hours based on grow rate of 13.7 mg/l (Stahl Europe B.V. (i)). It can be assumed that the tested substance was the sodium salt of Acid Black 210, but it was not possible to recover the original report, therefore the full composition of the substance is unknown, as well as many details on the study like the physical form of the applied substance and if the eye has been rinsed or not.

Another study (Dirk Scheerbaum, 2011) was performed on Acid Black 210 potassium salt (read across) according to OECD series on testing and assessment Number 23 (2000): “Guidance document on aquatic toxicity testing of difficult substances and mixtures”, paragraph 3.8, Coloured substances, as indicated into the Guidance on the Application of the CLP Criteria v 4.0, section 4.1.3.2.2 and also in the IR/CSA Guidance, Chapter R.7b, Appendix 7.8.1

The NOEC is > 1 mg/l and EC_{50} is between 10 and 100 mg/l (nominal).

The observed algae toxicity is reasonably not referred to the counter ion but is due to the shadowing effect of the substance in the tested medium. Several studies on algae conducted on dark dyes, including those with a modified test system for coloured substances, showed that the growth inhibition is not due to a toxic effect of the dye, but to the light absorption of the stained water. Modified test system is usually conducted putting the dye above the algae testing solution, in a different vessel and not into contact with the alga. The same toxicity expressed as grow rate and yield

inhibition has been observed like when in the same condition the algae is into contact with the dye. It has been deduced that the observed toxicity was related to the shading effect of the dye. This method has some limitation because it focuses on the shadow effect but gives no information on the real potential toxicity for algae of the tested substance.

At present new information is available on aquatic toxicity. A Lemna growth inibition test has been conducted following OECD TG 221 on Acid Black 210 potassium salt (Alexa Caduff, 2012) showing no effect up to the highest tested concentration of 2000 mg/l.

Lemna is an aquatic plant that develops his leaves on the surface of the water, while nourishing substances are taken from the water solution. With this test the observed effect is only related to the potential toxicity of the substance and not to the potential shading effect of a classical Alga study. A deviation to the protocol has been applied to the test recommended for dyes (M. Cleuvers, 2002), i.e. beakers will be incubated on a black non-reflecting surface; additionally, the walls of the incubation chambers will also be covered with black fabric in order to avoid reflection. This study has been conducted with Acid Black 210 potassium salt, but the result is applicable to Acid Black 210 sodium salt as well.

According to a broad agreement by EU Competent Authorities the Lemna test is a suitable alternative to an algal test for strongly coloured substances, as mentioned in the introduction to the method C.26 "Lemna sp. Growth inhibition test" of the European Commission Regulation No 761/2009 of 23 July 2009. "This method is equivalent to OECD TG 221 (2006). The EU authorities' agreement refers to the Manual of Decision (EU Manual of Decisions dated July 2006, at *http://tsar.jrc.ec.europa.eu/documents/Manual_of_decisions.pdf* 13.5.3 Alternatives to the algae growth inhibition test with coloured substances). This method is also in conformity with the content of the Guidance on information requirements and chemical safety assessment — Chapter R.7b: Endpoint specific guidance; Table 7.8.3 Summary of difficult substance testing issues, available at: *https://echa.europa.eu/documents/10162/13632/information_requirements_r7b_en.pdf*, as indicated in the Guidance on the Application of the CLP Criteria v 4.0, section 4.1.3.2.2.

Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, Regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, paragraph 5.2.1.3., reports that "where it can be demonstrated in the case of highly coloured substances that algal growth is inhibited solely as a result of a reduction in light intensity, then the 72h EC_{50} for algae should not be used as a basis for classification. " *http://ec.europa.eu/environment/archives/dansub/pdfs/annex6_en.pdf.*

For highly light absorbing substances, the modified standard algae growth inhibition test is not recommended. With these particular substances, a modified standard Lemna-test (OECD TG 221) is recommended. The following standard modification to the standard Lemna test has to be applied: the test has to be performed on a black, non-reflecting surface (M. Cleuvers, 2002).

While for the same substance Acid Black 210 potassium salt the alga test according to OECD TG 201 has provided a result of EC_{50} (72 h): > 10 — < 100 mg/l (Dirk Scheerbaum, 2011), with Lemna no toxicity up to the maximum tested level of 2000 mg/l on the active substance has been observed.

The Dirk Scheerbaum study (Dirk Scheerbaum, 2011) has been rated Klimish 2 because it is a valid study, well performed, giving information about the behaviour of the substance (shadowing effect), but as stated above it should not be used as a basis for classification.

Therefore the toxicity of the substance for aquatic plants can be assessed at EC_{50} > 2000 mg/l and the already performed studies following OECD TG 201 can be disregarded.

Acute toxicity on Fish has been tested on the sodium and potassium salt of Acid Black 210, while Daphnia Magna acute toxicity has been tested only on the sodium salt and they don't reveal any toxicity at high levels of dosing.

Furthermore, a short summary is available of a study performed and submitted in the framework of the notification of the substance S124668 (Acid Black 210, sodium salt) under DSD in 1997 (JH Moore, MHI Comber, 1997). The summary indicates that a long term reproductive study on Daphnia has been performed on the substance. The result is indicating that the substance has a NOEC long term > 1 mg/l.

8.4.6. Comparison with criteria for environmental hazards (sections 8.4.1 – 8.4.4)

The core classification system for substances consists of one acute classification category and three chronic classification categories. The acute and the chronic classification categories are applied independently. The criteria for classification of a substance in Category Acute 1 are defined on the basis of acute aquatic toxicity data only (EC_{50} or LC_{50} < 1 mg/l).

Summarizing the results for acute toxicity:

LC_{50} (96 h) Fish (*Poecilia reticulata*)= 1890 mg/l

EC_{50} (48 h) Crustacea (*Daphnia magna*) > 150 mg/l

E_rC_{50} (7 d) Aquatic plants (*Lemna minor*) > 2000 mg/l

According to the criteria none of the performed acute tests on aquatic species indicates a potential for classification for Aquatic Acute Toxicity.

No effects have been reported at the highest tested concentrations for all species, therefore no real $E(L)C_{50}$ can be established and no identification of the most sensitive species can be performed.

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if available information on chronic toxicity merits long-term hazard classification.

According to CLP Annex I, Fig. 4.1.1, considering adequate chronic toxicity data available for at least one trophic level, the criteria to be referred to are given in Table 4.1.0 (b) (i):

Chronic Category 2

Chronic NOEC or ECx (for fish) > 0.1 to ≤ 1 mg/l and/or

Chronic NOEC or EC x (for crustacea) > 0.1 to ≤ 1 mg/l and/or

Chronic NOEC or EC x (for algae or other aquatic plants) > 0.1 to ≤ 1 mg/l

Based on the result of Daphnia Reproduction study (NOEC (21 d): ca. 2.5 mg/l, (JH Moore, MHI Comber, 1997)) no classification for Chronic Aquatic Toxicity is proposed under Regulation 1272/2008.

In absence of adequate chronic toxicity data, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data). According to fig. 4.1.1, criteria follows table 4.1.0 (b)(iii):

Chronic Category 3

96 hr LC_{50} (for fish) > 10 to ≤ 100 mg/l and/or

48 hr EC_{50} (for crustacea) > 10 to ≤ 100 mg/l and/or

72 or 96 hr ErC_{50} (for algae or other aquatic plants) > 10 to ≤ 100 mg/l

All the reliable studies on the three trophic levels report an EC_{50} > 100 mg/l

8.4.7. Conclusions on classification and labelling for environmental hazards (sections 8.4.1 – 8.4.4)

Classification for acute aquatic toxicity is based on the following acute data:

LC_{50} (96 h) Fish (*Poecilia reticulata*)= 1890 mg/l

EC_{50} (48 h) Crustacea (*Daphnia magna*) > 150 mg/l

ErC_{50} (7 d) Aquatic plants (*Lemna minor*) > 2000 mg/l

No effects have been reported at the highest tested concentrations for all species; in a conservative approach the lowest EC_{50} can be considered the EC_{50} (48h) *Daphnia Magna* (150 mg/l), outside the described classification criteria for acute aquatic toxicity, as described above. In this respect Daphnia Magna will also be considered the most sensitive species.

For classification for chronic aquatic toxicity adequate chronic data for the 3 throphic levels are not available, therefore the main assessment is performed based on acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data), according to CLP, Annex I, fig. 4.1.1, and table 4.1.0 (b)(iii);

Since Acid Black 210 Sodium salt is non biodegradable, and the lowest EC_{50} is > 100 mg/l, no classification for chronic aquatic toxicity is proposed under Regulation 1272/2008.

This conclusion is supported by the fact that adequate chronic toxicity data is available for at least one trophic level nalso related to the mnost sensitive specie based on acute results, therefore according to CLP Annex I, Fig. 4.1.1, the criteria given in Table 4.1.0 (b) (i) can be applied.

Based on the result of Daphnia Reproduction study (NOEC (21 d): ca. 2.5 mg/l, (JH Moore, MHI Comber, 1997)), > 1 mg/l as indicated by the abovementioned criteria, no classification for chronic aquatic toxicity is proposed under Regulation 1272/2008.

RAC evaluation of aquatic hazards (acute and chronic)
Summary of the Dossier Submitter's proposal ABl210-Na is used in water-based formulations mainly for industrial leather dyeing but also in textile and paper formulation. The substance currently has a harmonised environmental classification as Aquatic Chronic 3; H412 in Annex VI of the CLP Regulation. The DS proposed

to remove this classification due to data from new studies. The DS has taken into consideration the structural analogue potassium salt. The current aquatic classification is based on a 72 hour EC_{50} of 13.7 mg/L for algae. There is no original test report available and consequently there are uncertainties about e.g. the actual tested substance and whether nominal or measured concentrations have been reported. The substance is difficult to test with algae because of the colouring effect. The DS provided new information on a *Lemna* growth inhibition test for ABl210-K resulting in an EC_{50} greater than 2000 mg/L (active ingredient) to be used instead of algae data. Based on this data and on acute toxicity for fish and *Daphnia* (LC_{50} 1890 and 150 mg/L respectively), the substance being not rapidly degradable and non-bioaccumulative, the DS concluded that no classification is needed for ABl210-Na. They also note that the long-term NOEC for *Daphnia* of ca. 2.5 mg/L does not fulfil the criteria for chronic classification.

The typical concentration of ABl210-Na is ca. 66.4% (w/w). The impurities, mainly sodium chloride, sodium sulphate and water, are considered not to affect the environmental classification. The impurities in ABl210-K include potassium chloride and sulphate. The substance is in its salt form, and is completely dissociated in water.

Degradation

There is one hydrolysis study performed according to the EU Method C.7 available for ABl210-K with purity ca. 65%. The half-life was > 1 year at pH 4.08, 6.91 and 8.8. No transformation products were detected. There is no information on photolysis or phototransformation in water.

There is no ready biodegradability test available. In an inherent biodegradability test (OECD TG 302B) with ABl210-K (purity ca. 65%) 38% degradation was observed after 28 days. The substance is considered not rapidly degradable.

Bioaccumulation

There is no bioconcentration test available. The log Kow is -1.73 at 20 °C (EU A.8) for ABl210-K (purity 67%) and - 3.1 at 25 °C (EU A.8) for ABl210-Na (purity unknown).

Aquatic toxicity

The aquatic toxicity tests available are presented in the table below.

Table: Aquatic toxicity tests on ABl210-Na and ABL210-K as presented by the DS.

Test substance	Method	Result	Remarks	Reference in the CLH Report
Acute toxicity to fish				
ABl210-K purity ca. 65%	*Poecilia reticulata*, ISO 7346-1 Part 1, static, not GLP	48 h LC_{50} > 2000 mg/L test material (nominal) 96 h LC_{50} ca. 1890 mg/L test material (estimated)	Key study	Dolezalova, 1996
ABl210-Na, purity unknown	*Oncorhynchus mykiss*, EU C.1, GLP, semi-static, limit test	96 h LC_{50} > 120 mg/L	supporting study/not taken into account for classification purposes	Stahl Europe B.V., 1996b

Chronic toxicity to fish - no information				
Acute toxicity to aquatic invertebrates				
ABl210-Na, purity unknown	*Daphnia Magna*, EU C.2, GLP, limit test	48 h EC_{50} > 150 mg/L (measured)	key study	Stahl Europe B.V., 1996c
Chronic toxicity to aquatic invertebrates				
ABl210-Na, notified S124668, purity unknown	*Daphnia magna*, OECD TG 211	21 d, NOEC (reproduction, growth) ca. 2.5 mg/L (measured concentration 78% of the nominal)	weight of evidence	Moore and Comber, 1997
Toxicity to algae and plants				
ABl210-K, purity ca. 65%	*Lemna minor*, OECD TG 221, GLP, static	7 d E_rC_{50} > 2000 mg/L active ingredient (nominal) corresponding to 7 d E_rC_{50} > 3080 mg/L test material 7 d NOE_rC < 30.8 mg/L test material (frond number and dry weight) test concentrations not measured	key study	Caduff, 2012
ABl210-K, purity ca. 65%*	*Desmodesmus subspicatus*, OECD TG 201, static, test performed in duplicate; 1. in Erlenmeyer flasks 2. Microplate 96-well with plane bottom where: light intensity 60-120 µE m-2s-1, 200 µL per well, rotary shaker 100 rpm	72 h E_rC_{50} > 10 - < 100 mg/L (measured) 72 h E_rC_{10} ca 10.8 mg/L, initial measured concentration 105% of the nominal; 98% after 72 hr comparable toxicity with both methods	disregarded test conc. 1, 10 and 100 mg/L	Scheerbaum, 2011
ABl210-Na, purity unknown	*Desmodesmus subspicatus*, EU C.3, GLP	72 h, E_rC_{50} 13.7 mg/L (nominal) 72 h, NOE_rC < 1.9 mg/L (nominal)	disregarded study	Stahl Europe B.V. (i)

* it is not clear which is the test material; sodium salt as indicated in the CLH report or potassium salt as per DS' reply to a PC comment.

There are two acute fish toxicity studies available. The test with ABl210-K giving a 96 h LC_{50} value of ca. 1890 mg/L is taken into account for classification. The other fish test on *Oncorhynchus mykiss* is disregarded mainly because of the lack of measured test substance concentrations and unknown purity of the test substance.

There is one acute, and one chronic *Daphnia* study with ABl210-Na available; in both studies, the purity of the test substance is unknown and the study description is insufficient. In the acute limit test, the 48 h EC_{50} was ≥ 150 mg/L. The chronic *Daphnia* study was submitted in the framework of the notification of the substance S124668 (ABl210-Na). The 21 d NOEC was ca. 2.5 mg/L.

There is one *Lemna* study available. *Lemna* is an aquatic plant that develops its leaves on the surface of the water, while nutrients are taken from the water. With this test, the observed effect is related to the potential toxicity of the substance and not to the potential shading effect due to the colour of the test substance as in an algae study. A deviation to the protocol was noted by the DS, i.e. beakers were incubated on a black non-reflecting surface; additionally, the walls of the incubation chambers were also covered with black fabric to avoid reflection. The 7 d E_rC_{50} for the test material is > 3080 mg/L (2000 mg/L active ingredient). The NOEC value (with respect to growth rate, NOE_rC) was < 30.8 mg/L.

There are two algae studies available. In the Scheerbaum study (2011) the 72 h E_rC_{50} was 10-100 mg/L, whereas in the Stahl Europe B.V. (i) study the 72 h E_rC_{50} was 13.7 mg/L. The chronic 72 h E_rC_{10} and NOE_rC were ca. 10.8 mg/L, and < 1.9 mg/L respectively. The DS is of the opinion that these tests should be disregarded because of the shadowing effect. In addition, the purity of the test substance is unknown in the Stahl Europe B.V. (i) test.

The DS is of the opinion that *Lemna* test is a suitable alternative to an algae test for strongly coloured substances as presented in the introduction to the method C.26 of the European Commission Regulation No 761/2009 of 23 July 2009. This method is listed in ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7b, in Table 7.8-3 as refinement in case of algae studies on coloured substances.

Comments received during public consultation

One MSCA noted that the CLH report has limited information to evaluate environmental data and that the given reliability of the studies seems uncertain due to missing data such as purity and concentration of the test substance. The DS explained that it was not possible to find the original reports for the old studies. For the new studies, necessary information was given.

One MSCA did not agree to remove the classification. They felt that more details are needed to prove that toxicity is due to a shading effect in the Scheerbaum (2011) study. Another MSCA also had concerns regarding the protocol of this study. It is noted in the CLH Report that the study was performed according to the OECD series of testing and assessment Number 23, paragraph 3.8 Coloured substances, which list a few strategies to minimise the shading effect. It is not clear, however, how this was considered. Moreover, they wondered if the conclusion that the toxic effects were only caused by light absorption were noticed in this study or from observation with other substances. The DS explained that the test had been performed in duplicate, using sterile Erlenmeyer flasks and with Microplate 96-well plates with flat bottom following the method proposed in the OECD 23. Microplates were used to reduce the light path by reducing the depth or the volume. However, the toxicity to algae was comparable in both tests indicating that the method was not adequate. The DS believes an indirect demonstration of the light effect is given by an old test summary recently found in a Stahl archive performed on the notified substance S124668. The test has been performed in the Zeneca Speciality Blackley testing laboratory in duplicate using the same concentration of test material; one flask is normal, the other is divided in two sections. The above section contained the dye solution, the section below the algae that were not in contact with the dye,

but were shaded from the light by the dye solution. The results showed that values and shape of the curves from both flasks are practically identical showing that the toxicity is generated by the light shading effect and not by the intrinsic toxic effect of the dye. Similar tests are available for several black or brown dyes.

One MSCA is of the opinion that the chronic *Daphnia* study and the discarded chronic algae study (72 h NOE_rC < 1.9 mg/L) suffer from the same deficiencies. They also point out that there is data lacking in the Scheerbaum (2011) study report. The DS agreed to disregard also the chronic *Daphnia* study.

One MSCA agreed with the use of read-across. They consider that insufficient details are presented for the key *Lemna* study by Caduff (2012). The DS stated that the test guideline validity "The doubling time of frond number in the control must be less than 2.5 days (60 h) corresponding to approximately a 7-fold increase in 7 days and an average specific growth rate of 0.275 d^{-1}" was met but did not present any calculations to confirm this. The MSCA also encouraged the use of chronic E_rC_{10} values from the *Lemna* study.

Assessment and comparison with the classification criteria

RAC agrees that it is appropriate to take into account the results of tests performed with the structural analogue potassium salt to assess the environmental hazard of ABl210-Na.

Degradation

RAC agrees with the DS that the substance is not rapidly degradable based on its hydrolytic stability, a 38% degradation after 28 days in an inherent biodegradability test and the absence of a ready biodegradability test.

Bioaccumulation

RAC agrees with the DS, that the substance does not have potential to bioaccumulate based on the log K_{OW} -1.73 and log K_{OW} -3.1 for ABl210-K and ABl210-Na, respectively.

Aquatic toxicity

RAC considers that aquatic tests, where the test substance purity is unknown and which have been performed under the pre-REACH regime for regulatory purposes, can be used as supporting evidence for the classification. In the case of Acid Black 210 sodium and potassium salts, measured concentrations in aquatic tests in general seem to be so close to the nominal concentrations that the reliability of the tests is not questioned in case the data on measured concentration is missing. It can, also, be expected that the purity of the previously notified substance of the same name would not differ much from the purity of the substance in question.

Short-term

RAC considers that there are reliable data on fish, algae and *Lemna*. The 96 hour LC_{50} for *Poecilia reticulata* is ca. 1890 mg/L. The 72 hour E_rC_{50} for *Desmodesmus subspicatus* is between 10 to 100 mg/L and the 7 day E_rC_{50} for *Lemna minor* is greater than 3080 mg/L. RAC disagrees with the DS to disregard the Scheerbaum (2011) algae test. There is not enough information to prove that toxicity reported is caused only by a shading effect. The test aimed to take into account the principles proposed in the OECD series of testing and assessment 23 for coloured substances, which are also listed in the ECHA Guidance on Information requirements and Chemical Safety Assessment Chapter R.7b Table R.7.8-3. The test was performed in duplicate, using sterile Erlenmeyer flasks and with Microplate 96-well plates with

flat bottom. The following adjustments to the standard algae growth inhibition test (OECD TG 201) should be applied for coloured substances: the irradiation should be in the highest end of the range in the method (i.e. 120 μEm^{-2} s^{-1} or higher), the light path should be shortened by reduction of the volume of the test solutions (in the range of 5-25 mL) and sufficient agitation should be performed in order to obtain a high frequency of exposure of the algae to high irradiation at the surface of the culture. It is not clear to which extent these adjustments were included in the Scheerbaum (2011) study using Erlenmeyer flasks. In the Microplate 96-well with flat bottom test, that was performed in addition to the Erlenmeyer flask test, the parameters were: light intensity 60-120 μEm^{-2} s^{-1}, volume 200 µL per well, rotary shaker 100 rpm. However, the toxicity to algae was comparable in both tests. RAC concludes that even it is unclear how much the growth inhibition is caused by shading or intrinsic toxicity the 72 hour E_rC_{50} and E_rC_{10} would not be expected to be lower than 10-100 mg/L in any case.

The 96 h LC_{50} > 120 mg/L for fish, 48 hour EC_{50} > 150 mg/L for *Daphnia magna* and 72 hour E_rC_{50} 13.7 mg/L for *Desmodesmus subspicatus* are considered as supporting evidence.

Long-term

RAC is of the opinion that there are two reliable long-term study results available for classification namely the 7 day NOE_rC < 30.8 mg/L for *Lemna minor* and the 72 hour E_rC_{10} of ca. 10.8 mg/L for *Desmodesmus subspicatus*. Even through the algae test does not give a definitive toxicity value to ABl210-Na because of the possible shading effect, it is considered reliable enough to be used in this weight of evidence classification. The 21 day NOEC of ca. 2.5 mg/L for *Daphnia magna* and the 72 hour NOE_rC < 1.9 mg/L are considered as supportive evidence. There is no long-term data for fish.

Comparison with the criteria

There are reliable acute data on fish, *Lemna* and algae. There are uncertainties of the effect of shading in the algae test but RAC sees the results are, however, applicable for classification purposes. The *Daphnia* test result is used as supportive evidence because the purity of the test substance in the acute *Daphnia* test is not confirmed. Consequently, the lowest reliable acute toxicity value is an algae E_rC_{50} in between 10 to 100 mg/L. There are reliable chronic data on *Lemna* and algae. The results from another algae test and the *Daphnia* test are used as supportive evidence. The lowest reliable chronic toxicity value is an E_rC_{10} ca 10.8 mg/L for algae. The surrogate system based on acute fish data and non-biodegradability of the substance is used to account for the missing chronic data on fish.

These values do not fulfil the classification criteria for aquatic hazards. Consequently, RAC agrees with the DS proposal to **remove the current classification 'Aquatic Chronic 3; H412: Harmful to aquatic life with long lasting effects'**.

It is however clear to RAC that the information related to test conditions presented in the CLH Report is barely enough to make a weight of evidence conclusion on classification for aquatic toxicity. In the event that more reliable information will be available in the future the classification may need to be revisited.

8.5. Other information

8.5.1. Toxicity to aquatic micro-organisms

The results are summarised in the following table:

Table 18. Overview of effects on micro-organisms

Method	Results	Remarks	Reference
activated sludge freshwater static OECD TG 209 (Activated Sludge, Respiration Inhibition Test)	EC_{50} (3 h): > 100 mg/l test mat. (nominal) based on: respiration rate	1 (reliable with restrictions) key study read-across from supporting substance (structural analogue or surrogate **Test material (Common name): Acid Black 210 potassium salt** **Purity: ca. 65 %** Impurities: sodium sulfate, sodium chloride, potassium chloride	FJ Carrion (1997b)
activated sludge freshwater static equivalent or similar to OECD TG 209 (Activated Sludge, Respiration Inhibition Test)	EC_0 (3 h): ca. 10000 mg/l test mat. (nominal) based on: respiration rate	2 (reliable with restrictions) supporting study experimental result **Test material (commercial name): Luganil Schwarz NT Stucke P 20/87** Purity (dye content as sodium salt content): 77% wt sodium chloride: 11% wt potassium chloride: 5% wt water: 7% wt	BASF SE (1988)

Discussion

A study (FJ Carrion (1997b) was performed on a preparation containing Acid Black 210 potassium salt. The tested substance did not show any respiration inhibition and the EC_{50} (equal to NOEC) is > 100 mg/l.

Another study (BASF SE, 1988) tested a preparation containing Acid Black 210 sodium salt. The item was tested up to 10g/l without showing any respiratory inhibition with NOEC > 10g/l.

It has to be taken into account that the substance is not biodegradable and not hydrolysable, but it is very soluble with logKow of -3.1, therefore no bioaccumulation is foreseen. According to the ECHA guidance on information requirements and chemical safety assessment R7b version 3, February 2016, section R.7.8.21.3, the available information have been enough to derive the $PNEC_{stp}$ and according to the CSR $PEC/PNEC_{stp} < 1$, then no further tests have been performed

The following information is taken into account for effects on aquatic micro-organisms:

EC_{50} > 100 mg/l, nominal

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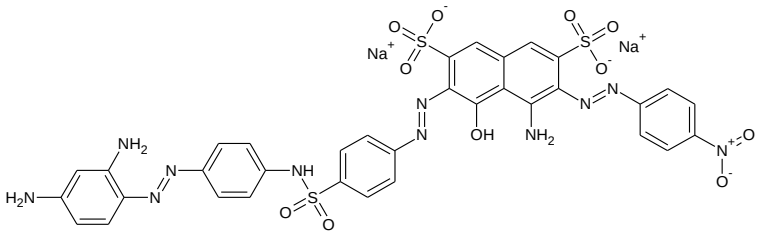
10. ANNEX I – JUSTIFICATION FOR READ ACROSS

ACID BLACK 210 Na salt (ABl210-Na)

Justification of the Read Across from K salt.



Introduction

Article 13 of REACH requires that: *"Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out in Annex XI are met. In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, in vitro methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across)*".

On this basis, as also described in Annex XI of REACH a read-across or category approach may be used to fulfil REACH information requirements and, thus, adapt the standard testing regime.

Hypothesis for the analogue approach

The substances Acid Black 210 sodium salt (EC 421-880-6 – ABl210-Na) has been evaluated taking into consideration the structural analogue potassium salt (EC: 286-384-2 – ABl210-K). The read across approach has been used for two purposes:

- in order to support and confirm the outcomes from the existing studies on the ABl210-Na

- in order to predict and assess endpoint information for the target substance ABl210-Na, avoiding unnecessary studies.

According to the ECHA guidance about the read across approach[1], the substance similarity can be based on common functional groups and common precursors and/or the likelihood of common breakdown products via physical and biological processes, which result in structurally similar chemicals. In the actual case, the whole chromophore molecule can be regarded as the common function group, which toxicological property in water solution is bound to the common dissociated form. Chemical-physical properties, environmental fate and pathway, eco- and toxicological information related to the ABl210-Na and ABl210-K are detailed below.

Substance identity and salification variability

Both ABl210-Na and ABl210-K are acid, trisazo, water-soluble anionic dyes (Anionic Azo Dyes). Anionic dyes include many compounds from the most varied classes of dyes, which exhibit characteristic differences in structure but possess as a common feature water-solubilising, ionic substituents.

<u>The manufacture technological process – Common precursors</u>

Both ABl210-Na and ABl210-K undergo to the same manufacture process. The starting materials are the following:

water (CAS: 7732-18-5; EC: 231-791-2)

4-amino-N-(4-aminophenyl)benzenesulfonamide (EC: 240-834-4; CAS: 16803-97-7)

4-amino-5-hydroxynaphthalene-2,7-disulfonic acid (EC: 201-975-7; CAS: 90-20-0. Other name: H Acid)

p-nitroaniline (EC: 202-810-1; CAS: 100-01-6)

m-phenylendiamine (EC: 203-584-7; CAS: 108-45-2)

sodium nitrite (EC: 231-555-9; CAS:7632-00-0) for diazotisation

chloridric acid (EC: 231-791-2; CAS: 7732-18-5) for diazotisation

sodium hydroxide (EC: 215-185-5; CAS: 1310-73-2) for Acid Black 210Na

[1] ECHA. Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals. Guidance for the implementation of REACH. May 2008.

potassium hydroxide (EC: 215-181-3; CAS: 1310-58-3) for Acid Black 210 K

sulfamic acid (EC: 226-218-8; CAS: 5329-14-6)

Reaction process steps:

step 1. 4-amino-N-(4-aminophenyl)benzenesulfonamide is first dissolved in water at room temperature, then the reaction vessel temperature is cooled down (0-5 °C) and diazotised by reaction with $NaNO_2$ and 30 % aqueous HCl to obtain the diazonium salt. Under stirring conditions, sulfamic acid is added to convert the excess of $NaNO_2$ into N_2 and H_2SO_4, which is finally neutralised with NaOH to Na_2SO_4.

step 2. 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid (H Acid) is dissolved in slightly alkaline water solution at room temperature, then the reaction vessel temperature is cooled down (0-5 °C)and then added to the diazotised solution of 4-amino-N-(4-aminophenyl)benzenesulfonamide for the coupling reaction (product A)

step 3. p-nitroaniline is dissolved in water solution at room temperature, then the reaction vessel temperature is cooled down (0-5 °C) and diazotised by means of $NaNO_2$ and HCl. The excess of $NaNO_2$ is once again reacted with sulfamic acid. (Product B)

step 4. Product A and product B are mixed together for coupling reaction (Product C).

step 5. m-phenylendiamine is dissolved in water solution at room temperature, then the reaction vessel temperature is cooled down (0-5 °C) and diazotised by means of $NaNO_2$ and HCl. The excess of $NaNO_2$ is once again reacted with sulfamic acid (Product D)

step 6. Product D and Product C are mixed together for coupling reaction (Product E).

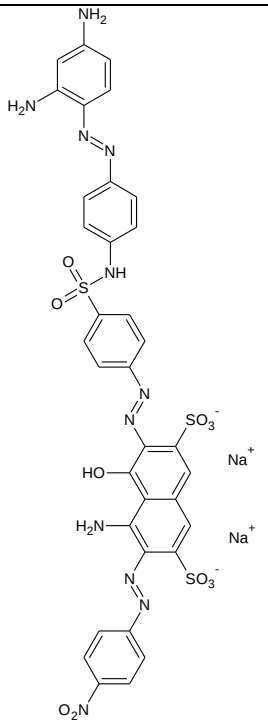
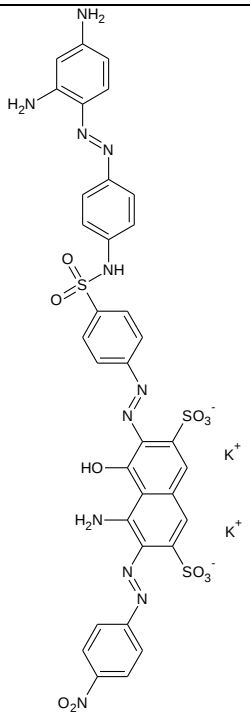
step 7. Either NaOH (to produce the sodium salt form) or KOH (to produce the potassium salt form) are then used to neutralize the substance, which is then isolated and dried.

Both the manufacturing of ABl210-Na and ABl210-K are derived from the same precursors and involve the same process stages from 1 to 6. Only the final step is different because in the case of AB210-Na the substance neutralization is made using NaOH, while in the case of ABl210-K this result is obtained using KOH.

Structure similarity

Table 01: Substance identity

Common name	**Acid Black 210 Na salt (ABl210-Na)**	**Acid Black 210 K salt (ABl210-K)**
CAS number	/	85223-29-6
EC Number	421-880-6	286-384-2
IUPAC name	disodium 4-amino-6-[[4-(N-(4-((E)-(2,4-diaminophenyl)diazenyl)phenyl)sulfamoyl)phenyl)diazenyl)-5-hydroxy-3-((E)-(4-nitrophenyl)diazenyl)naphthalene-2,7-disulfonate	4-amino-6-[[4-[[[4-[(2,4-diaminophenyl)azo]phenyl]amino]sulphonyl]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]naphthalene-2,7-disulphonic acid, potassium salt

Molecular formula	$C_{34}H_{25}N_{11}Na_2O_{11}S_3$	$C_{34}H_{25}K_2N_{11}O_{11}S_3$
Molecular weight	905.8	938.0
Structure		

Acid dyes are derived from an acid base, giving anionic species in water; in fact, they are designed to form ionic bonds with the basic groups of the fibres (amino groups), as in the cases of silk, wool, polyamides or leather. Furthermore, the acid dyes are capable to form hydrogen bonds and other intermolecular interactions with the fibres.

ABl210-Na and ABl210-K share the same base structure, except for the salification. Sodium and potassium are both alkali metals, contiguous in the period table group. The alkali metals provide the best example of group trends in properties in the periodic table, with elements exhibiting well-characterized homologous behaviour. The Na and K chemistry is dominated by the loss of their lone valence electron to form the +1 oxidation state, due to the ease of ionizing this electron and the very high second ionization energy. Both sodium and potassium can form monocharged positive ion. Despite the difference in terms of ionic radius, they are both relatively small ions and tend to form very water soluble compounds (salts) like halides, sulphates, nitrates etc., completely dissociated in water.

Because potassium and sodium are chemically very similar, their salts were not at first differentiated: the existence of multiple elements in their salts was suspected in 1702 (Marggraf, 1761) and this was proven in 1807 when potassium and sodium were individually isolated from different salts by electrolysis.

Taken into account that the dyes in aqueous media are dissociated into the anionic base and the relative counter-ions (i.e. Na^+ and K^+) and the great analogy between sodium and potassium, it is expected that all the characteristics and behaviour of the substance can be attributed to the base structure. Since the dyes properties mainly depend on the organic moiety and in minimal part on the counter-ions, in this case, the difference between the two ions can be neglected. Due to the size, at a minimum extent potassium has potentially less solvatation property, it is slightly less electronegative. In the case of sulphated salts, potassium can form the monoacid form more easily than the sodium.

Typical compositions

As abovementioned, ABl210-Na and ABl210-K have same precursors and manufacturing process, thus it is expected that the typical commercial batches presents very similar characteristics.

The main component ABl210-Na is commonly greater/equal than 60 %. The remaining composition is constituted by sodium chloride (0-15 %), sodium sulphate (0-3 %), water (0-20 %), organic impurities (0-3.9 %) and isomer of the main component (0-4 %).

A complete analytical characterization of the ABl210-K is not available, nevertheless the variability of the tested lot compositions used in the current REACH registration dossier describes the following possible composition ranges:

- main component ABl210-K: ca 65 – 77 %
- sodium chloride: 0-15 %
- potassium chloride: 0-10 %
- water content: 0-15 %

Table 02: Purity/Impurity profile

	ABl210-Na	**ABl210-K**
Main component	> 60 %	65 - 77 %
Sodium chloride	0-15 %	0-15 %
Water	0-20 %	0-15 %
Organic impurities	0-8 %*	n.a.
sodium sulphate	0-3 %	-
potassium chloride	-	0-10 %

*The isomer of the main component was here included.

It can be noted that the main component is expected to be greater than 60 % in both the cases; common typical impurities are sodium chloride and water (in similar concentrations), on the contrary the sodium sulphate can be found only in the ABl210-Na composition, while the potassium chloride in the case of ABl210-K.

Sodium sulphate has been extended studied and both experimental and literature data are available[2], which sustain the non-dangerous (eco)toxicological profile of this substance.

[2] The substance has been registered under REACH Regulation (1907/2006) and the related dossiers can be consulted online, on the ECHA Registered substance database (Registration 100000-1000000 t/a and registration 1000-10000 t/a). Sodium sulphate has been submitted to the OECD SIDS testing program (OECD SIDS, 2005) and to the HERA review (HERA, 2006). Furthermore, the Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion on the safety of sodium sulphate (EFSA, 2010).

In addition, concerning the case of the potassium chloride many experimental and literature data exist, which trace a non-dangerous profile[3].

The role as impurity has no significant impact neither on the bioavailability nor on the (eco)toxicological characterization of the main component; furthermore, considering the similar characteristics of sodium sulphate and potassium chloride, it is not expected that these two impurities may determine a different bioavailability or behaviour of the main component.

In the cases of the ABl210-Na, further organic impurities were determined under 5 %. More than one impurity are involved. They are non-detrimental substances and have no relevant role in the substance characterization.

In conclusion, the typical compositions of ABl210-Na and ABl210-K are comparable and they are expected to be not significant responsible of an eventual different (eco)toxicological characterization of ABl10-Na and ABl210-K.

Further details are investigated case by case.

[3] The substance has been registered under REACH Regulation (1907/2006) and the related dossier can be consulted online, on the ECHA Registered substance database (Registration 100000-1000000 t/a). Furthermore, sodium sulphate has been submitted to the OECD SIDS testing program (OECD SIDS, 2001).

Physical-chemical properties

Data about ABl210-K are reported in supporting role for the following endpoints:

Melting point/freezing point

Density

Partition coefficient

Water solubility

The comparison of the physico-chemical properties of the two substances is made in the following table:

Table 03: Measured physic-chemical properties

	ABl210-Na	**ABl210-K**
Melting/boiling point	> 330 °C	Decomposition starting from 200 °C
Reference	NONS Stahl	Acid Black 210 Consortium, 2011
Relative density	1.43 at 20 °C	1.29 at 20 °C
Reference	NONS Stahl	Acid Black 210 Consortium, 2011
Water solubility	270 g/l at 20 °C and pH ca 8.7	183 g/l at 20 °C and pH ca 9
Reference	NONS Stahl	Acid Black 210 Consortium, 2011
Log Kow	- 3.1 at 25 °C	-1.73 at pH 8.64
Reference	NONS Stahl	Acid Black 210 Consortium, 2011

Both ABl210-Na and ABl210-K are solid, powders at 20 °C and 1013 hPa, thus vapour pressure and flash point can be neglected.

All the parameters investigated appears well comparable.

For completeness sake, the same properties mentioned above were also estimated using the EPA EPISuite tool[4]. This calculation has not a value in itself, since EPIWEB is not able to read and model disconnected structures, but it is representative of the influence of the couple sodium/potassium as a variable within the molecule.

[4]The Estimation Programs Interface (EPI) SuiteTM was developed by the US Environmental Protection Agency's Office of Pollution Prevention and Toxics and Syracuse Research Corporation (SRC).

Table 04: **Calculated physic-chemical properties (EPIWEB 4.1)**

	ABl210-Na	**ABl210-K**
Smile notation	Nc6ccc(/N=N/c1ccc(cc1)NS(=O)(=O)c2ccc(cc2)/N=N/c5c(O)c4c(cc(c(/N=N/c3ccc(cc3)[N+]([O-])=O)c4N)S(=O)(=O)O[Na])cc5S(=O)(=O)O[Na])c(N)c6	Nc6ccc(/N=N/c1ccc(cc1)NS(=O)(=O)c2ccc(cc2)/N=N/c5c(O)c4c(cc(c(/N=N/c3ccc(cc3)[N+]([O-])=O)c4N)S(=O)(=O)O[K])cc5S(=O)(=O)O[K])c(N)c6
Melting point	349.84 °C	349.84 °C
Boiling point	1436 °C	1436 °C
Water solubility	0.08845 mg/l at 25 °C	0.05338 mg/l at 25 °C
Log Kow	1.59 at 25 °C	1.59 at 5 °C
Vapour pressure	10^{-39} Pa at 25 °C	10^{-39} Pa at 25 °C

It can be noticed that a slight difference is reported both in the measured and in the calculated water solubility, indicating as the sodium derivative the more soluble representative.

The difference in the measured data seems greater: this outcome may be attribute to the impurity profile and/or to it can be more likely attributable to the method of the analysis.

Considering that the main difference in the lot tested compositions is the presence of the sodium sulphate (in the case of ABl210-Na), instead the potassium chloride (ABl210-K) it is expected that the difference in the water solubility potential would be small. On the contrary, the method of analysis may explains this difference. In the case of ABl210-Na it is not known how was determined the water solubility, nevertheless in the case of ABl210-K this property was determined using the UV analysis, of which result is known to be concentration and substance extinction coefficient dependent.

However, despite the differences, both substances are highly soluble in water, completely dissociated and completely bioavailable; both salts derived from strong acids, thus it is expected that they would be completely dissociated and stable in water in that form.

Read across approach

The following endpoints were investigated in a read across approach, using only the available data on the analogue.

Particle size distribution (Granulometry)

Oxidising properties

Dissociation constant

Particle size distribution is expected to be well representative, taken into account that both ABl210-Na and ABl210-K are manufactured starting from the same compound and following the same procedures. Both the products are marked for the same applications and the same roles and they can be used alternatively; as consequence, they have very similar physical-chemical characteristics.

The oxidising properties can be accessed on the basis of the structure, thus the read across approach using ABl210-K can be considered as reliable and appropriated.

In the case of the dissociation constant, it is expected that the differences that may occur would be so minimal that they would be negligible. In fact, as previously mentioned, ABl210-Na and ABl210-K share the same anionic base and the pka value depends to this moiety. The value recorded in the test has been assigned to the protonation of amino groups; the sulpho groups $–SO_3X$, in form of potassium salts, were not protonated back during the titration, thus cannot affect the pKa value.

All the tests taken here into account were performed with the same ABl210-K lot and the composition agrees with the typical one mentioned in table 02. Concerning the impurities profile, there are no reason to suppose a possible impact on the outcomes for the endpoints assessed.

Environmental fate and pathway and ecotoxicological information

Table 05: **Environmental fate and pathway and aquatic toxicity**

	ABl210-Na	**ABl210-K**
Hydrolysis	n.a.	Half life > 1 y at 25 °C
Reference		Acid Black 210 Consortium, 2011
Biodegradation in water: screening tests	n.a.	inherently biodegradable
Reference		Clariant Productos SA, 1997
Adsorption / desorption	n.a.	log Koc: ca 1.1 at 20 °C
Reference		Acid Black 210 Consortium, 2011
Short-term toxicity to fish	LC50 (96h) > 120 mg/l	LC50 (96h): 1890 mg/l
Reference	NONS Stahl	Synthesia AS, 1996
Short-term toxicity to aquatic invertebrates	LC50 (48h) > 150 mg/l	n.a.
Reference	NONS Stahl	
Long-term toxicity to aquatic invertebrates	NOEC (21d): 2.5 mg/l	n.a.
Reference	NONS Stahl, 1997	
Toxicity to aquatic algae and cyanobacteria	ECb50 (72h): 3.8 mg/l* ECr50 (72h): 13.7 mg/l*	ECr50 (72h) > 1 - < 100 mg/l*
Reference	NONS Stahl	Acid Black 210 Consortium, 2011
Toxicity to aquatic plants other than algae	n.a.	ECr50 (7d) > 2000 mg/l (nominal) ECb50 (7d) > 2000 mg/l (nominal)
Reference		Acid Black 210 Consortium, 2012
Toxicity to microorganisms	EC50 (3h) > 100 mg/l EC50 (3h) > 1000 mg/l	n.a.
Reference	BASF, 1988	Clariant Iberica, 1997

* Disregarded study

The environmental fate and pathway has been assessed taken into account the experimental data available on the potassium salt.

Both ABl210-Na and ABl210-K are completely miscible with water and they dissociate readily in water to anionic base and sodium/potassium ions. Considering the hydrolysis potential, the most sensitive functional group present in the common anionic base is the sulphonamide, thus the salification with sodium or potassium does not involve a diverse hydrolysis potential. An analogous pathway concern the biodegradability in water.

Based on the physicochemical properties both the sodium and potassium salts can be expected to have a low potential for adsorption; they have a low octanol water partition coefficient. As in the case of the aquatic phase, the ABl210-Na ANS ABl210-K adsorption/desorption ability is mainly due to the anionic base and the different salification can be neglected.

The composition of the ABl210-K lot tested in the hydrolysis and in the adsorption/desorption assays agree with that reported in table 02; while the purity of the substance used in the biodegradability test was stated by the data owner at approximately 65 - 70 %; the remaining composition was identified as a mixture of sodium sulphate, sodium chloride and potassium chloride (acting as cutting agents).

In both cases, the impurities are not expected to impact the test outcomes.

Concerning the aquatic toxicity, there are experimental data related to the ABl210-Na on the three trophic aquatic levels commonly investigated. Further information on the ABl210-K has been taken into account about the aquatic plants tests: both algae and lemna.

The algae toxicity has been judged as mainly due to the shadowing effect of the substances in the tested media and it has been judged as not related to the sodium or potassium ions.

In order to exclude possible doubts related to the counterion impact, it has been taken into account if sodium or potassium as such, or better, as counter-ions of other molecules well studies, have ever demonstrated a different behaviour regarding algae toxicity.

Table 06: **Algae toxicity (EC50 at 72 hours)**

	Sodium salt	**Potassium salt**
Sulphate	1900 mg/l (120 h) 10228 mg/l (32d)	1430-2900 mg/l
Reference	ECHA database OECD SIDS, 2005	ECHA database
Chloride	2430 mg/l (120 h) 4800 mg/l (time unspecified)	greater 100 mg/l up to 2500 mg/l
Reference	ECHA database	ECHA database OECD SIDS, 2001
Acetate	greater 1000 mg/l	greater than 100 mg/l
Reference	ECHA database	ECHA database

As previously mentioned, both the dyes in aqueous media are dissociated into the common anionic base and the relative counter-ions (i.e. Na^+ and K^+); considered the great analogy between sodium and potassium, it is expected that all the characteristics and behaviour of the substance can be attributed to the base structure. In addition, the ABl210-K composition of the lots tested follow in the ranges reported in table 02.

Based on the structure similarity, the chemical-physical characteristics and the comparable typical composition, ABl210-Na and ABl210-K are expected undergoing to the same environmental pathway. Furthermore, the potential reactivity is also expected to be the same, as well as the possible degradation/transformation products.

In conclusion, the read across approach to evaluate the environmental fate and the aquatic toxicity can be considered as appropriated and reliable.

Toxicological information

Table 07: **Toxicological information**

	ABl210-Na	**ABl210-K**
Acute Oral	LD50 > 2000 mg/kg bw (rat)	LD0 > 2000 mg/kg bw
Reference	NONS Stahl	Clariant Products S.A., 1997
Acute Inhalation	negligible	negligible
Acute Dermal	LD50 > 2000 mg/kg bw (rat)	n.a.
Reference	NONS Stahl	
Skin irritation/corrosion	not irritating	not irritating
Reference	NONS Stahl	Clariant Products S.A., 1997
In vivo eye irritation/corrosion	inconclusive*	not irritating
Reference	NONS Stahl	Clariant Products S.A., 1997 BASF AG, 1984
In vitro eye irritation/corrosion	not corrosive	n.a.
Reference	REACH&Colours Kft, 2104	
Skin sensitisation	not sensitising	not sensitising
Reference	NONS Stahl	Acid Black 210 Consortium, 2011
Repeated Dose (subacute)	NOAEL: 150 mg/kg bw/day (rat)	NOAEL: ca 150 mg/kg bw/day (rat) (OECD 422)
Reference	NONS Stahl	Acid Black 210 Consortium, 2011
Bacterial reverse mutation assay (e.g. Ames test) in vitro	positive with metabolic activation negative without metabolic activation	n.a.
Reference	NONS Stahl	
Mammalian cell gene mutation assay in vitro	negative	n.a.
Reference	NONS Stahl	
Mammalian chromosomal aberration in vitro	n.a.	negative
Reference		Acid Black 210 Consortium, 2011
Reproductive and Developmental *Toxicity Fertility*	n.a.	NOAEL(P): ca 150 mg/kg bw/day (rat) NOAEL(F1): ca 150 mg/kg bw/day (rat) (OECD 422)

Reference		Acid Black 210 Consortium, 2011
Developmental Toxicity	n.a.	NOAEL $_{mat}$: ca 150 mg/kg bw/day (rat) NOAEL $_{dev}$: ca 150 mg/kg bw/day (rat) (OECD 422)
Reference		Acid Black 210 Consortium, 2011

* Disregarded study

The results obtained in the acute dyes investigations available are comparable and both substance are not irritating to skin. In addition, the subacute investigation showed analogous outcomes: in the studies available on both the substances macroscopical changes of spleen (hypertrophy and colour change), pigmentation of some organs (kidneys, liver, spleen, brain), changes in urine properties (colour, parameters) and blood chemistry changes (AST and ALT activity) were recorded at the highest doses tested (1000 mg/kg bw/day and 450 mg/kg bw/day in ABl210-Na and ABl210-K, respectively).

On the basis of the chemical structure and the chemical-physical characterization, ABl210-Na and ABl210-K are expected undergoing to the same pathway in biological organisms. The adsorption potential is expected to be the same for both the substances, as well as the excretion rate.

After oral intake in many cases the extent of absorption via the gastrointestinal tract is determined by the lipophilicity of the substance, which can be stated to be same for ABl210-Na and ABl210-K. The oral mucosa has a thin epithelium and rich vascularity, which favour absorption; however, contact is usually too brief for substantial absorption. The following step regards the stomach, in which the pH is at about 1-3 and both the dyes are completely dissociated into the anionic based and the counterion

About the inhalation route, both ABl210-Na and ABl210-K physical state and trade forms lead to the conclusion that inhalation is not an appropriate route of exposure.

The extend of percutaneous absorption of a substance depends largely on the physical and chemical properties of the substance itself. In particular, factors like the degree of ionization, molecular size and water and lipid solubilities influence penetration through the skin. Based on the chemical structure and chemical-physical properties, the dermal absorption of the two analogous sodium and potassium salts can be considered comparable.

After absorption, the following metabolic pathway is expected to be the same for both ABl210-Na and ABl210-K: the distribution, metabolism and impact is expected to be qualitatively and quantitatively analogous.

The toxicological data available sustain the validity and the reliability of the read across approach; in particular, the outcomes from the subacute tests performed on ABl210-Na and ABl210-K underlined an analogous pathway of the two substances. From this point of view, the reproductive toxicity potential can be regarded as comparable. Dose descriptors can be considered as unaffected by the read across approach, thus there is no need for any adaptation.

The chromosomal aberration potential too can be assessed by read across approach, without significant adaptations.

The purity of the ABl210-K used in the acute oral toxicity, skin and eye irritation tests was stated by the data owner at approximately 65 - 70 %; the remaining composition was identified as a mixture of sodium sulphate, sodium chloride and potassium chloride (acting as cutting agents).

The lots tested in the chromosomal aberration assay and in the Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD 422) have a composition characterized by the specifications given in the table 02.

Thus, the composition can be considered to be not a discriminating factors.

Eye irritation endpoint

The eye irritation potential of ABl210-Na was investigated integrating the available experimental information on the sodium salt, with those available on the potassium analogue.

The compositions of the ABl210-K lots tested are in both cases agree with the typical composition described in the table 02.

Concerning the eye irritation potential testing system, relevant aspects that can impact the assay's outcomes are the form and amount of the substance tested. In the case of solid substances, the reference OECD guideline updated (OECD 405) recommends a volume of 0.1 ml or a weight of not more than 100 mg; furthermore, in the guideline is reported that 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. These procedures are directed to reduce the effects related to the powder form.

Thus, this aspect results discriminating in order to a evaluate and compare the eye irritation potential test outcomes.

Either dyes (the sodium and the potassium salt) are highly soluble and they dissolve into the lachrymal fluid, that is mainly composed of water and salts. In this respect, it can be evaluated if sodium or potassium as such, or better, as counterions of other molecules well studies have ever demonstrated a different behaviour regarding eye irritation.

Table 08: Eye irritation

	Sodium salt	**Potassium salt**
Sulphate	Slightly irritant (GHS) Not irritating (CLP)	Not irritant to severe irritant/corrosive due to the $KHSO_4$ impurity $KHSO_4 < 1$ %: not irritating (CLP) $KHSO_4 \geq 1 < 3$ %: eye irrit 2 (CLP) $KHSO_4 \geq 3$ %: eye dam 1 (CLP)
Reference	ECHA database OECD SIDS, 2005	ECHA database
Chloride	Slightly irritant (GHS) depending on the concentration Not irritating (CLP)	Mild irritation Not irritating (CLP)

Reference	ECHA database	ECHA database OECD SIDS, 2001
Acetate	Not irritant (CLP)	Not irritant (CLP)
Reference	ECHA database	ECHA database

Based on the tested substances an effect of eye irritation has never been attributed to the presence of sodium or potassium salts.

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