Committee for Risk Assessment

RAC

Annex 2
Response to comments document (RCOM) to the Opinion proposing harmonised classification and labelling at EU level of

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

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

CLH-O-0000001412-86-165/F

Adopted
22 September 2017

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DISODIUM 4-AMINO-6-((4-((4-(2,4-DIAMINOPHENYL)AZO)PHENYLSULFAMOYL)PHENYL)AZO)-5-HYDROXY-3-((4-NITROPHENYL)AZO)NAPHTHALENE-2,7-DISULFONATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance 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
Dossier submitter: Italy

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
16.01.2017	Germany		MemberState	1
Comment received				
Substance ID: In the current entry in Annex IV of CLP (Index No. 611-159-00-6) the substance "disodium 4-amino-6-((4-((4- (2,4-diaminophenyl)azo)phenylsulfamoyl)phenyl)azo)-5-hydroxy-3-((4-nitrophenyl)azo)naphthalene-2,7-disulfonate" is identified by the corresponding ELINCS No. 421-880-6, no CAS No. is given. In the reference substance in the IUCLID dossier the same identifiers are given (EC No. 421-880-6, CAS No. -). Deviating from this, in the CLH report the substance is identified using the CAS No. 201792-73-6 in addition EC No. 421-880-6. This inconsistency should be amended/clarified. In this context, we like to address the aspect that the degree of purity of the substance is >= 60.0 - < 100.0 % (w/w) (Part A, Section 1.1, Table 1 of the CLH report). Based on this low purity, the substance might not be a mono-constituent substance and could therefore not be identified by the given CAS No. 201792-73-6 for the substance 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). Please clarify this issue. Environmental hazards: With respect to classification for Environmental hazards (pages 25 - 37 of the CLH dossier) there are some uncertainties. For details see below.				

Health hazards:
With respect to classification for eye irritation/damage (pages 18 - 23 of the CLH dossier) there are major concerns. For details see below.
The conclusion to remove the current classification as Eye Dam. 1 (H318) cannot be supported. The current classification should be maintained.

Dossier Submitter's Response

Thank you for the comment.
Regarding the substance identity:
There is no CAS number in Annex IV of CLP and at the time of submission of the inquiry and within the registration dossier no CAS number was indicated in the EC inventory (therefore in the downloaded Referece Substance is missing), but after the inquiry of the substance in order for the Lead Registrant to Register in 2011, the Lead Registrant received this information from ECHA on 1 August 2011:

"Please include EC number 421-880-6 in the EC number field of your dossier.
Please include the CAS number 201792-73-6 and the 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) in the appropriate field in your reference substance.
We consider that these identifiers correspond best to your substance."

Regarding the substance purity:
The substance was presented as monocontituent in the framework of DPD and ECHA recognised the substance presented by the Lead Registrant in 2011 as the same substance based on the analytical identification presented during the inquiry process. For dyes it is not uncommon to consider as monoconstituent substances purities < 80 % according to a deviation rule. In fact many of them contain variable percentages of inorganic salts that are coming from the synthetic process, but they are not contributing to the functional, toxicological and eco-toxicological properties of the substance; they are therefore considered as impurities.

RAC's response

Thank you for the additional clarifications regarding the substance identity. The issue of the substance purity was approached in the ODD.

Date	Country	Organisation	Type of Organisation	Comment number
16.01.2017	Belgium		MemberState	2

Comment received

The limited information reported in the CLH report makes a thorough and objective evaluation of the Human health/Environmental data and thus interpretation of the results very difficult.(f.i. no info on test concentrations used, validity criteria met or not?, possible deviations from the guideline and the impact on the results, …)
Furthermore we have strong doubts about the given reliability of most of the studies (Reliability 2 is given for studies where purity/impurities are unknown, measured or nominal concentrations unknown, Env : read across substance : assumed purity of 65%,…).

Dossier Submitter's Response

Thank you for the comment.

We are aware that there are not clear and no enough details on the tested substance related to the existing classification, since it was not possible to recover the original reports presented in the framework of DPD. Despite this it is known the exact composition of the tested substances for the new presented data, test concentrations used are known and reported within the IUCLID dossier and this CLH report, concentrations are measured in the ecotoxicological testing, validity criteria are met and deviations from the guideline, when present, are described.

It has not to be a confounding factor the purity of the substance, either as sodium or potassium salt.
In both cases they are indicated as < than 80 % and about 65 %. As explained in comment 1, this is typical of dyes to consider them as monoconstituents even if their purities are below 80 %, because the main “byproducts” are inorganic salts like i.e. sodium sulphate potassium sulphate, sodium chloride, potassium chloride, and they are not considered as contributing to the functional, toxicological and eco-toxicological properties of the substance.

More in details:

The “reference” composition of the substance today considered as 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: 201792-73-6, EC number: 421-880-6 is presented at page 12 of the CLH dossier:

Acid Black 210 (Na salt):	66.4 %
Sodium chloride:	7.4 %
Sodium sulphate:	1.86 %
Water:	16 %

For Eye irritation/corrosion:
Pag 22 and 23 of the CLH dossier are reporting the compositions for the considered dyes:

<u>For the BASF SE 1984 study:</u>

Acid Black 210 (Li/K salt)	67 %
Litium chloride	3 %
Water	30 %

<u>For the J. Zapatero 1997 study</u>

Acid Black 210 (Na/K salt)	65 %
Sodium chloride	
Sodium sulphate	
Potassium Chloride	
Water	

<u>For the in vitro test according to OECD TG 437, S. Cinelli, 2014 study:</u>

Acid Black 210 (Na salt):	66.4 %
Sodium chloride:	7.4 %
Sodium sulphate:	1.86 %
Water:	16 %

For Aquatic toxicity:
<u>For the Dirk Scheerbaum, 2011 study:</u>

Acid Black 210 (Na salt): 66.4 % Sodium chloride: 7.4 % Sodium sulphate: 1.86 % Water: 16 % For the Alexa Caduff, 2012 study: Acid Black 210 (K salt): 66.5 % Potassium chloride and sulphate 10 % Water: 20 % For both the considered endpoints the indicated impurities (water and inorganic salts) have no influence on the test results or on the applicability of the Read Across or on the reliability of the studies.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
04.01.2017	France		MemberState	3
Comment received				
France agrees with the conclusion to remove the classification for severe eye damage/eye irritation. Page 19 Can IT confirm that the last 3 scores of the table deals with the chemosis score and not the conjunctivae score?				
Dossier Submitter's Response				
Thank you for the comment. Yes, it is confirmed: the headline of the report says: Conjunctiva oedema				
RAC's response				
The position of the France MS was noted. The additional confirmation is appreciated.				

Date	Country	Organisation	Type of Organisation	Comment number
16.01.2017	Germany		MemberState	4
Comment received				
With respect to classification for eye irritation/damage (pages 18 – 23 of the CLH dossier) there are major concerns. The argumentation provided by the dossier submitter for classification (no classification required) is mainly based on assumptions made on the basis of a read-across approach, although information on 2 studies assessing the eye damaging/irritating potency of the actual test substance (CAS: 201792-73-6) is available, which contradicts the assumptions obtained by the read-across approach. Moreover, the substance used as source substance in the read across approach (Acid Black 210-K; CAS: 85223-29-6) itself is classified as Eye Dam. 1 by 30 notifiers and as Eye Irrit. 2 by 5 further notifiers in the C&L inventory, indicating that there are studies available for this source substance, which suggest that this chemical is also				

irritating/damaging to the eyes, but which are not considered by the dossier submitters.

Moreover, the objections the dossier submitter cites as reason for disregarding the in vivo study by Stahl Europe B.V. (page 22 of the CLH dossier) using the actual test substance are in most cases negligible and/or also true for the presented studies with Acid Black 210-K used for read-across:

1) The dossier submitter stated that basic information for a correct classification of Acid Black 210 Na is missing. This statement is not entirely correct, since the testing method (EU method B.5) is reported as well as the number of animals used and the detailed individual results.

2) They further criticised that no information is reported whether the eyes of the test animals were washed or not. However, results obtained without washing eyes are handled and interpreted identically compared to results obtained from animals whose eyes were washed when using the EU method B.5 (“At 24 hours a washout may be used if considered appropriate.”). Furthermore, the reported read-across studies also do not report whether the test substance was washed or not. Hence, this objective regarding the basic studies performed on the test substance is also applicable to the two selected read-across studies used for declassification of Acid Black 210-Na.

3) The dossier submitter also stated that no information is given whether the substance has been added directly in powder form. These issues are admittedly not reported but might also be negligible since the EU Method B.5 (Acute Toxicity: Eye Irritation) protocol gives detailed instructions how to test and apply solid chemicals, including powders. Moreover, also when testing solid substances, washing of eyes is optional (“…the eye may be rinsed with saline or distilled water.”).

4) The dossier submitter also annotated that no information on the purity of the test substance and the identities of potential impurities are given, which is correct. But as the dossier submitter states in the CLH report, Acid Black 210-Na is manufactured via defined precursors and thus, it is “expected that the typical commercial batches present very similar characteristics” (page 45).

Furthermore, a typical purity/impurity profile for this substance is given on page 45, suggesting a similar purity/impurity profile of the test substance used by Stahl Europe B.V.

In addition, the purity of the source substances Acid Black 210-K, which is used in both referred read-across studies is rather low (~ 65%) and the identity of impurities in at least one of the two read-across studies (BASF SE, 1984) is also not reported.
It is further stated on page 45 that a complete analytical characterisation of Acid Black 210-K is not available and that only ranges of composition can be reported.

Thus, another of the mentioned objectives stated by the dossier submitter regarding the basic studies using the actual test substance are also applicable to one or both of the selected read-across studies used for declassification of Acid Black 210-Na.

In addition, the BCOP-test performed according to OECD TG 437 by Cinelli et al. (2014) using Acid Black 210-Na yielded in a mean in vitro irritation score (IVIS) of approximately 25.5 (page 20 of the CLH dossier). The BCOP test can only be used to identify chemicals inducing serious eye damage as defined by CLP, i.e. chemicals to be classified as CLP

Category 1 (IVIS > 55) or chemicals that do not require classification for eye irritation or serious eye damage under the CLP classification system (IVIS ≤ 3). However, the BCOP test method is not recommended for the identification of test chemicals that should be classified as (mildly) irritating to the eyes (CLP Category 2). Based on a 3 < IVIS ≤ 55 obtained in a BCOP test, no accurate/ reliable prediction on the actual eye irritating/damaging potency of the test substance can be made. Based on a 3 < IVIS ≤ 55 the necessity of classification cannot be excluded.

The dossier submitter further stated that the test item used in the BCOP test was coloured and thus the mean opacity value was probably affected by the substance remaining on the corneal surface (page 8 and 23 of the CLH dossier) leading to higher, overestimated cornea opacity values. However, only based on an assumption like this an indisputable CLP classification (no classification necessary) cannot be made, especially considering that an appropriate (coloured) negative control substance should have been used for comparison and evaluation of the obtained results (not reported; study not assignable) and that the colour of the test substance could have also led to underestimated cornea opacity values.
It was concluded by the dossier submitter that the results of the BCOP test do not “lead to a clear conclusion about the classification regarding irritating properties [of Acid Black 210-Na], but clearly exclude serious eye damage” (page 8 of the CLH report). This statement, however, is also not correct, as the BCOP test method when used to identify chemicals inducing serious eye damage only has an overall accuracy of 79% and a false negative rate of 14%. Thus, there is still a significant probability of Acid Black 210-Na eliciting serious eye damage, although the obtained IVIS was > 3 and ≤ 55.

Since the data used in a hazard or risk assessment should be relevant, reliable and sufficient for the regulatory purpose, it is necessary to base the assessment on the totality of available information, i.e. to apply Weight of Evidence (WoE) considerations. Moreover, it is particularly important to adequately justify read-across of negative findings. The results of the two studies performed according to validated test guidelines and using Acid Black 210-Na cannot be excluded from this WoE evaluation, taking into account that the presented tests using a read-across substance elicit similar objectives as the studies performed on the actual test substance. Hence, the conclusion of the dossier submitter that the test substance does not meet the criteria for classification cannot be supported.

Moreover, the substance used as source substance in the read across approach (Acid Black 210-K) itself is classified as Eye Dam. 1 or Eye Irrit. 2 by various notifiers in the C&L inventory, indicating that there might be further studies available for Acid Black 210-K which indicate that this source substance is also irritating/damaging to the eyes but which were not considered by the dossier submitters.

Since the CLP guidance document states that if “discrimination between Serious Eye Damage Cat. 1 and Eye Irritant Cat. 2 is not possible, Serious Eye Damage Cat. 1 must be chosen”, the current classification of Acid Black 210-Na as Eye Dam. 1 (H318) should be maintained, especially in regard of the irreversibility of the colouration of the third eyelid detected in one animal after 28 days of observation in the in vivo study by Stahl Europe B.V., which, moreover, prevented a proper assessment of conjunctival erythema until day 14 (page 21 of the CLH report) and thus might have led to an underestimation of the observed adverse effects.

Dossier Submitter’s Response

Thank you for the comment.

The specific paragraph can be commented as following:

- *The argumentation provided by the dossier submitter for classification (no classification required) is mainly based on assumptions made on the basis of a read-across approach, although information on 2 studies assessing the eye damaging/irritating potency of the actual test substance (CAS: 201792-73-6) is available, which contradicts the assumptions obtained by the read-across approach.*

Just one study is available on the sodium derivative with results regarding eye damage that can be discussed.

- *Moreover, the substance used as source substance in the read across approach (Acid Black 210-K; CAS: 85223-29-6) itself is classified as Eye Dam. 1 by 30 notifiers and as Eye Irrit. 2 by 5 further notifiers in the C&L inventory, indicating that there are studies available for this source substance, which suggest that this chemical is also irritating/damaging to the eyes, but which are not considered by the dossier submitters.*

It has to be noticed that there are also 155 notifications as “not classified” in the C&L inventory. On the other side it is not clear when it is said that 5 notifiers indicated that there are studies available not considered by the lead registrant: always “no available data” is reported in all sections of the C&L dissemination website. A regular SIEF survey has been performed and tests have been requested according to article 30: no study except the considered ones has been presented to the SIEF or to the Lead Registrant.

- *Moreover, the objections the dossier submitter cites as reason for disregarding the in vivo study by Stahl Europe B.V. (page 22 of the CLH dossier) using the actual test substance are in most cases negligible and/or also true for the presented studies with Acid Black 210-K used for read-across:*

The reasons indicated at pg 22 listing the deficiencies for the “in vivo” study are the following:

1. purity of the tested substance: this is known for all the other presented studies except for the Stahl studies
2. identities of impurities: this is really important, and as indicated in the comment to the first comment the composition is well known for almost all the newly presented studies except for the Stahl studies
3. if the eyes have been rinsed or not: agreed
4. and if the substance has been added directly in powder form: no preparation of the sample is described, while it is in the Zaptero study

It is not clear how it can be considered “negligible” the fact that it is not known the composition of the tested substance.

1) The dossier submitter stated that basic information for a correct classification of Acid Black 210 Na is missing. This statement is not entirely correct, since the testing method (EU method B.5) is reported as well as the number of animals used and the detailed individual results.

It is criticised several time within this document that the composition is one of the key factor for reliability in a study; this information is completely missing up to now for the Stahl study, therefore there is reasons to state that basic information is missing.

2) They further criticised that no information is reported whether the eyes of the test animals were washed or not. However, results obtained without washing eyes

are handled and interpreted identically compared to results obtained from animals whose eyes were washed when using the EU method B.5 ("At 24 hours a washout may be used if considered appropriate."). Furthermore, the reported read-across studies also do not report whether the test substance was washed or not. Hence, this objective regarding the basic studies performed on the test substance is also applicable to the two selected read-across studies used for declassification of Acid Black 210-Na.

Washing of the eye in method B.5 is described for solids in paragraph 1.4.2.3.2, where testing for solids is described. In this case the substance must be checked after 1 hour from the application to check for residuals that have not been removed by physiological mechanisms.

3) The dossier submitter also stated that no information is given whether the substance has been added directly in powder form. These issues are admittedly not reported but might also be negligible since the EU Method B.5 (Acute Toxicity: Eye Irritation) protocol gives detailed instructions how to test and apply solid chemicals, including powders. Moreover, also when testing solid substances, washing of eyes is optional ("…the eye may be rinsed with saline or distilled water.").

4) The dossier submitter also annotated that no information on the purity of the test substance and the identities of potential impurities are given, which is correct. But as the dossier submitter states in the CLH report, Acid Black 210-Na is manufactured via defined precursors and thus, it is "expected that the typical commercial batches present very similar characteristics" (page 45).

The description in pg 45 is a general description to support the Read Across, but when coming to the testing evaluation or specific evaluation of read across for any single endpoint, then also any Authority always agrees that the identification of the tested specific substance must be taken into consideration

- *Furthermore, a typical purity/impurity profile for this substance is given on page 45, suggesting a similar purity/impurity profile of the test substance used by Stahl Europe B.V.*

Page 45 is describing a general decription of a process; it does not suggest a specific composition or purity/impurity profile, that can depend on a specific production plant and lot number

- *In addition, the purity of the source substances Acid Black 210-K, which is used in both referred read-across studies is rather low (~ 65%) and the identity of impurities in at least one of the two read-across studies (BASF SE, 1984) is also not reported.*

The "low" purity, in the case the impurities are identified in quality and quantity and recognised as not influencing a specific result, is not a relevant element.
For one study there is no impurity description, but the fact that the result is negative is more relevant. In general an impurity can cause a toxicological effect, but it is really difficult that if an effect is related to the substance, then the impurity will remove it.

- *It is further stated on page 45 that a complete analytical characterisation of Acid Black 210-K is not available and that only ranges of composition can be reported.*

For complete analytical characterisation we refer to a report to be presented compliant with REACH requirements, with all spectra like IR, NMR, UV. But the composition, if reported, has been verified to provide the reported data.

- *In addition, the BCOP-test performed according to OECD TG 437 by Cinelli et al. (2014) using Acid Black 210-Na yielded in a mean in vitro irritation score (IVIS) of approximately 25.5 (page 20 of the CLH dossier). The BCOP test can only be used to identify chemicals inducing serious eye damage as defined by CLP, i.e. chemicals to be classified as CLP Category 1 (IVIS > 55) or chemicals that do not require classification for eye irritation or serious eye damage under the CLP classification system (IVIS ≤ 3). However, the BCOP test method is not recommended for the identification of test chemicals that should be classified as (mildly) irritating to the eyes (CLP Category 2). Based on a 3 < IVIS ≤ 55 obtained in a BCOP test, no accurate/ reliable prediction on the actual eye irritating/damaging potency of the test substance can be made. Based on a 3 < IVIS ≤ 55 the necessity of classification cannot be excluded.*

We agree, it can also reasonably be excluded that the substance will be considered as corrosive.

- *The dossier submitter further stated that the test item used in the BCOP test was coloured and thus the mean opacity value was probably affected by the substance remaining on the corneal surface (page 8 and 23 of the CLH dossier) leading to higher, overestimated cornea opacity values. However, only based on an assumption like this an indisputable CLP classification (no classification necessary) cannot be made, especially considering that an appropriate (coloured) negative control substance should have been used for comparison and evaluation of the obtained results (not reported; study not assignable) and that the colour of the test substance could have also led to underestimated cornea opacity values.*
  It was concluded by the dossier submitter that the results of the BCOP test do not "lead to a clear conclusion about the classification regarding irritating properties [of Acid Black 210-Na], but clearly exclude serious eye damage" (page 8 of the CLH report). This statement, however, is also not correct, as the BCOP test method when used to identify chemicals inducing serious eye damage only has an overall accuracy of 79% and a false negative rate of 14%. Thus, there is still a significant probability of Acid Black 210-Na eliciting serious eye damage, although the obtained IVIS was > 3 and ≤ 55.

This result must be evaluated for incertitude with all the other presented studies.

- *Since the data used in a hazard or risk assessment should be relevant, reliable and sufficient for the regulatory purpose, it is necessary to base the assessment on the totality of available information, i.e. to apply Weight of Evidence (WoE) considerations. Moreover, it is particularly important to adequately justify read-across of negative findings. The results of the two studies performed according to validated test guidelines and using Acid Black 210-Na cannot be excluded from this WoE evaluation, taking into account that the presented tests using a read-across substance elicit similar objectives as the studies performed on the actual test substance. Hence, the conclusion of the dossier submitter that the test substance does not meet the criteria for classification cannot be supported.*
  Moreover, the substance used as source substance in the read across approach (Acid Black 210-K) itself is classified as Eye Dam. 1 or Eye Irrit. 2 by various notifiers in the C&L inventory, indicating that there might be further studies available for Acid Black 210-K which indicate that this source substance is also irritating/damaging to the eyes but which were not considered by the dossier submitters.

There are also 155 notifications as "not classified" in the C&L inventory. On the other side it is not clear when it is said that 5 notifiers indicated that there are studies available not

considered by the lead registrant: "no available data" is always reported in all sections of the C&L dissemination website. A regular SIEF survey has been performed and tests have been requested according to article 30: no study except the considered ones has been presented to the SIEF or to the Lead Registrants. Based on extensive experience C&L notifications unfortunately is a non reliable source of information.

- *Since the CLP guidance document states that if "discrimination between Serious Eye Damage Cat. 1 and Eye Irritant Cat. 2 is not possible, Serious Eye Damage Cat. 1 must be chosen", the current classification of Acid Black 210-Na as Eye Dam. 1 (H318) should be maintained, especially in regard of the irreversibility of the colouration of the third eyelid detected in one animal after 28 days of observation in the in vivo study by Stahl Europe B.V., which, moreover, prevented a proper assessment of conjunctival erythema until day 14 (page 21 of the CLH report) and thus might have led to an underestimation of the observed adverse effects.*

Many elements can help in distinguish between Serious Eye Damage Cat 1 and Eye Irritant Cat 2 in this case, and even if it can be discussed if a precautionary "Eye Irritation Cat 2" can be maintained for this substance it is completely excluded an Eye Damage in all presented studies.

1) In fact , also considering as valid the Stahl in vivo study, the only reported effect not reversible in 28 days is the colouration of the third eyelid in one animal, that was impairing a complete evaluation of the cornea until day 14 (not for 28 days and not seriuously impairing vision). In this respect the definition of eye damage is considering this classification when *"..... the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application"*. , therefore not applicable to this study result. The official parameters for the evaluation of eye irritation/corrosion,(therefore sight impairement) have been reported, i.e. corneal opacity, iritis, conjunctival redness, conjunctiva oedema. In almost all cases this evaluation has been performed and it is valid for non classification. In just one animal for one parameter this measurement can be doubted, but since it is consistent with the others, it is reasonable to suppose that it is reliable like the others.

2) The in vitro BCOP study is clearly excluding eye corrosion based on IVIS < 55

3) The Zapatero study is excluding also eye irritation.

4) The BASF study is excluding also eye irritation.

RAC's response

Thank you for the detailed comments and answers regarding the eye irritation/corrosion endpoint. The position of the German MS was noted. RAC recognizes the difficulties raised by the impurities and the staining capacity of the test substance. Therefore, the ODD takes into account the substance purity, the staining capacity and the usage of the original study of Stahl BV (1996). With respect to the finding on which the classification should be maintained it is to be noted that the evaluation of the nictitating membrane is not included either in the regulatory criteria or in its associated methods of investigation. Also, the relevance to humans is questionable. Humans don't have fully developed third eye lids; the equivalent *plica semilunaris* is only a small fold of conjunctiva representing a vestigial remnant.

Date	Country	Organisation	Type of Organisation	Comment number
16.01.2017	Belgium		MemberState	5
Comment received				
See General comment				

Dossier Submitter's Response
Noted.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
04.01.2017	France		MemberState	6
Comment received				
France does not support the proposal to remove classification of Acid Black 210 Na from the actual entry Aquatic Chronic 3, H412. More details concerning the algae test of Scheerbaum (2011) (page 33) are needed in order to prove that toxicity reported in the test is caused by a shading effect of the dye and not by a real toxicity effect of the substance tested.				
Dossier Submitter's Response				
Thank you for the comment. Furher details are reported following on the algae studies that can be taken into consideration (see response to comment 7).				
RAC's response				
RAC has taken into account the comment and additional information given by the Dossier Submitter in the Opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
16.01.2017	Germany		MemberState	7
Comment received				
The proposal for removal of the classification with Aquatic Chronic 3 is based on: a new test with Lemna showing no effect up to 2000 mg/L, an algae study which is disregarded because of an unknown exact composition of the test material (ErC50= 13.7 mg/L) and an algae study (ErC50 > 10 - < 100 mg/L) which is not used for classification because other tests with dark dyes show that the growth inhibition is not due to a toxic effect but to the light absorption of the stained water. In the CLP report page 32 it is written that the latter study "was performed … according to OECD series on testing and assessment Number 23 … paragraph 3.8, Coloured substances". It is not clear from the description of the study in which way this OECD Guidance was taken into account and if the conclusion that the toxic effects of the substance were only caused by light absorption was deduced from the study or from observation with other substances. This should be explained more clearly to decide that the study could not be used for classification.				
Dossier Submitter's Response				
Thank you for the comment. The test performed on algae of Dirk Scheerbaum, 2011 has been performed in duplicate, on the Sterile erlenmeyer flasks, volume: 250 mL, and with Microplate, 96-well with plane bottom, according to the method proposed in the OECD series on testing and assessment Number 23 … paragraph 3.8, Coloured substances", where it is specified that one of the general strategies for discriminating between effects resulting from toxicity and light absorption is reducing the light path by reducing the depth or the volume. Therefore it				

was proposed to use the microplates with this purpose. Unfortuantely Acid Black shows a comparable green alga toxicity when tested in Erlenmeyer flasks and microplates, respectively, indicating that this method was not adequate for the substance.
An indirect demonstration of the effect of the light on Acid Black 210 is given by an old test summary, recently found in a Stahl archive performed on the notified substance S124668, Acid Black 210 sodium salt. The test has been performed in Zeneca in duplicate, according to a method in which one flask is normal, the other is divided in two sections, the above section contains the dye solution, the below section contains the algae, that are not into contact with the dye, but are shaded from the light by the dye solution.

The result is that in the regular flask the ErC50 72 hours = 6.0 mg/l, in the “special shaded” flask, the Er50 72 hours = 3.9 mg/l. The values are practically identical and the summary reports that the shape of the curves are equal too, demonstrating that the toxicity is generated by the shading in itself and not by the presence of the dye.

Similar tests are available for several black or brown dyes.

The test with the Lemna, taken in a weight of evidence is instead demonstrating that when the dye is into contac with the aquatic plant, but the shading influence has no effect because the Leamna is developing the leaves on the surface, the dye has no toxic effect.

RAC’s response

RAC concludes that the Scheerbaum study can be used in this weight of evidence classification although the contributions from toxicity and shading can not be specified. Please see the opinion for further details. RAC is of the opinion that the Stahl archive study and similar tests are based on ETAD method used in the late 90s which is more recently thought to be too simplistic (ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b Table R.7.8-3).

Date	Country	Organisation	Type of Organisation	Comment number
16.01.2017	Belgium		MemberState	8

Comment received

In our opinion, no reliable acute aquatic data are available for the substance itself. But considering these data and those on the read across substance Acid Black 210 potassium salt, results point to no classification for acute aquatic toxicity as all LC50>1mg/l.
At present, however, no registration dossier is available for the structural analogue and in depth information hereof is missing in the CLH report.

Concerning aquatic chronic toxicity, the dossier submitter disregards the chronic algae study (Scenedesmus subspicatus, 72hNOErC<1.9 mg/l) on the substance itself a.o.t. on the basis of the unknown composition and whether it is unknown if results are reported as nominal or measured concentrations. However the NOEC Daphnia study used in the reasoning for chronic toxicity based on NOEC-values suffers also from such deficiencies .

No chronic toxicity data are available for all 3 trophic level, thus also the surrogate approach shall be considered. As already mentioned, reliability of the acute toxicity data on the substance itself is questionable.
In the registration dossier also a read across with ABl210-K is available for algae with a 72hErC50 between >10 and <100mg/l, although no info is given on the purity and if the result is expressed as nominal or measured concentration.

Furthermore it is mentioned for the read across substance ABl210-K for the other trophic levels that purity was “assumed” 65%.

Dossier Submitter’s Response

Thank you for the comment.

Specific comments are given to the specific paragraph below:

- *In our opinion, no reliable acute aquatic data are available for the substance itself. But considering these data and those on the read across substance Acid Black 210 potassium salt, results point to no classification for acute aquatic toxicity as all LC50>1mg/l.*
  At present, however, no registration dossier is available for the structural analogue and in depth information hereof is missing in the CLH report.

All available studies are reportd in this CLH report, no further information is available for the potassium salt

- *Concerning aquatic chronic toxicity, the dossier submitter disregards the chronic algae study (Scenedesmus subspicatus, 72hNOErC<1.9 mg/l) on the substance itself a.o.t. on the basis of the unknown composition and whether it is unknown if results are reported as nominal or measured concentrations. However the NOEC Daphnia study used in the reasoning for chronic toxicity based on NOEC-values suffers also from such deficiencies .*

In fact the study can also been disregarded since for very dark dyes the method is not suitable, as the Lemna test is demonstrating

- *No chronic toxicity data are available for all 3 trophic level, thus also the surrogate approach shall be considered. As already mentioned, reliability of the acute toxicity data on the substance itself is questionable.*
  In the registration dossier also a read across with ABl210-K is available for algae with a 72hErC50 between >10 and <100mg/l, although no info is given on the purity and if the result is expressed as nominal or measured concentration.

The purity of the substance is known, it is reported also in page 3:

<u>For the Dirk Scheerbaum, 2011 study:</u>

Acid Black 210 (Na salt):	66.4 %
Sodium chloride:	7.4 %
Sodium sulphate:	1.86 %
Water:	16 %

And the results are expressed as measured concentrations

- *Furthermore it is mentioned for the read across substance ABl210-K for the other trophic levels that purity was “assumed” 65%.*

The composition of potassium salt is known as reported above.

RAC’s response

Please see the Opinion for RAC conclusions on these issues. In the CLH Report the Dirk Scheerbaum study is presented to be performed with Acid Black 210, potassium salt.

Date	Country	Organisation	Type of Organisation	Comment number
23.12.2016	United Kingdom		MemberState	9

Comment received

The CLH proposal presents a clear case for read-across (RAX) between the Acid Black 210 Na (target substance) and Acid Black 210 K (source substance). In addition, some relevant information is presented to support the assumption that acute effects seen in the standard OECD 201 algal growth inhibition study are due to light absorption not a toxic response.

However, insufficient details are presented for the key source substance Lemna study (Alexa Caduff, 2012) to consider if it is a valid study for RAX. The CLH report considers the study as Reliability 1 (reliable without restrictions), although the IUCLID and online registration consider it Reliability 2 (reliable with restrictions). The reliability score should be confirmed. In doing so, it should be clarified if:
- test guideline validity criteria were met
- a positive reference control was conducted/valid
- analytical verification of exposure concentrations are available and if not, evidence to support endpoints based on nominal concentrations

To evaluate aquatic chronic classification, chronic study endpoints need to be considered. Based on the IUCLID, it appears effects were observed at all treatments and NOErC values are considered ≤ 30.8 mg/l. Based on the levels of growth rate inhibition, it would appear ErC10 values would be useful for classification purposes.

Dossier Submitter's Response

Thank you for the comment.
It has to be underlined that the study reliability in IUCLID and the disseminated dossier is indicated as 2 because of the rule of presenting Read Across studies with IUCLID (See ECHA guidance "Practical guide: How to use alternatives to animal testing to fulfil your information requirements for REACH registration , version 2, July 2016, section 4.4, pg 39, additional tips: a Klimisch score of 1 (reliable without restrictions) should normally not be used for results derived from read-across.

To confirm the Reliability score the following can be confirmed:
1) Test guideline validity criteria were met: "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."
2) According to the OECD guidance itt is advisable to test a reference substance at least twice a year or, where testing is carried out at a lower frequency, in parallel to the determination of the toxicity of a test substance.
3) The endpoints results are based on nominal concentrations; this is reliable since based on all performed tests with sodium or potassium salt, it is demonstrated that the substance is very stable in water and never discrepancies between nominal and measured concentrations has been revealed

RAC's response

RAC agrees with the proposed read-across. RAC also welcomes the additional information given by the Dossier Submitter. RAC also agrees to the use of chronic values from the algae test.