Committee for Risk Assessment

RAC

Opinion
proposing harmonised classification and labelling at EU level of

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

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

CLH-O-0000001412-86-165/F

Adopted

22 September 2017

22 September 2017

CLH-O-0000001412-86-165/F

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

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

Chemical name:	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

The proposal was submitted by Italy and received by RAC on 10 October 2016.

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

PROCESS FOR ADOPTION OF THE OPINION

Italy has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at *http://echa.europa.eu/harmonised-classification-and-labelling-consultation/* on 21 November 2016. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by 16 January 2017.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC:	Radu Branisteanu
Co-Rapporteur, appointed by RAC:	Riitta Leinonen

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on 22 September 2017 by consensus.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	611-159-00-6	disodium 4-amino-6-((4-((4-(2,4-diaminophenyl)azo)phenylsulfamoyl)phenyl)azo)-5-hydroxy-3-((4-nitrophenyl)azo)naphthalene- 2,7-disulfonate	421-880-6	-	Eye Dam. 1 Aquatic Chronic 3	H318 H412	GHS05 Dgr	H318 H412			
Dossier submitters proposal	611-159-00-6	disodium 4-amino-6-((4-((4-(2,4-diaminophenyl)azo)phenylsulfamoyl)phenyl)azo)-5-hydroxy-3-((4-nitrophenyl)azo)naphthalene- 2,7-disulfonate	421-880-6	201792-73-6	Remove Eye Dam. 1 Aquatic Chronic 3	Remove H318 H412	Remove GHS05 Dgr	Remove H318 H412			
RAC opinion	611-159-00-6	disodium 4-amino-6-((4-((4-(2,4-diaminophenyl)azo)phenylsulfamoyl)phenyl)azo)-5-hydroxy-3-((4-nitrophenyl)azo)naphthalene- 2,7-disulfonate	421-880-6	201792-73-6	Remove Eye Dam. 1 Aquatic Chronic 3	Remove H318 H412	Remove GHS05 Dgr	Remove H318 H412			
Resulting Annex VI entry if agreed by COM	611-159-00-6	disodium 4-amino-6-((4-((4-(2,4-diaminophenyl)azo)phenylsulfamoyl)phenyl)azo)-5-hydroxy-3-((4-nitrophenyl)azo)naphthalene- 2,7-disulfonate	421-880-6	201792-73-6							

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Background: current classification and read-across

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

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

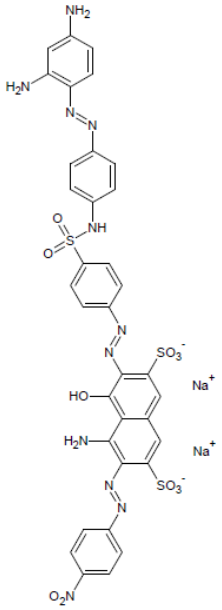
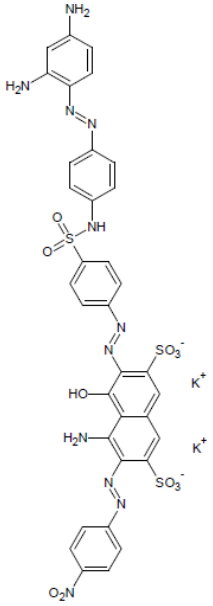
Justification for the Read Across from Acid Black 210 Potassium salt

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

a) Structural similarity

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

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

Common name	ABI210-Na	ABI210-K
Molecular formula	$C_{34}H_{25}N_{11}Na_2O_{11}S_3$	$C_{34}H_{25}K_2N_{11}O_{11}S_3$
Structure		

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

b) *Common precursors*

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

c) Typical composition

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

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

	ABI210-Na	ABI210-K
Main component	> 60%	65-77%
Sodium chloride	0-15%	0-15%
Water	0-20%	0-15%
Organic impurities	0-8% *	n.a.

Sodium sulphate	0-3%	-
Potassium chloride	-	0-10%

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

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

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

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

d) Physico-chemical properties

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

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

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

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

e) Toxicological profile

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

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

Conclusion

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

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

Zapatero – 1997 (Key study)

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

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

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

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

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

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

BASF SE – 1984 (supportive study)

This study was performed on 3 rabbits (1 male and 2 female) according to a protocol stated as being "equivalent or similar" to *OECD TG 405 method – Acute Toxicity: Eye irritation/Corrosion*. The test material was well identified and a certificate was provided with the exact composition: Acid Black 210 lithium/potassium salt ca. 67%, 3% inorganic salt (lithium chloride) and 30% water. No ratio of the lithium vs. potassium salts of the organic moiety is given. The material (0.1 mL) was instilled without further preparation since the test material was a water solution.

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

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

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

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

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

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

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

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

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

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

In vitro study

The Bovine Corneal Opacity and Permeability (BCOP) study performed by Cinelli (2014) was cited by the DS as a supporting study and rated as Klimisch 1. The test followed the protocol of OECD TG 437; the test material was Acid Black 210 sodium salt of 66.4% purity. Following the Public Consultation, specific concentrations of the impurities were added as follows: sodium sulphate 1.86%, sodium chloride 7.4% and water 16%. The test material was applied at a concentration of 20% w/v using physiological saline as the vehicle. The IVIS score computed according to the method protocol was of 25.5. It is noted that the mean opacity value was affected by the coloration of the corneal surfaces. In addition, when compared to the negative control, no relevant increase in corneal permeability was recorded following the application of the test substance.

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

The staining properties

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

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

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

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

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

The information provided in the original study

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

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

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

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

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

The new studies

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

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

Conclusion

RAC considers that findings from a study using ABl210-Na and read-across from ABl210-K is appropriate for the evaluation of eye irritation/eye damage of ABl210-Na. The substances do not have any known physico-chemical properties that would support classification. No human data were available for the assessment. The animal data showed that both substances are mild eye irritants but the severity and reversibility indices had numerical values below the threshold for

classification. Both substances and the equivalent lithium salt exhibited staining capacity of the conjunctival membrane; however this finding cannot be used for classification.

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

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

ABl210-Na is used in water-based formulations mainly for industrial leather dyeing but also in textile and paper formulation. The substance currently has a harmonised environmental classification as Aquatic Chronic 3; H412 in Annex VI of the CLP Regulation. The DS proposed to remove this classification due to data from new studies. The DS has taken into consideration the structural analogue potassium salt. The current aquatic classification is based on a 72 hour EC_{50} of 13.7 mg/L for algae. There is no original test report available and consequently there are uncertainties about e.g. the actual tested substance and whether nominal or measured concentrations have been reported. The substance is difficult to test with algae because of the colouring effect. The DS provided new information on a *Lemna* growth inhibition test for ABl210-K resulting in an EC_{50} greater than 2000 mg/L (active ingredient) to be used instead of algae data. Based on this data and on acute toxicity for fish and *Daphnia* (LC_{50} 1890 and 150 mg/L respectively), the substance being not rapidly degradable and non-bioaccumulative, the DS concluded that no classification is needed for ABl210-Na. They also note that the long-term NOEC for *Daphnia* of ca. 2.5 mg/L does not fulfil the criteria for chronic classification.

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

Degradation

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

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

Bioaccumulation

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

Aquatic toxicity

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

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

Test substance	Method	Result	Remarks	Reference in the CLH Report
Acute toxicity to fish				
ABl210-K purity ca. 65%	*Poecilia reticulata*, ISO 7346-1 Part 1, static, not GLP	48 h LC_{50} > 2000 mg/L test material (nominal) 96 h LC_{50} ca. 1890 mg/L test material (estimated)	Key study	Dolezalova, 1996
ABl210-Na, purity unknown	*Oncorhynchus mykiss*, EU C.1, GLP, semi-static, limit test	96 h LC_{50} > 120 mg/L	supporting study/not taken into account for classification purposes	Stahl Europe B.V., 1996b
Chronic toxicity to fish - no information				
Acute toxicity to aquatic invertebrates				
ABl210-Na, purity unknown	*Daphnia Magna*, EU C.2, GLP, limit test	48 h EC_{50} > 150 mg/L (measured)	key study	Stahl Europe B.V., 1996c
Chronic toxicity to aquatic invertebrates				
ABl210-Na, notified S124668, purity unknown	*Daphnia magna*, OECD TG 211	21 d, NOEC (reproduction, growth) ca. 2.5 mg/L (measured concentration 78% of the nominal)	weight of evidence	Moore and Comber, 1997
Toxicity to algae and plants				
ABl210-K, purity ca. 65%	*Lemna minor*, OECD TG 221, GLP, static	7 d E_rC_{50} > 2000 mg/L active ingredient (nominal) corresponding to 7 d E_rC_{50} > 3080 mg/L test material 7 d NOE_rC < 30.8 mg/L test material (frond number and dry weight) test concentrations not measured	key study	Caduff, 2012
ABl210-K, purity ca. 65%*	*Desmodesmus subspicatus*, OECD TG 201, static, test performed in duplicate; 1. in Erlenmeyer flasks 2. Microplate 96-well with plane bottom where: light intensity 60-120 µE m-2s-1,	72 h E_rC_{50} > 10 - < 100 mg/L (measured) 72 h E_rC_{10} ca 10.8 mg/L, initial measured concentration 105% of the nominal; 98% after 72 hr comparable toxicity with both methods	disregarded test conc. 1, 10 and 100 mg/L	Scheerbaum, 2011

	200 µL per well, rotary shaker 100 rpm			
ABI210-Na, purity unknown	*Desmodesmus subspicatus*, EU C.3, GLP	72 h, E_rC_{50} 13.7 mg/L (nominal) 72 h, NOE_rC < 1.9 mg/L (nominal)	disregarded study	Stahl Europe B.V. (i)

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

There are two acute fish toxicity studies available. The test with ABI210-K giving a 96 h LC_{50} value of ca. 1890 mg/L is taken into account for classification. The other fish test on *Oncorhynchus mykiss* is disregarded mainly because of the lack of measured test substance concentrations and unknown purity of the test substance.

There is one acute, and one chronic *Daphnia* study with ABI210-Na available; in both studies, the purity of the test substance is unknown and the study description is insufficient. In the acute limit test, the 48 h EC_{50} was ≥ 150 mg/L. The chronic *Daphnia* study was submitted in the framework of the notification of the substance S124668 (ABI210-Na). The 21 d NOEC was ca. 2.5 mg/L.

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

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

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

Comments received during public consultation

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

One MSCA did not agree to remove the classification. They felt that more details are needed to prove that toxicity is due to a shading effect in the Scheerbaum (2011) study. Another MSCA also had concerns regarding the protocol of this study. It is noted in the CLH Report that the study was performed according to the OECD series of testing and assessment Number 23, paragraph 3.8 Coloured substances, which list a few strategies to minimise the shading effect. It is not clear, however, how this was considered. Moreover, they wondered if the conclusion that

the toxic effects were only caused by light absorption were noticed in this study or from observation with other substances. The DS explained that the test had been performed in duplicate, using sterile Erlenmeyer flasks and with Microplate 96-well plates with flat bottom following the method proposed in the OECD 23. Microplates were used to reduce the light path by reducing the depth or the volume. However, the toxicity to algae was comparable in both tests indicating that the method was not adequate. The DS believes an indirect demonstration of the light effect is given by an old test summary recently found in a Stahl archive performed on the notified substance S124668. The test has been performed in the Zeneca Speciality Blackley testing laboratory in duplicate using the same concentration of test material; one flask is normal, the other is divided in two sections. The above section contained the dye solution, the section below the algae that were not in contact with the dye, but were shaded from the light by the dye solution. The results showed that values and shape of the curves from both flasks are practically identical showing that the toxicity is generated by the light shading effect and not by the intrinsic toxic effect of the dye. Similar tests are available for several black or brown dyes.

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

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

Assessment and comparison with the classification criteria

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

Degradation

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

Bioaccumulation

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

Aquatic toxicity

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

Short-term

RAC considers that there are reliable data on fish, algae and *Lemna*. The 96 hour LC_{50} for *Poecilia reticulata* is ca. 1890 mg/L. The 72 hour E_rC_{50} for *Desmodesmus subspicatus* is between 10 to

100 mg/L and the 7 day E_rC_{50} for *Lemna minor* is greater than 3080 mg/L. RAC disagrees with the DS to disregard the Scheerbaum (2011) algae test. There is not enough information to prove that toxicity reported is caused only by a shading effect. The test aimed to take into account the principles proposed in the OECD series of testing and assessment 23 for coloured substances, which are also listed in the ECHA Guidance on Information requirements and Chemical Safety Assessment Chapter R.7b Table R.7.8-3. The test was performed in duplicate, using sterile Erlenmeyer flasks and with Microplate 96-well plates with flat bottom. The following adjustments to the standard algae growth inhibition test (OECD TG 201) should be applied for coloured substances: the irradiation should be in the highest end of the range in the method (i.e. 120 $\mu Em^{-2}\ s^{-1}$ or higher), the light path should be shortened by reduction of the volume of the test solutions (in the range of 5-25 mL) and sufficient agitation should be performed in order to obtain a high frequency of exposure of the algae to high irradiation at the surface of the culture. It is not clear to which extent these adjustments were included in the Scheerbaum (2011) study using Erlenmeyer flasks. In the Microplate 96-well with flat bottom test, that was performed in addition to the Erlenmeyer flask test, the parameters were: light intensity 60-120 $\mu Em^{-2}\ s^{-1}$, volume 200 µL per well, rotary shaker 100 rpm. However, the toxicity to algae was comparable in both tests. RAC concludes that even it is unclear how much the growth inhibition is caused by shading or intrinsic toxicity the 72 hour E_rC_{50} and E_rC_{10} would not be expected to be lower than 10-100 mg/L in any case.

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

Long-term

RAC is of the opinion that there are two reliable long-term study results available for classification namely the 7 day NOE_rC < 30.8 mg/L for *Lemna minor* and the 72 hour E_rC_{10} of ca. 10.8 mg/L for *Desmodesmus subspicatus*. Even through the algae test does not give a definitive toxicity value to ABI210-Na because of the possible shading effect, it is considered reliable enough to be used in this weight of evidence classification. The 21 day NOEC of ca. 2.5 mg/L for *Daphnia magna* and the 72 hour NOE_rC < 1.9 mg/L are considered as supportive evidence. There is no long-term data for fish.

Comparison with the criteria

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

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

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

ANNEXES:

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

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