# 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,**

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# 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.)<br>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<br>Aquatic Chronic 3 | H318<br>H412 | GHS05<br>Dgr | H318<br>H412 | |

| Specific Concentration Limits and M Factors: none |
|---|
| Pictogram(s) |
|  <br>Corrosion |

## 2.4. Current self-classification and labelling

No classification

# 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

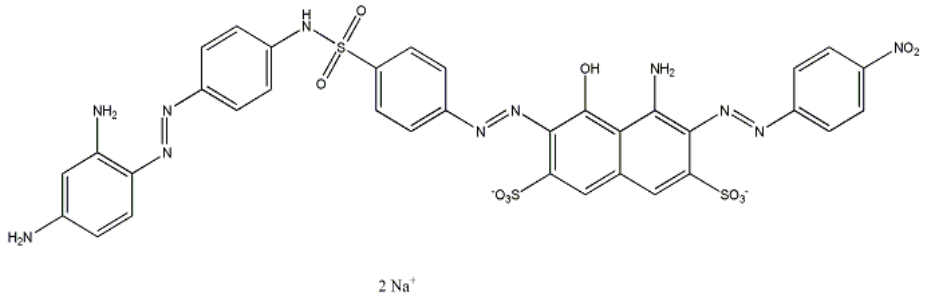
## 4. IDENTITY OF THE SUBSTANCE

### 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<br>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) | |
| sodium chloride<br>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 |

| Impurity | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| sodium sulfate<br>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<br>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<br><br>Acid Black 210 sodium salt<br><br>Purity: Unknown | Solid: black odourless powder | Qualitative assessment |
| Melting / freezing point<br><br>Acid Black 210 sodium salt<br><br>Purity: Unknown<br><br>EU Method A.1: Kofler hot bar | Decomposition > 200 °C | Stahl Europe B.V. (a)<br><br>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<br><br>Acid Black 210 potassium salt<br><br>Purity: 67 %<br><br>EU Method A.1: Differential Scanning Calorimetry | Decomposition > 200 °C | Michal Bartos (2011a)<br><br>The onset of a thermal decomposition was observed at 200°C. No peak corresponding to melting point was observed. |
| Boiling point<br><br>Acid Black 210 potassium salt | Decomposition > 200 °C | Michal Bartos (2011b)<br><br>The onset of a thermal decomposition was observed at 200°C. No peak corresponding to |

| Property | Value | Reference |
|---|---|---|
| Purity: 67 %<br><br>EU Method A.1: Differential Scanning Calorimetry | | melting/boiling point was observed. |
| Relative density<br><br>Acid Black 210 sodium salt<br><br>Purity: unknown<br><br>EU Method A.3 | 1.43 at 20 °C | Stahl Europe B.V. (b) |
| Relative density<br><br>Acid Black 210 potassium salt<br><br>Purity: 67 %<br><br>EU Method A.3: pycnometer | 1.286 at 20 °C | Michal Bartos (2011c) |
| Vapour pressure<br><br>Acid Black 210 acid form | 10E-45 Pa at 25 °C | Estimated with SPARC v4.6 |
| Surface tension<br><br>Acid Black 210 sodium salt<br><br>Purity: unknown<br><br>EU Method A.5: | 71.8 mN/m at 26°C at 1 g/L | Stahl Europe B.V. (c)<br><br>Concentration of the solution: 1000 mg/l |
| Water solubility<br><br>Acid Black 210 sodium salt<br><br>Purity: unknown<br><br>EU Method A.6: flask method | 270 g/l at pH 8.7 and 25 °C | Stahl Europe B.V. (d) |
| Water solubility<br><br>Acid Black 210 potassium salt<br><br>Purity: 67 %<br><br>EU Method A.6: flask method | 183 g/L at pH 9 and 20 °C | Michal Bartos (2011d) |

| Property | Value | Reference |
|---|---|---|
| Partition coefficient n-octanol/water (log value)<br><br>Acid Black 210 sodium salt<br>Purity: unknown<br>EU Method A.8: shake flask method | -3.1 at 25 °C | Stahl Europe B.V. (e) |
| Partition coefficient n-octanol/water (log value)<br><br>Acid Black 210 potassium salt<br>Purity: 67 %<br>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<br><br>Acid Black 210 sodium salt<br>Purity: unknown<br>EU Method A.10, A.12, A.13 | Non flammable | Stahl Europe B.V. (f) |
| Explosive properties<br><br>Acid Black 210 sodium salt<br>Purity: unknown<br>EU Method A.14 | Not explosive | Stahl Europe B.V. (g) |
| Self-ignition temperature<br><br>Acid Black 210 sodium salt<br>Purity: unknown<br>EU Method A.16 | 215°C at 1013 hPa | Stahl Europe B.V. (h) |
| Oxidising properties | Not oxidising properties | Michal Bartos (2011f) |

| Property | Value | Reference |
|---|---|---|
| Acid Black 210 potassium salt<br>Purity: 67 %<br>EU Method A.17 | | |
| Granulometry<br><br>Acid Black 210 potassium salt<br>Purity: 67 %<br>EU Method ISO 13320 | D50=52.51 µm | Michal Bartos (2011g)<br><br>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.<br>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<br><br>Acid Black 210 potassium salt<br>Purity: 67 %<br>Method: OECD TG 112 | 7.62 | Michal Bartos (2011h)<br><br>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<br><br>Vehicle: water<br><br>Amount: 0.1 ml<br><br>EU Method B.5 (Acute Toxicity: Eye Irritation / Corrosion) | not irritating<br><br>Cornea score:<br><br>0 of max. 4 (animal #1) (Time point: mean of 24, 48 and 72 h)<br><br>0 of max. 4 (animal #2) (Time point: mean of 24, 48 and 72 h)<br><br>0.33 of max. 4 (animal #3) (Time point: mean of 24, 48 and 72 h) (fully reversible within: 48 h)<br><br>Iris score:<br><br>0 of max. 4 (animal #1) (Time point: mean of 24, 48 and 72 h) | 1 (reliable without restrictions)<br><br>key study<br><br>read-across from supporting substance (structural analogue or surrogate)<br><br>**Test material (Common name): Acid Black 210 potassium salt**<br><br>**Purity: ca. 65 %**<br><br>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)<br><br>0 of max. 4 (animal #3) (Time point: mean of 24, 48 and 72 h)<br><br>Conjunctivae score:<br><br>0.78 of max. 4 (animal #1) (Time point: mean of 24, 48 and 72h) (fully reversible within: 72 h)<br><br>0.78 of max. 4 (animal #2) (Time point: mean of 24, 48 and 72 h) (fully reversible within: 72 h)<br><br>1 of max. 4 (animal #3) (Time point: mean of 24, 48 and 72 h) (fully reversible within: 6 d)<br><br>0 of max. 4 (animal #1) (Time point: mean of 24, 48 and 72 h)<br><br>0.33 of max. 4 (animal #2) (Time point: mean of 24, 48 and 72 h) (fully reversible within: 48 h)<br><br>0 of max. 4 (animal #3) (Time point: mean of 24,48 and 72 h)<br><br>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<br><br>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.<br><br>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.<br><br>In the reading made 48 hours after administration, the observed lesions had | | |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | 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 additional reading made 6 days after administration | | |
| rabbit (Vienna White): 3 animals, 1 male, 2 female<br><br>Vehicle: none<br><br>equivalent or similar to OECD TG 405 (Acute Eye Irritation / Corrosion) | not irritating<br><br>Cornea score:<br><br>ca. 0 of max. 4 (mean (of the 3 tested animals) over the 3 time point: 24, 48 and 72 hour<br><br>Iris score:<br><br>ca. 0 of max. 2 (mean (of the 3 tested animals)) over the 3 time point: 24, 48 and 72 hour<br><br>Conjunctivae score:<br><br>ca. 0 of max. 3 (mean (of the 3 tested animals)) over the 3 time point: 24, 48 and 72 hour<br><br>Chemosis score:<br><br>ca. 0 of max. 4 (mean (mean of the 3 tested animals)) over the 3 time point: 24, 48 and 72 hour<br><br>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)<br><br>supporting study<br><br>read-across from supporting substance (structural analogue or surrogate)<br><br>**Test material (Common name): Acid Black 210 litium/potassium salt**<br><br>**Purity: ca. 67 %**<br><br>Form: solution | BASF SE (1984) |
| in vitro study<br><br>Bovine eyes<br><br>Vehicle: physiol. saline<br><br>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<br><br>: ca. 25.5 of max. 100 (mean IVIS = mean opacity score + (15 x mean permeability OD 490 score)) | 1 (reliable without restriction)<br><br>supporting study<br><br>experimental result<br><br>**Test material (Common name): Acid Black 210 sodium salt**<br><br>**Purity: ca. 66.4 %**<br><br>Impurities: sodium sulfate, sodium chloride | S. Cinelli (2014) |
| rabbit (New Zealand White): 3 animals<br><br>EU Method B.5 (Acute Toxicity: Eye Irritation / | not classified<br><br>Cornea score:<br><br>0 (animal #1) (Time point: mean at 24, 48 and 72h) | 4 (non assignable)<br><br>disregarded study<br><br>experimental result<br><br>**Test material (Common** | Stahl Europe B.V. (1996a) |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Corrosion) | 0 (animal #2) (Time point: mean at 24, 48 and 72h)<br><br>0.3 (animal #3) (Time point: mean at 24, 48 and 72h)<br><br>Iris score:<br><br>0 (animal #1) (Time point: mean at 24, 48 and 72h)<br><br>0 (animal #2) (Time point: mean at 24, 48 and 72h)<br><br>0.3 (animal #3) (Time point: mean at 24, 48 and 72h)<br><br>Conjunctivae score:<br><br>(Max. duration: 28h.Max. value at the end of observation period:. (related to al animals))<br><br>Chemosis score:<br><br>0 (animal #1) (Time point: mean at 24, 48 and 72h)<br><br>0 (animal #2) (Time point: mean at 24, 48 and 72h)<br><br>0.3 (animal #3) (Time point: mean at 24, 48 and 72h)<br><br>Other effects:<br><br>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).<br>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. | **name): Acid Black 210 sodium salt**<br><br>Form: powder<br><br>**Purity: unknown**<br><br>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

#### 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)<br><br>Screening study – HPLC method with UV detector | Half-life (DT50):<br>t1/2 (pH 4.08): > 1 yr at 25 °C<br>t1/2 (pH 6.91): > 1 yr at 25 °C<br>t1/2 (pH 8.8): > 1 yr at 25 °C<br>Recovery (in %):<br>pH 4.08: ca. 97 at 50 °C after ca. 5 d<br>pH 6.91: ca. 96.15 at 50 °C after ca. 5 d<br>pH 8.8: ca. 92.35 at 50 °C after ca. 5 d<br>Transformation products: no | 1 (reliable without restrictions)<br>key study<br>read-across from supporting substance (structural analogue or surrogate)<br>**Test material (Common name): Acid Black 210 potassium salt**<br>**Purity: ca. 65 %**<br>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<br><br>Aerobic, sewage, industrial, non-adapted<br><br>OECD TG 302 B (Inherent biodegradability: Zahn-Wellens/EMPA Test) | Inherently biodegradable<br><br>% Degradation of test substance:<br><br>38 after 28 d (TOC removal)<br><br>BOD5*100/COD = 1.3 % | 1 (reliable without restrictions)<br><br>key study<br><br>read-across from supporting substance (structural analogue or surrogate)<br><br>**Test material (Common name): Acid Black 210 potassium salt**<br><br>**Purity: ca. 65 %**<br><br>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)<br><br>HPLC estimation method<br><br>EU Method C.19 (Estimation of the Adsorption Coefficient (KOC) on Soil and Sewage Sludge Using High Performance Liquid Chromatography (HPLC)) | Adsorption coefficient:<br><br>log Koc (soil, pH=5.6): ca. 2.42 at 25 °C<br><br>log Koc (sludge, pH=5.6): ca. 2.39 at 25 °C<br><br>log Koc (soil, pH=7.4): ca. -0.07 at 25 °C<br><br>log Koc (sludge, pH=7.4): ca. -0.52 at 25 °C | 1 (reliable without restrictions)<br><br>key study<br><br>read-across from supporting substance (structural analogue or surrogate)<br><br>**Test material (Common name): Acid Black 210 potassium salt**<br><br>**Purity: ca. 65 %**<br><br>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*<br>freshwater<br>static | $LC_{50}$ (48 h): > 2000 mg/l test mat. (nominal)<br>$LC_{50}$ (96 h): ca. 1890 mg/l test mat. (estimated) | 1 (reliable without restrictions)<br>key study<br>read-across from | 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) | | supporting substance (structural analogue or surrogate)<br><br>**Test material (Common name): Acid Black 210 potassium salt**<br><br>**Purity: ca. 65 %**<br><br>Impurities: sodium sulfate, sodium chloride, potassium chloride | |
| *Rainbow trout (Oncorhynchus mykiss)*<br><br>semi-static<br><br>EU Method C.1 (Acute Toxicity for Fish) | $LC_{50}$ (24 h): > 120 mg/l<br><br>$LC_{50}$ (48 h): > 120 mg/l<br><br>$LC_{50}$ (72 h): > 120 mg/l<br><br>$LC_{50}$ (96 h): > 120 mg/l | 2 (reliable with restrictions)<br><br>supporting study<br><br>experimental result<br><br>**Test material: (Common name): Acid Black 210 sodium salt**<br><br>**Purity: unknown**<br><br>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*<br><br>EU Method C.2 (Acute Toxicity for Daphnia) | $EC_{50}$ (48 h): > 150 mg/l<br><br>Based on measured concentration | 2 (reliable with restrictions)<br><br>key study<br><br>experimental result<br><br>**Test material: (Common name): Acid Black 210 sodium salt**<br><br>**Purity: unknown**<br><br>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*<br><br>freshwater<br><br>OECD TG 211 (Daphnia magna Reproduction Test) | NOEC (21 d): ca. 2.5 mg/l (meas. (not specified)) based on: reproduction<br><br>NOEC (21 d): ca. 2.5 mg/l (meas. (not specified)) based on: growth<br><br>LOEC (21 d): ca. 8 mg/l (meas. (not specified)) based on: growth<br><br>LOEC (21 d): ca. 8 mg/l | 2 (reliable with restrictions)<br><br>weight of evidence<br><br>experimental result<br><br>**Test material: (Common name): Acid Black 210 sodium salt** | JH Moore, MHI Comber (1997) |

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

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | | sulfate, sodium chloride, potassium chloride | |
| *Scenedesmus subspicatus (new name: Desmodesmus subspicatus)* (algae)<br><br>EU Method C.3 (Algal Inhibition test) | $EC_{50}$ (72 h): 3.8 mg/l based on: biomass<br><br>$EC_{50}$ (72 h): 13.7 mg/l based on: growth rate<br><br>NOEC (72 h): < 1.9 mg/l based on: growth rate<br><br>NOEC (72 h): < 1.9 mg/l based on: biomass | 4 (not assignable)<br><br>disregarded study<br><br>experimental result<br><br>**Test material: (Common name): Acid Black 210 sodium salt**<br><br>**Purity: unknown**<br><br>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 28$^{th}$ 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.

## 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<br><br>freshwater<br><br>static<br><br>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)<br><br>key study<br><br>read-across from supporting substance (structural analogue or surrogate<br><br>**Test material (Common name): Acid Black 210 potassium salt**<br><br>**Purity: ca. 65 %**<br><br>Impurities: sodium sulfate, sodium chloride, potassium chloride | FJ Carrion (1997b) |
| activated sludge<br><br>freshwater<br><br>static<br><br>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)<br><br>supporting study<br><br>experimental result<br><br>**Test material (commercial name): Luganil Schwarz NT Stucke P 20/87**<br><br>Purity (dye content as sodium salt content): 77% wt<br><br>sodium chloride: 11% wt<br><br>potassium chloride: 5% wt<br><br>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

# 9. REFERENCES

Alexa Caduff (2012). Direct Black 19 Growth inhibition test with Lemna minor SCREENING TEST. Testing laboratory: BMG Engineering Ltd. Report no.: A12-00233. Owner company: Reach&Color Kft. Report date: 2012-04-23.

BASF SE (1984). Report on the acute irritation to the eye of the withe rabbit. Testing laboratory: BASF Department of Toxicology. Report no.: 83/259. Owner company: BASF SE. Report date: 1984-03-01.

BASF SE (1988). Oxygen consumption following ROBRA. Report no.: 10/1175/88. Owner company: BASF SE. Report date: 1988-12-14

Dirk Scheerbaum (2011) Acid Black 210 Alga, Growth Inhibition test with Desmodesmus subspicatus, 72 hours acc. to OECD TG 201. Testing laboratory: Dr. U. Noack-Laboratorien. Owner company: Acid Black 210 Consortium.

FJ Carrion (1997a). Ecotoxicological tests according to ETAD recommendation: biodegradation method according ot 302B OECD. Testing laboratory: Laboratory of the environmental contamination of the textile research institute and industry cooperation of Terrassa. Report no.: 2699/005. Owner company: Clariant Iberica SA.

FJ Carrion (1997b) Inhibition activity of waste water bacteria. Testing laboratory: Laboratory of the environmental contamination of the textile institute and industry. Report no.: 2699/005. Owner company: Clariant Iberica SA. Report date: 1997-10-05.

J. Zapatero (1997). Primary eye irritation in rabbits test substance: Carbon Derma AF 135. Testing laboratory: CENTRO DE INVESTIGACION Y DESAROLLO APLICADO S. A. L. Report no.: CD-96/5305T-1. Owner company: Clariant Iberica SA.

Jana Netusilova (2010). Acid Black 210 Hydrolysis as a function of pH. Testing laboratory: Výzkumný ústav organických syntéz a. s. Research Institute for Organic Syntheses Inc. Centre of Ecology, Toxicology and Analytics. Report no.: 10-345. Owner company: Acid Black 210 consortium.

JH Moore, MHI Comber (1997). Chronic toxicity to daphnia magna. Testing laboratory: Brixham Environmental Laboratory. Owner company: Stahl Europe B. V.

Karel Cizek (2011). Acid Black 210-Estimation of the adsorption coefficient using HPLC. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 11-273. Owner company: Acid Black 210 Consortium.

Ludmila Dolezalova (1996). Korostan Black BN3 testing on ecotoxicity. Testing laboratory: VUOS. Report no.: 4220/5A. Owner company: Synthesia AS.

Michal Bartos (2011a). Determination of physical-chemical properties of Acid Black 210-Melting Point. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Michal Bartos (2011b). Determination of physical-chemical properties of Acid Black 210-Boiling point. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Michal Bartos (2011c). Determination of physical-chemical properties of Acid Black 210-Relative density. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Michal Bartos (2011d). Determination of physical-chemical properties of Acid Black 210-Water solubility. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Michal Bartos (2011e). Determination of physical-chemical properties of Acid Black 210-Partition coefficient. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Michal Bartos (2011f). Determination of physical-chemical properties of Acid Black 210-Oxidizing properties. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Michal Bartos (2011g). Determination of physical-chemical properties of Acid Black 210-Granulometry. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Michal Bartos (2011h). Determination of physical-chemical properties of Acid Black 210-Dissociation constant. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Michael Cleuvers, Hans-Toni Ratte, Phytotoxicity of coloured substances: is Lemna Duckweed an alternative to the algal growth inhibition test? Chemosphere 49 (2002) 9–15)

Stahl Europe B. V. (a). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl Europe B. V, Melting point, Missing information from migrated NONS file.

Stahl Europe B. V. (b). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl Europe B. V, Relative density, Missing information from migrated NONS file.

Stahl Europe B. V. (c). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl Europe B. V, Surface tension, Missing information from migrated NONS file.

Stahl Europe B. V. (d). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl Europe B. V, Water solubility, Missing information from migrated NONS file.

Stahl Europe B. V. (e). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl Europe B. V, Partition coefficient, Missing information from migrated NONS file.

Stahl Europe B. V. (f). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl Europe B. V, Flammability, Missing information from migrated NONS file.

Stahl Europe B. V. (g). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl Europe B. V, Explosive properties, Missing information from migrated NONS file.

Stahl Europe B. V. (h). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl Europe B. V, Self ignition temperature, Missing information from migrated NONS file.

Stahl Europe B. V. (i)Testing laboratory: RCC, Research and consulting company AG, 4452 Itingen. Owner company: Stahl Europe B. V, Algal Inhibition test. Missing information from migrated NONS file. .

Stahl Europe B. V (1996a). Testing laboratory: Zeneca Specialties Blackley, Manchester, Owner company: Stahl Europe B. V, Eye irritation/corrosion, Missing information from migrated NONS file.

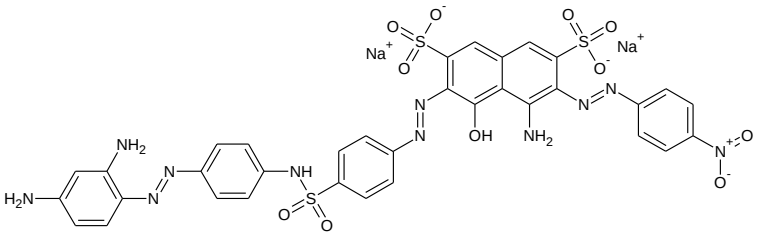
Stahl Europe B. V (1996b). Testing laboratory: Zeneca Brixham Environmental Laboratory, Zeneca Ltd Brixham, Devon, TQ5 8BA . Owner company: Stahl Europe B. V, Fish acute toxicity, Missing information from migrated NONS file.

Stahl Europe B. V (1996c). Testing laboratory: Zeneca Brixham Environmental Laboratory, Zeneca Ltd Brixham, Devon, TQ5 8BA, Owner company: Stahl Europe B. V, Daphnia Magna, Missing information from migrated NONS file.

# 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

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

[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.

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).

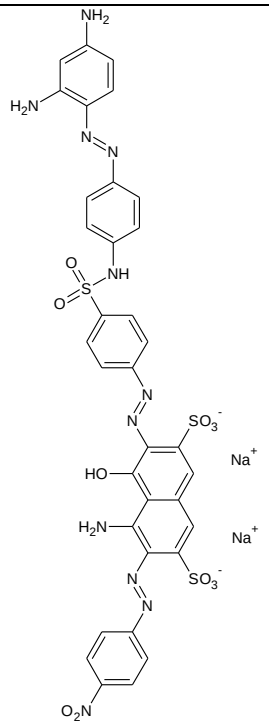
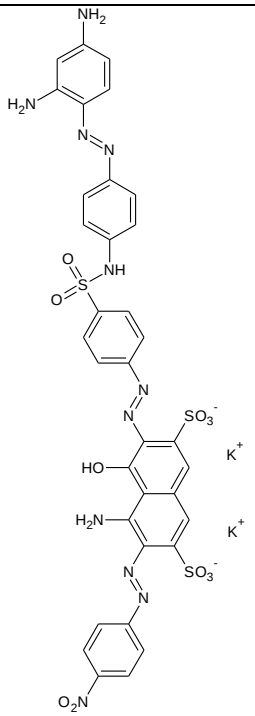
step 7. Either NaOH (to produce the sodium salt form) or KOH (to produce the potassium salt form) are then used to neutralize the substance, which is then isolated and dried.

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.

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

[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).

[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).

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.

## 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.

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

| | **ABl210-Na** | **ABl210-K** |
|---|---|---|

[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).

| | | |
|---|---|---|
| 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*<br>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)<br>ECb50 (7d) > 2000 mg/l (nominal) |
| *Reference* | | Acid Black 210 Consortium, 2012 |
| Toxicity to microorganisms | EC50 (3h) > 100 mg/l<br>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)<br>10228 mg/l (32d) | 1430-2900 mg/l |
| *Reference* | ECHA database<br>OECD SIDS, 2005 | ECHA database |
| Chloride | 2430 mg/l (120 h)<br>4800 mg/l (time unspecified) | greater 100 mg/l<br>up to 2500 mg/l |
| *Reference* | ECHA database | ECHA database<br>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<br>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<br>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<br>*Toxicity Fertility* | n.a. | NOAEL(P): ca 150 mg/kg bw/day (rat)<br>NOAEL(F1): ca 150 mg/kg bw/day (rat)<br>(OECD 422) |
| *Reference* | | Acid Black 210 Consortium, 2011 |
| *Developmental Toxicity* | n.a. | NOAEL $_{mat}$: ca 150 mg/kg bw/day (rat)<br>NOAEL $_{dev}$: ca 150 mg/kg bw/day (rat)<br>(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)<br>Not irritating (CLP) | Not irritant to severe irritant/corrosive due to the $KHSO_4$ impurity<br>$KHSO_4 < 1$ %: not irritating (CLP)<br>$KHSO_4 \geq 1 < 3$ %: eye irrit 2 (CLP)<br>$KHSO_4 \geq 3$ %: eye dam 1 (CLP) |

| | | |
|---|---|---|
| *Reference* | ECHA database<br>OECD SIDS, 2005 | ECHA database |
| Chloride | Slightly irritant (GHS)<br>depending on the concentration<br>Not irritating (CLP) | Mild irritation<br>Not irritating (CLP) |
| *Reference* | ECHA database | ECHA database<br>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.

**Reference**

Alexa Caduff (2012). Grown inhibition test with Lemna Minor. Testing laboratory: BMG Engineering Ltd. Report no.: A12-00882. Owner company: Acid Black 210 Consortium. Report date: 2012-12-12.

Alexa Caduff (2012). Growth inhibition test with Lemna Minor. Testing laboratory: BMG Engineering Ltd. Report no.: A12-00233. Owner company: REACH&Colours Kft.

BASF AG (1984). Report on the acute irritation to the eye of the withe rabbit. Testing laboratory: BASF Department of Toxicology. Report no.: 83/259. Owner company: BASF AG. Report date: 1984-03-01.

Dirk Scheerbaum (2011). Acid Black 210 Alga, Growth Inhibition test with Desmodesmus subspicatus, 72 hours acc. to OECD 201. Testing laboratory: Dr. U. Noack-Laboratorien. Report no.: SSO14515N. Owner company: Acid Black 210 Consortium. Report date: 2011-12-14.

ECHA database of the registered substances. EC: 204-822-2, CAS: 127-08-2. Name: potassium acetate. Registration type: full. Submission type: joint Submission. Tonnage band: 10,000 - 100,000 tonnes per annum. ECHA database of the registered substances. EC: 204-823-8, CAS: 127-09-3. Name: sodium acetate. Registration type: full. Submission type: joint Submission. Tonnage band: 10,000 - 100,000 tonnes per annum.

ECHA database of the registered substances. EC: 231-211-8, CAS: 7447-40-7. Name: potassium chloride. Registration type: full. Submission type: joint Submission. Tonnage band: 10,000 - 100,000 tonnes per annum.

ECHA database of the registered substances. EC: 231-598-3, CAS: 7647-14-5. Name: sodium chloride. Registration type: full. Submission type: joint Submission. Tonnage band: 100,000 – 1,000,000 tonnes per annum.

ECHA database of the registered substances. EC: 231-820-9, CAS: 7757-82-6. Name: sodium sulphate. Registration type: full. Submission type: joint Submission. Tonnage band: 100,000 – 1,000,000 tonnes per annum.

ECHA database of the registered substances. EC: 231-820-9, CAS: 7757-82-6. Name: sodium sulphate. Registration type: full. Submission type: joint Submission. Tonnage band: 1,000 – 10,000 tonnes per annum.

ECHA database of the registered substances. EC: 231-915-5, CAS: 7778-80-5. Name: potassium sulphate. Registration type: full. Submission type: joint Submission. Tonnage band: 1,000,000 – 10,000,000 tonnes per annum.

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the use of potassium sulphate and sodium sulphate as sources of respectively potassium and sodium added for nutritional purposes to food supplements. EFSA Journal 2010;8(12):1940 [12 pp.]. doi:10.2903/j.efsa.2010.1940. Available online: www.efsa.europa.eu

Federica Cattapan (2010). OECD 487-In vitro mammalian cell micronucleus test (MNvit). Testing laboratory: CHELAB. Report no.: 131/2010. Owner company: Acid Black 210 Consortium.

Human and Environmental Risk Assessment on ingredients of Household Cleaning Products. Substance: Sodium sulpfate (CAS# 7757-82-6) Edition 1.0. January 2006. Available online: www.heraproject.com

J. Zapatero (1997). Primary skin irritation in rabbits. Test substance: Carbon Derma AF 135. Testing laboratory: Centro De Investigacion Y Dearollo Aplicado Sal. Report no.: 96/5304T-1. Owner company: Clariant Products S. A.

J. Zapatero (1997). Primary eye irritation in rabbits. Test substance: Carbon Derma AF 135. Testing laboratory: Centro De Investigacion Y Dearollo Aplicado Sal. Report no.: CD-96/5305T-1. Owner company: Clariant Products S. A.

J. Zapatero (1997). Acute Oral Toxicity test in Rats. Fixed Dose Method. Test Substance: Carbon Derma AF 135. Testing laboratory: Centro De Investigacion Y Dearollo Aplicado Sal. Report no.: CD-96/5303T-1. Owner company: Clariant Products S. A. Report date: 1997-07-03.

JH Moore, MHI Comber (1997). Chronic toxicity to daphnia magna. Testing laboratory: Brixham Environmental Laboratory. Owner company: Stahl.

Jana Netusilova (2010). Acid Black 210 Hydrolysis as a function of pH. Testing laboratory: Výzkumný ústav organických syntéz a. s. Research Institute for Organic Syntheses Inc. Centre of Ecology, Toxicology and Analytics. Report no.: 10-345. Owner company: Acid Black 210 consortium.

Karel Cizek (2011). Acid Black 210-Estimation of the adsorption coefficient using HPLC. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 11-273. Owner company: Acid Black 210 Consortium.

Ludmila Dolezalova (1996). Korostan Balck BN3 Testing of ecotoxicity. Testing laboratory: VUOS. Report no.: 4220/5A. Owner company: Synthesia AS.

Marggraf, Andreas Siegmund (1761). Chymische Schriften. p. 167.

Michal Bartos (2011). Determination of physical-chemical properties of Acid Black 210-Oxidising properties. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 65/195/09. Owner company: Acid Black 210 consortium. Report date: 2011-11-21.

Missing information from migrated NONS file (1996). Testing laboratory: Zeneca Specialities Blackley, Manchester. Owner company: Stahl.

Missing information from migrated NONS file (1996). Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl.

Missing information from migrated NONS file (1996). Testing laboratory: Zeneca Central toxicology laboratory Macclesfiled, Cheshire SK10 4TJ. Owner company: Stahl.

Missing information from migrated NONS file (1996). Testing laboratory: Zeneca Specialties Blackley Laboratory Maaclsfield, Cheshire SK10 4TJ. Owner company: Stahl.

Missing information from migrated NONS file (1996). Testing laboratory: Brixham Environmental Laboratory, Zeneca Ltd. Brixham, Devon, TQ5 8BA. Owner company: Stahl.

Missing information from migrated NONS file (1996). Testing laboratory: Brixham Environmental Laboratory, Zeneca Ltd. Brixham, Devon, TQ5 8BA. Owner company: Stahl.

Missing information from migrated NONS file Testing laboratory: Zeneca Specialties Blackley, Manchester. Owner company: Stahl.

OECD SIDS. Potassium chloride CAS N°: 7447-40-7. UNEP Publications. 2001

OECD SIDS. Sodium sulphate CAS N°: 7757-82-6. UNEP Publications. 2005

Prof. Carrion Inhibition activity of waste water bacteria. Testing laboratory: Laboratory of the environmental contamination of the textile institute and industry. Report no.: 2699/005. Owner company: Clariant Iberica. Report date: 1997-10-05.

Prof. FJ Carrion (1997). Ecotoxicological tests according to ETAD recommendation: biodegradation method according ot 302B OECD. Testing laboratory: Laboratory of the environmental contamination of the textile research institute and industry cooperation of Terrassa. Report no.: 2699/005. Owner company: Clariant Products SA.

S. Cinelli (2014). Acid Black 210 Na Assessment of ocular corrosion/severe irritation bovine corneal opacity/permeability (BCOP) Assay. Testing laboratory: Research Toxicology Centre S. p. A. Report no.: 98470. Owner company: REACH&Colours Kft.

Zdenka Rösslerová (2011). Acid Black 210. Assessment of Toxicokinetic Behaviour. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 11-338. Owner company: Acid Black 210 Consortium.

Zuzana Roznovska Acid Black 210 Skin sensitisation: Local Lymph Node Assay. Testing laboratory: VUOS. Report no.: 10-370. Owner company: Consortium Acid Black 210. Report date: 2011-10-02.

Zuzana Rožnovská (2011). Acid Black 210 – Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. Testing laboratory: Výzkumný ústav organických syntéz a. s. Center of Ecology, Toxicology and Analytics. Report no.: 11 - 162. Owner company: Acid Black 210 Consortium.