~~Confidential~~

Helsinki, 17 November 2022

Addressees
Registrants of JS_EC_421-880-6 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
14/06/2021

Registered substance subject to this decision ("the Substance")
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

Decision number: Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***26 May 2026***.

Requested information must be generated using the Substance unless otherwise specified.

Information required from all the Registrants subject to Annex VII of REACH

1. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays, also requested below (triggered by Annex VII, Section 8.4., column 2)

Information required from all the Registrants subject to Annex VIII of REACH

2. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays, also requested below (triggered by Annex VIII, Section 8.4., column 2)

3. Simulation testing on ultimate degradation in surface water also requested below (triggered by Annex VIII, Section 9.2.)

4. Identification of degradation products also requested below (triggered by Annex VIII, Section 9.2.)

5. Bioaccumulation in aquatic species also requested below (triggered by Annex I, Sections 0.6.1. and 4; Annex XIII, Section 2.1.)

Information required from all the Registrants subject to Annex IX of REACH

6. In vivo genetic toxicity study (triggered by Annex IX, Section 8.4., column 2): Transgenic rodent somatic and germ cell gene mutation assay (test method: OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; germ cells and duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive; OR In vivo mammalian alkaline comet assay

(test method: OECD TG 489) in rats, or if justified, in other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

7. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 408) by oral route, in rats

8. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit)

9. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: EU C.47./OECD TG 210)

10. Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.; test method: EU C.25./OECD TG 309) at a temperature of 12°C. Non-extractable residues (NER) must be quantified and a scientific justification of the selected extraction procedures and solvents must be provided.

11. Identification of degradation products (Annex IX, 9.2.3.; test method: EU C.25./OECD TG 309)

12. Bioaccumulation in aquatic species (Annex IX, Section 9.3.2; test method: EU C.13./OECD TG 305)

The reasons for the decision(s) are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressees of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

In the requests above, the same study has been requested under different Annexes. This is because some information requirements may be triggered at lower tonnage band(s). In such cases, only the reasons why the information requirement is triggered are provided for the lower tonnage band(s). For the highest tonnage band, the reasons why the standard information requirement is not met and the specification of the study design are provided. Only one study is to be conducted; all registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the others under Article 53 of REACH.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report, where** relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4. In addition, the studies relating to biodegradation and bioaccumulation are necessary for the PBT assessment. However, to determine the testing

needed to reach the conclusion on the persistency and bioaccumulation of the Substance you should consider the sequence in which these tests are performed and other conditions described in this Appendix.

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the decision
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons for the decision

Contents

Reasons related to the information under Annex VII of REACH

1. In vivo mammalian alkaline comet assay or Transgenic rodent somatic and germ cell gene mutation assays

1 Under Annex VII Section 8.4., column 2 of REACH, further mutagenicity studies must be considered in case of a positive result in an in vitro gene mutation study in bacteria.

2 Your dossier contains positive results (OECD 471; 1996 & 2020) for the in vitro gene mutation study in bacteria which raise the concern for gene mutation that must be investigated.

3 The examination of the information provided, as well as the selection of the requested test and the test design are addressed under Request 6.

Reasons related to the information under Annex VIII of REACH

2. In vivo mammalian alkaline comet assay or Transgenic rodent somatic and germ cell gene mutation assays

4 Under Annex VIII, Section 8.4, column 2 of REACH, the performance of an appropriate in vivo somatic cell genotoxicity study must be considered if there is a positive result in any of the in vitro genotoxicity studies in Annex VII or VIII.

5 Your dossier contains positive results (OECD 471; 1996 & 2020) for the in vitro gene mutation study in bacteria which raise the concern for gene mutation that must be investigated.

6 The examination of the information provided, as well as the selection of the requested test and the test design are addressed under Request 6.

3. Simulation testing on ultimate degradation in surface water

7 Further degradation testing must be considered if the chemical safety assessment (CSA) according to Annex I indicates the need to investigate further the degradation of the substance (Annex VIII, Section 9.2., Column 2).

3.1. Triggering of further degradation testing

8 This information requirement is triggered in case the chemical safety assessment (CSA) indicates the need for further degradation investigation (Annex I, Section 4; Annex XIII, Section 2.1), such as if the substance is a potential PBT/vPvB substance (Guidance on IRs and CSA, Section R.11.4.). This is the case if the Substance itself or any of its constituent or impurity present in concentration ≥ 0.1% (w/w) or relevant transformation/degradation product meets the following criteria:

- it is potentially persistent or very persistent (P/vP) as:
  - it is not readily biodegradable (*i.e.* <60/70% degradation in an OECD 301),
- it is potentially bioaccumulative or very bioaccumulative (B/vB) as:
  - for some groups of substances (e.g., organometals, ionisable substances, surfactants) other partitioning mechanisms may drive bioaccumulation (e.g., binding to protein/cell membranes) and high potential for bioaccumulation cannot be excluded solely based on its potential to partition to lipid;
- it meets the T criteria set in Annex XIII: NOEC or EC10 < 0.01 mg/L or classification as carc. 1A or 1B, muta. 1A or 1B, repro. 1A, 1B or 2, or STOT RE 1 or 2.

3.2. Information provided

9 Your registration dossier provides the following:

- The Substance is not readily biodegradable (QSAR estimation with QSAR dyes RC 2.0 model and based on Expert Judgement);
- The Substance is not inherently biodegradable (38 % degradation after 28 days in OECD TG 302B for the analogue EC 286-384-2);
- The Substance is ionisable substance and therefore high potential for bioaccumulation cannot be excluded based on available information;

10 Furthermore, the information in your dossier is currently incompliant and therefore:

- for the reasons explained in request 12 of this decision, it is not possible to conclude

on the bioaccumulation potential of the Substance, and

- for the reasons explained in requests 1, 2 and 6 to 9 of this decision, it is not possible to conclude on the toxicity of the Substance;

In addition, under section 2.3 of your IUCLID dossier and section 8 of your CSR ('PBT assessment'), you conclude that the Substance is P but not B. In support of your conclusion, you provide the following justification: "*The substance is not biodegradable and not hydrolysable, but it is very soluble with Kow of -3.1, therefore no bioaccumulation is foreseen. Furthermore a quick photolitic degradation both in water and in the air has to be considered*".

11 However, Log Kow is not a valid descriptor of the bioaccumulation potential because the substance is ionised under environmentally relevant pH.

12 Based on the above, the available information on the Substance indicates that it is a potential PBT/vPvB substance. Further, the additional information from your PBT assessment is not adequate to conclude on the PBT/vPvB properties of the Substance.

13 Therefore, the chemical safety assessment (CSA) indicates the need for further degradation investigation.

3.3. Information provided on further degradation

14 The examination of the available information or adaptations, your comments to the draft decision, as well as the selection of the requested test and the test design are addressed respectively in Request 10.

4. Identification of degradation products

15 Further degradation testing must be considered if the chemical safety assessment (CSA) according to Annex I indicates the need to investigate further the degradation of the substance (Annex VIII, Section 9.2., Column 2).

4.1. Triggering of identification of degradation products

16 This information requirement is triggered in case the chemical safety assessment (CSA) indicates the need for further degradation investigation (Annex I, Section 4; Annex XIII, Section 2.1), such as if the substance is a potential PBT/vPvB substance (Guidance on IRs and CSA, Section R.11.4.).

17 As already explained in Request 3, the Substance is a potential PBT/vPvB substance.

18 Therefore, the chemical safety assessment (CSA) indicates the need for further degradation investigation.

4.2. Information provided on identification of degradation products

19 The examination of the available information or adaptations, your comments to the draft decision, as well as further information on the selection of the approach to generate this information are addressed in Request 11.

5. Bioaccumulation in aquatic species

20 Bioaccumulation in aquatic species is required for the purpose of PBT/vPvB assessment (Annex I, Sections 0.6.1 and 4 to REACH).

5.1. Triggering of Bioaccumulation in aquatic species

21 This information requirement is triggered in case the chemical safety assessment (CSA) indicates the need for further investigation on bioaccumulation in aquatic species (Annex I, Section 4; Annex XIII, Section 2.1), such as if the substance is a potential PBT/vPvB substance (Guidance on IRs and CSA, Section R.11.4.).

22 As already explained in Request 3, the Substance is a potential PBT/vPvB substance.

23 Therefore, the chemical safety assessment (CSA) indicates the need for further investigation on bioaccumulation in aquatic species.

5.2. Information provided on Bioaccumulation in aquatic species

24 The examination of the available information or adaptations, your comments to the draft decision as well as the selection of the requested test and the test design are addressed in Request 12.

Reasons related to the information under Annex IX of REACH

6. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays

25 An appropriate in vivo somatic cell genotoxicity is an information requirement under Annex IX to REACH (Section 8.4., Column 2) if (1) there is a positive result in any of the in vitro genotoxicity study under Annex VII or VIII to REACH and (2) there are no results available from an in vivo study.

6.1. Triggering of in vivo mutagenicity studies

26 As already explained under Requests 1 and 2 your dossier contains positive results for the in vitro gene mutation study in bacteria which raise the concern for gene mutation that must be investigated.

6.2. Information provided

27 Your dossier contains a negative in vivo micronucleus study (OECD 474; 1996) with the Substance.

28 You have also adapted this information requirement by using a Grouping of substances and read-across approach; you propose read-across from an analogue substance, acid black 234, (EC 605-104-5, CAS 157577-99-6) ('source substance').

6.3. Assessment of the information provided

6.3.1. The provided in vivo study on the Substance is not appropriate

29 According to Guidance on IRs & CSA, Chapter R.7a, Section R.7.7.6.3., in order to be appropriate, the *in vivo* somatic cell genotoxicity study must address the specific concern raised by the *in vitro* positive result.

30 However, the *in vivo* micronucleus study (OECD 474) provided is not addressing the gene mutation concern raised by the *in vitro* data. Therefore, the provided *in vivo* test is not appropriate to investigate the gene mutation concern identified *in vitro*.

31 ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern(s) identified *in vitro*.

32 On this basis, the information requirement is not fulfilled

6.3.2. Your read-across adaptation is rejected

33 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

34 Additional information on what is necessary when justifying a read-across approach can be found in the Guidance on IRs and CSA, Chapter R.6. and related documents (RAAF, 2017; RAAF UVCB, 2017).

35 You provide the following reasoning for the prediction of this information requirement: "*Acid Black 234, EC 605-104-5 is in fact, considered as representative of the mutagenic behaviour of Acid Black 210 in a worst-case scenario approach, as the Ames test in Acid Black 234*

can be considered as most conservative substance in relation to Acid Black 210, because the positivity is seen in three strain (TA 98, TA100 and TA 1537)". You state that "Moreover one metabolite of ABK234 is Aniline. Aniline is classified as a category 3 mutagen". You conclude that "however the Ames test on this substance arises no concern, therefore the positivity that can be found in Acid Black 234 Ames test seems not to be involved with the release of Aniline in this case. Therefore, the substance, Acid Black 210 is considered not mutagenic for the time being. The classification will be confirmed with the results of the comet assay".

36 ECHA understands that you predict the properties of the Substance using a read-across adaptation which assumes that different compounds have the same type of effects. The properties of your Substance are predicted based on a worst-case approach.

37 We have identified the following issue(s) with the prediction of toxicological properties:

6.3.2.1. Missing supporting information to substantiate worst-case consideration

38 Annex XI, Section 1.5 of the REACH Regulation states that "physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)". For this purpose, "it is important to provide supporting information to strengthen the rationale for the read-across" (Guidance on IRs and CSA R.6, Section R.6.2.2.1.f.). The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

39 Supporting information must include bridging studies to compare properties of the Substance and source substance to confirm your claimed worst-case prediction.

40 As indicated above, your read-across hypothesis is based on the assumption that the source substance constitutes a worst-case for the prediction of the property under consideration of the Substance. In this context, relevant, reliable, and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm a conservative prediction of the properties of the Substance from the data on the source substance(s). Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and for the source substance(s).

41 For the source substance, your read-across justification or the registration dossier does not include any robust study summaries or descriptions of data for the source substance or bridging studies that would confirm a conservative prediction of the properties of the Substance.

42 In the absence of such information, you have not established that the source substance constitutes a worst-case for the prediction of the property under consideration of the Substance. Therefore, you have not provided sufficient supporting information to strengthen the rationale for the read-across.

6.3.2.2. Missing supporting information on the impact of non-common compounds

43 As already explained above, the set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

44 Supporting information must include information to compare properties of the metabolites of the Substance and of the source substance to confirm your claimed worst-case prediction on the impact of metabolites on the prediction.

45 As indicated above, your read-across hypothesis is based on the (bio)transformation of the Substance and of the source substance(s) to a common compound(s). In this context, exposure to the Substance and of the source substance(s) may also lead to exposure to

other compounds than the common compound of interest. The impact of exposure to these non-common compounds on the prediction of properties of the target needs to be assessed to ensure that a reliable prediction can be made.

46 You have not provided information characterising the exposure to the non-common compounds from exposure to the Substance and to the source substances resulting (e.g., p-nitroaniline and aniline respectively). No experimental data or other adequate and reliable information addressing the impact of exposure to these non-common compounds is included in the documentation of your read-across approach.

47 In the absence of such information, you have not established that a reliable prediction of the property under consideration of the Substance can be derived on the basis of your read-across hypothesis. Therefore, you have not provided sufficient supporting information to strengthen the rationale for the read-across.

6.3.2.3. Conclusion on the read-across approach

48 For the reasons above, you have not established that relevant properties (in vivo genetic toxicity) of the Substance can be predicted from data on the source substance(s). Your read-across approach under Annex XI, Section 1.5. is rejected.

49 In the comments to the draft decision, you agree to perform the requested study.

6.4. Test selection

50 According to the Guidance on IRs & CSA R.7a, Section R.7.7.6.3, the transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) and the in vivo mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive in vitro result on gene mutation.

6.5. Specification of the study design

6.5.1. TGR assay

51 In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats and the test substance is usually administered orally.

52 Based on OECD TG 488, you are requested to follow the 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals.

53 According to the test method OECD TG 488, the test must be performed by analysing tissues from liver as slowly proliferating tissue and primary site of xenobiotic metabolism, glandular stomach and duodenum as rapidly proliferating tissue and site of direct contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

6.5.2. Comet assay

54 In case you decide to perform the comet assay according to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified (OECD TG 489, para. 23).

55 Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

56 In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

6.5.3. Germ cells

57 A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483, depending on the concern raised by the substance) may still be required under Annex IX/X of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

6.5.3.1. TGR assay

58 Therefore, in case you decide to perform the TGR, you must collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, in order to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA, to decide on the need for assessment of mutation frequency in the collected germ cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

6.5.3.2. Comet assay

59 In case you decide to perform the comet assay, you may consider collecting the male gonadal cells collected from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

7. Sub-chronic toxicity study (90-day)

60 A sub-chronic toxicity study (90 day) is an information requirement under Annex IX to REACH (Section 8.6.2.).

7.1. Information provided

(i) An OECD 407 study (1996) with the Substance.

(ii) An OECD 422 study (2011) with the Substance.

7.2. Assessment of the information provided

61 (Eco)toxicological studies must comply with a recognised test method (Art. 13(3) of REACH), in this case the OECD TG 408. Such study must cover the key parameters of the corresponding OECD test guideline (Art. 13(3) of REACH).

62 The studies (i) and (ii) are described as a sub acute (28-day) and a combined repeated dose toxicity screening study.

63 These studies are not conducted using the recognised method OECD TG 408 for a sub-chronic toxicity study (90-day). In addition, the studies do not cover the key parameters of the OECD TG 408 such as an exposure duration of at least 90 days; clinical and functional observations at week 11 or after; body weight and food/water consumption measurements; haematology and clinical biochemistry; as well as gross necropsy and histopathology of the organs listed in the OECD TG 408 at the end of the study.

64 Based on the above, the information you provided do not fulfil the information requirement.

65 In the comments to the draft decision, you agree to perform the requested study.

66 On this basis, the information requirement is not fulfilled.

7.3. Specification of the study design

67 Following the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity of the Substance; Guidance on IRs and CSA, Section R.7.5.6.3.2.

68 According to the OECD TG 408, the rat is the preferred species.

69 Therefore, the study must be performed in rats according to the OECD TG 408, in rats and with oral administration of the Substance.

8. Pre-natal developmental toxicity study in one species

70 A pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is an information requirement under Annex IX to REACH (Section 8.7.2.).

8.1. Information provided

71 You have adapted this information requirement in the IUCLID dossier by providing the justification: "the study does not need to be conducted because relevant human exposure can be excluded in the relevant exposure assessment".

72 ECHA understands that you intended to adapt this information requirement on the basis of Annex XI, Section 3.

73 You have also provided the following study:

(i) OECD 422 study with the Substance (2011)

8.2. Assessment of the information provided

8.2.1. Exposure based waiving not in line with the conditions specified in Annex XI, Section 3

74 As stated in Annex XI, Section 3, testing in accordance with Sections 8.6 and 8.7 of Annex VIII and in accordance with Annexes IX and X may be omitted based on the exposure scenario(s) developed in the CSR, by providing an adequate and scientifically-supported justification based on a thorough and rigorous exposure assessment in accordance with

Section 5 of Annex I and by communicating the specific conditions of use through the supply chain. Any one of the following criteria 3.2.(a), (b) or (c) shall be met.

- Under 3.2 (a), the manufacturer or importer demonstrates and documents that all of the following conditions are fulfilled,

  i. the results of the exposure assessment covering all relevant exposures throughout the life cycle of the substance demonstrate the absence of or no significant exposure in all scenarios of the manufacture and all identified uses as referred to in Annex VI section 3.5.;

  However, you did not provide adequate and reliable documentation demonstrating the "absence of or no significant exposure in all scenarios of the manufacture and all identified uses". The registered substance is used as a dye in leather and textile applications. Several exposure scenarios for formulation, industrial and professional uses ([redacted]) demonstrate potential exposure for workers in your provided chemical safety report (CSR).

  ii. a suitable DNEL or a PNEC can be derived from results of available test data for the Substance taking full account of the increased uncertainty resulting from the omission of the information requirement, and that DNEL or PNEC is relevant and appropriate both to the information requirement to be omitted and for risk assessment purposes;

  However, the worker long-term systemic DNEL, which you derived in your CSR, is based on Repeated Dose 28-day Oral Toxicity Study in Rodents (OECD 407). ECHA underlines that such DNEL is not relevant nor appropriate both for the information requirement to be omitted and for risk assessment purposes. A Repeated Dose 28-day Oral Toxicity Study does not investigate effects on mating, fertility, pregnancy, the foetuses' sex and body weight/external, skeletal and soft tissue alterations (variations and malformations)/number of resorptions and or live foetuses/ measurement of anogenital distance in live rodent foetuses. For these reasons, a DNEL derived from a Repeated Dose 28-day Oral Toxicity Study is not appropriate to omit a PNDT study. A screening study is neither appropriate to omit a PNDT study for the purpose of subparagraph 3.2(a)(ii) as stated in its note (1)[2].

  iii. the comparison of the derived DNEL or PNEC with the results of the exposure assessment shows that exposures are always well below the derived DNEL or PNEC.

  Since the DNEL is considered not appropriate, it follows that the third criterion 3.2(a)(iii), exposure results are to be well below the derived DNEL, cannot be fulfilled.

75 Moreover, for substances satisfying the PBT and vPvB criteria of Annex XIII, PNEC and PECs cannot be derived with sufficient reliability to demonstrate that the ratio between PECs and the PNEC are always well below 1 (conditions (ii) and (iii) above). As explained under

[2] For the purpose of subparagraph 3.2(a)(ii), without prejudice to column 2 of Section 8.7 of Annexes IX and X, a DNEL derived from a screening test for reproductive/developmental toxicity shall not be considered appropriate to omit a prenatal developmental toxicity study or a two-generation reproductive toxicity study. For the purpose of subparagraph 3.2(a)(ii), without prejudice to column 2 of section 8.6 of Annexes IX and X, a DNEL derived from a 28-day repeated dose toxicity study shall not be considered appropriate to omit a 90-day repeated dose toxicity study.

Request 3, the information from your dossier currently does not allow excluding that the Substance may be PBT/vPvB. Therefore, 3.2 (a), is not applicable to potential PBT/vPvB substances.

- Under 3.2 (b), where the substance is not incorporated in an article the manufacturer or the importer demonstrates and documents for all relevant scenarios that throughout the life cycle strictly controlled conditions as set out in Art 18(4)(a) to (f) apply;

76 As mentioned above, in several exposure scenarios, the conditions of use and estimated exposure levels for the combined routes do not demonstrate strictly controlled conditions (SCC) as per Annex XI, section 3.2(b) and therefore criterion 3.2(b) for exposure-based adaptation is not satisfied. In particular, condition (a) as set out in Article 18(4) does not appear to be fulfilled because it has not been demonstrated that the substance is rigorously contained by technical means during its whole lifecycle.

- Under 3.2 (c), where the substance is incorporated in an article in which it is permanently embedded in a matrix or otherwise rigorously contained by technical means, it is demonstrated and documented that all of the following conditions i) to (iii) are fulfilled, where the first condition is,

  i. the substance is not released during its life cycle.
  ii. the likelihood that workers or the general public or the environment are exposed to the substance under normal or reasonably foreseeable conditions of use is negligible; and
  iii. the substance is handled according to the conditions set out in Article 18(4)(a) to (f) during all manufacturing and production stages including the waste management of the substance during these stages.

77 The substance is incorporated in a textile and a leather articles (AC 5 and 6) as a dye. However, no releases during its life cycle, nor strictly controlled conditions as set out in Article 18(4)(a) to (f) are demonstrated in the provided CSR.

78 Therefore, information you provided in the dossier does not meet the general rules for adaptation of Annex XI, Section 3, as none of the criteria of that adaptation are currently fulfilled. Therefore, the adaptation you provided is not in line with the conditions specified in Annex XI, Section 3).

79 Therefore, your adaptation is rejected, and the information requirement is not fulfilled

8.2.2. Study not adequate for the information requirement

80 (Eco)toxicological studies must comply with a recognised test method (Art. 13(3) of REACH), in this case OECD TG 414. Such study must cover the key parameters of the corresponding OECD test guideline (Art. 13(3) of REACH). Therefore, the following specifications must be met:

a) 20 female animals with implantation sites for each test and control group;
b) examination of the foetuses for external, skeletal and soft tissue alterations (variations and malformations) and measurement of anogenital distance in live rodent foetuses.

81 The study (ii) is described as "reproduction/ developmental toxicity screening test". This study has not been conducted in accordance with OECD TG 414 but with OECD TG 422 which is a screening tests rather than a conclusive developmental toxicity study. In any case, that study does not cover the key parameters of the OECD TG 414 such as:

a) a statistical power equivalent to the OECD TG 414, as the study provided was conducted on only 12 animals in each group;

b) skeletal and soft tissue alterations (variations and malformations) or measurement of anogenital distance in live rodent foetuses.

82 The study is not adequate for the information requirement.

83 In the comments to the draft decision, you agree to perform the requested study.

84 On this basis, the information requirement is not fulfilled.

8.3. Specification of the study design

85 A PNDT study according to the test method OECD TG 414 should be performed in rat or rabbit as preferred species.

86 The study shall be performed with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

87 Therefore, the study must be conducted in rats or rabbits with oral administration of the Substance.

9. Long-term toxicity testing on fish

88 Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

9.1. Information provided

89 In the registration dossier you have provided a justification to omit the study which you consider to be based on Annex IX, Section 9.1., Column 2. In support of your adaptation, you provided the following justification: "the chemical safety assessment, according to Annex I, does not indicate the need to investigate further the effects on aquatic organisms".

90 In the comments to the draft decision you provided a justification to adapt this information requirement by using substance-tailored exposure-driven testing under Annex XI, Section 3.2 (a).

9.2. Assessment of the information provided in the registration dossier

9.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

91 Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to fish under Column 1. It must be understood as a trigger for providing further information on long-term toxicity to fish if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

92 Your adaptation is therefore rejected.

9.3. Assessment of the information provided in the comments to the draft decision

9.3.1. The exposure based adaptation under Annex XI, Section 3.2 (a) is not valid

93 As already explained under Request 10, Annex XI, Section 3.2 (a) is not applicable to potential PBT/vPvB substances

94 Therefore, your adaptation is rejected.

95 On this basis, the information requirement is not fulfilled.

9.4. Study design and test specifications

96 To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (Guidance on IRs and CSA, Section R.7.8.2.).

10. Simulation testing on ultimate degradation in surface water

97 Simulation testing on ultimate degradation in surface water is an information requirement under Annex IX to REACH (Section 9.2.1.2.).

10.1. Information provided

98 An adaptation under Annex XI, Section 3 ('Substance-tailored exposure-driven testing'). In support of your adaptation, you provide the following statement: "Based on REACH regulation, annex XI.3 Testing in accordance with Annex IX and Annex X may be omitted, based on the exposure scenario(s) developed in the Chemical Safety Report. Based on the description of uses and the attached exposure scenario, water is not exposed to the substance".

10.2. Assessment of information provided

99 We have assessed this information and identified the following issue:

10.2.1. Annex XI, Section 3 (a) is not applicable to potential PBT/vPvB substances

100 Under Annex XI, Section 3(a), this information may be omitted based on the exposure scenario(s) developed in the Chemical Safety Report. The justification must be based on a rigorous exposure assessment in accordance with Annex I, Section 5 and must meet any one of the following criteria:

i. the absence or no significant exposure in all scenarios of the manufacture and all identified uses referred to in Annex VI, Section 3.5.,
ii. a predicted no effect concentration (PNEC) can be derived from available data, which:
   - must be relevant and appropriate both to the information requirement to be omitted and for risk assessment purposes and therefore must be based on reliable information on the hazardous properties of the substance on at least three trophic levels;
   - must take into account the increased uncertainty resulting from the omission of the information requirement, in this case by selecting an appropriate assessment factor (AF) as described in ECHA Guidance R.10.3, and
iii. the ratio between the results of the exposure assessment (PECs) and the PNEC are always well below 1.

101 For substances satisfying the PBT and vPvB criteria of Annex XIII, long-term effects and the estimation of the long-term exposure cannot be carried out with sufficient reliability (Annex I, Section 4.0.1). As a result, for such substances PNEC and PECs cannot be derived with sufficient reliability to demonstrate that the ratio between PECs and the PNEC are always well below 1 (conditions (ii) and (iii) above). Consequently, such information cannot be used to demonstrate that no significant exposure occurs in all scenarios of the manufacture and all identified uses referred to in Annex VI, Section 3.5 (condition (i) above).

102 As already explained under Request 3, the information from your dossier currently does not allow excluding that the Substance may be PBT/vPvB.

103 Therefore, you have not demonstrated that the ratio between the reported PECs and the currently available PNEC provide a reliable means to demonstrate the absence of significant

exposure of the environment. As a result, the conditions set out under Annex XI, Section 3.2 (a) are not met.

104 On this basis, the information requirement is not fulfilled, and your adaption is rejected.

10.2.2. The adaptations from Annex XI, Section 3.2 (b) or (c) are not justified

105 The required information may be omitted if the substance meets the conditions as specified in Annex XI, Section 3.2 (b) or (c) of Annex XI.

106 As explained under Request 8 the information you provided in the dossier does not meet the general rules for adaptation of Annex XI, Section 3.2 (b) and (c), as none of the criteria of that adaptation are currently fulfilled. The adaptation you provided is not in line with the conditions specified in Annex XI, Section 3.

107 Your adaptation is therefore rejected.

108 On this basis, the information requirement is not fulfilled.

109 In the comments to the draft decision, you state that "*the adaptation can be accepted if the PBT assessment will result in a lack of concern, according to the criteria of Annex XIII*" and that "*the substance is persistent, but not toxic and not bioaccumulative, therefore not considered either PBT or vPvB*". As explained under Request 12, it is not possible to conclude on the bioaccumulation potential of the Substance in aquatic species. Therefore, the information in your comments does not allow excluding that the Substance may be PBT/vPvB.

10.3. Study design and test specifications

110 Simulation degradation studies must include two types of investigations (Guidance on IRs and CSA, Section R.7.9.4.1.):

1) a degradation pathway study where transformation/degradation products are quantified and, if relevant, are identified, and
2) a kinetic study where the degradation rate constants (and degradation half-lives) of the parent substance and of relevant transformation/degradation products are experimentally determined.

111 You must perform the test, by following the pelagic test option with natural surface water containing approximately 15 mg dw/L of suspended solids (acceptable concentration between 10 and 20 mg dw/L) (Guidance on IRs and CSA, Section R.11.4.1.1.3.).

112 The required test temperature is 12°C, which corresponds to the average environmental temperature for the EU (Guidance on IRs and CSA, Table R.16-8) and is in line with the applicable test conditions of the OECD TG 309.

113 As specified in Guidance on IRs and CSA, Section R.7.9.4.1., the organic carbon (OC) concentration in surface water simulation tests is typically 2 to 3 orders of magnitude higher than the test material concentration and the formation of non-extractable residues (NERs) may be significant in surface water tests. Therefore, non-extractable residues (NER) must be quantified. The reporting of results must include a scientific justification of the used extraction procedures and solvents. By default, total NER is regarded as non-degraded Substance. However, if reasonably justified and analytically demonstrated a certain part of NER may be differentiated and quantified as irreversibly bound or as degraded to biogenic NER, such fractions could be regarded as removed when calculating the degradation half-life(s) (Guidance on IRs and CSA, Section R.11.4.1.1.3.). Further recommendations may be found in the background note on options to address non-extractable residues in regulatory persistence assessment available on the ECHA website.

114 Relevant transformation/degradation products are at least those detected at ≥ 10% of the applied dose at any sampling time or those that are continuously increasing during the study even if their concentrations do not exceed 10% of the applied dose, as this may indicate persistence (OECD TG 309; Guidance on IRs and CSA, Section R.11.4.1.).

11. Identification of degradation products

115 Identification of degradation products is an information requirement under Annex IX to REACH (Section 9.2.3.).

11.1. Information provided

116 You have provided no information on the identity of transformation/degradation products for the Substance in the registration dossier.

117 In the comments to the draft decision you have provided QSAR predictions of the identity of the potential degradation products with BIOWIN 4.10.

11.2. Assessment of information provided

118 To fulfil the information requirement, information on the identity of relevant transformation/degradation products must be provided (Annex XIII, fifth paragraph; Guidance on IRs and CSA, Section R.11.4.1.).

119 In the QSAR predictions provided in your comments, 6 potential transformation/degradation products were identified. However, you have not provided information on the stability, behaviour, and molar quantity of the degradation/transformation products relative to the Substance. Furthermore, in your comments to the draft decision, you state that "the adaptation can be accepted if the PBT assessment will result in a lack of concern, according to the criteria of Annex XIII" and that "the substance is persistent, but not toxic and not bioaccumulative, therefore not considered either PBT or vPvB". As explained under Request 12, it is not possible to conclude on the bioaccumulation potential of the Substance in aquatic species. Therefore, the information in your comments does not allow excluding that the Substance may be PBT/vPvB.

120 This information is required for the purpose of the PBT/vPvB assessment (Annex I, Section 4) and the risk assessment (Annex I, Section 6) of the Substance.

121 On this basis, the information requirement is not fulfilled.

11.3. Study design and test specifications

122 Identity, stability, behaviour, and molar quantity of the degradation/transformation products relative to the Substance must be evaluated and reported, when analytically possible. In addition, degradation half-life, log K_{ow} and potential toxicity of the transformation/degradation may need to be investigated. You must obtain this information from the degradation study requested in Request 10.

123 To determine the degradation rate of the Substance, the requested study according to OECD TG 309 (Request 10) must be conducted at 12°C and at a test concentration < 100 µg/L. However, to overcome potential analytical limitations with the identification and quantification of major transformation/degradation products, you may consider running a parallel test at higher temperature (but within the frame provided by the test guideline, e.g., 20°C) and at higher application rate (i.e., > 100 µg/L).

12. Bioaccumulation in aquatic species

124 Bioaccumulation in aquatic species is an information requirement under Annex IX to REACH (Section 9.3.2.).

12.1. Information provided

125 In the registration dossier, you have provided an adaptation under Annex IX, Section 9.3.2., Column 2 with the following justification: "*the study does not need to be conducted because the substance has a low potential for bioaccumulation based on log Kow <=3*".

126 In the comments to the draft decision, you provide a justification to adapt this information requirement by applying weight of evidence (WoE) adaptation in accordance with Annex XI, section 1.2:

i. LogBCF values on analogue substances derived from studies performed under the Japanese Regularoty framework;
ii. a scientific publication entitled "Use of partition coefficient as an indicator of bioaccumulation tendency of dyestuffs in fish" by [redacted] (1981);
iii. a survey report entitled "[redacted]" by Danish Environmental Protection Agency (1998);
iv. the test guideline for "[redacted] (2021);
v. QSAR predictions of BCF with BCF model (Meylan) 1.0.3 for the Substance;
vi. QSAR predictions of BCF with BCFBAF v.3.01 model of the potential main degradation products for the Substance;
vii. a presentation entitled "[redacted] (2016);
viii. LogD predictions with [redacted] Chemicalize model for the Substance;
ix. Information on the topological general characteristics of the Substance.

12.2. Assessment of information provided in the registration dossier

12.2.1. The log Kow is not a valid descriptor of the bioaccumulation potential of the Substance

127 Under Section 9.3.2., Column 2, first indent of Annex IX to REACH, the study may be omitted if the substance has a low potential for bioaccumulation and/or a low potential to cross biological membranes. A low log Kow (i.e., log Kow < 3) may only be used to support low potential for bioaccumulation if the partitioning to lipids is the sole mechanism driving the bioaccumulation potential of a substance. For some groups of substances (e.g., organometals, ionisable substances, surfactants) other partitioning mechanisms may drive bioaccumulation (e.g., binding to protein/cell membranes). For this reason, log Kow is not considered a valid descriptor of the bioaccumulation potential for such substances (Guidance on IRs and CSA, Appendix R.7.10-3).

128 Your registration dossier provides an adaptation stating that the log Kow is ≤ 3 without further explanation. The Substance is ionisable based on is structure and as indicated in section 4.21 of your registration dossier.

129 Therefore, log Kow is not a valid descriptor of the bioaccumulation potential of the Substance.

130 On this basis, the information requirement is not fulfilled.

12.3. Assessment of the information provided in the comments to the draft decision

12.3.1. Assessment of the weight of evidence approach

131 Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information enabling, through a reasoned justification, a conclusion on the information requirement, while the information from each single source alone is insufficient to fulfil the information requirement.

132 The justification must have regard to the information that would otherwise be obtained from the study that must normally be performed for this information requirement.

133 According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude on the corresponding information requirement.

134 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex IX, Section 9.3.2 includes similar information that is produced by the OECD TG 305. OECD TG 305 requires the study to investigate the following key elements:

1. the uptake rate constant (k1) and loss rate constants including the depuration rate constant (k2), and/or
2. the steady-state bioconcentration factor (BCFSS), and/or
3. the kinetic bioconcentration factor (BCFK), and/or
4. the biomagnification factor (BMF).

135 The source of information (iv) and (vii) do not provide relevant information on any of the key elements listed above. Source of information (iv) is consisting of the testing guideline text used for performing a test. As such, no reporting (e.g. methodology, conditions, results) on an actual test is provided in this source of information. The source of information (vii) is consisting of a general presentation on the use of read-across for the assessment of biodegradation and bioaccumulation potential of chemicals and does not provide any specific information on the Substance.

136 The sources of information (viii) and (ix) do not provide similar information that is produced by the OECD TG 305 and therefore they are considered as not relevant information within the context of the Weight of Evidence approach. However, these sources of information include relevant indicators for assessing low potential for bioaccumulation and low potential to cross biological membranes within the context of Annex IX, Section 9.3.2., column 2. Therefore, ECHA considers this information as relevant under Annex IX, Section 9.3.2., column 2 and as this information is assessed below.

137 The sources of information (i), (ii), (iii), (v) and (vi) provide relevant information on the key parameters 1 to 3 as listed above. However, the reliability of these sources of information is significantly affected by the following deficiencies:

12.3.1.1. Read-across adaptation rejected for the sources of information (i), (ii) and (iii)

138 ECHA understands that the sources of information (i), (ii) and (iii) included in your weight of evidence approach rely on grouping and read-across approach under Annex XI, Section 1.5. As you rely on a trend analysis to predict the properties of the Substance, ECHA understands that the selected substances follow a regular pattern as result of structural similarity and that you consider those as a group or 'category' of substances.

139 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

140 Additional information on what is necessary when justifying a read-across approach can be found in the Guidance on IRs and CSA, Chapter R.6. and related documents (RAAF, 2017; RAAF UVCB, 2017).

141 You do not provide a read-across justification document in your comments.

142 You define the the structural basis for the grouping as "azo-dyes" and "ionic dyestuffs". ECHA understands that this is the applicability domain of the grouping and will assess your predictions on this basis.

143 You predict the properties of the Substance from information obtained from the source substances listed in the respective information source (ii) and (iii). ECHA further noted that the source substances in the information source (i) are not reported.

144 You provide the following reasoning for the prediction of bioaccumulation in aquatic species: "*the applicability of logKow as a predictor of bioaccumulation* […] *in the case of ionic dyestuffs*" is justified.

145 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance based on an identified trend within the group.

146 We have identified the following issue(s) with the proposed scope of the grouping:.

12.3.1.1.1. Incomplete description of the applicability domain of the category

147 A category (grouping) hypothesis should address "the set of inclusion and/or exclusion rules that identify the ranges of values within which reliable estimations can be made for category members for the given endpoint" (Guidance on IRs and CSA, Section R.6.2.4.1.). Particularly, "the applicability domain of a (sub)category would identify the structural requirements and ranges of physico-chemical, environmental fate, toxicological or ecotoxicological properties within which reliable estimations can be made for the (sub)category members" (Guidance on IRs and CSA, Section R.6.2.1.2.). Therefore, to reliably predict properties within a category the applicability domain should be described including the borders of the category, for which chemicals the category does not hold and a justification for the inclusion and/or exclusion rules.

148 You describe the applicability domain of the substances covered by the grouping as: "azo-dyes" and "ionic dyestuffs".

149 This applicability domain does not introduce unambiguous inclusion/exclusion criteria which would identify the structural requirements and ranges of physico-chemical, environmental fate, toxicological or ecotoxicological properties within which reliable estimations can be made for the (sub)category members.

12.3.1.1.2. Absence of read-across documentation

150 Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must include a an explanation why the properties of the Substance may be predicted from information on the source substance(s).

151 You have not provided a read across justification and robust study summaries for the studies conducted with the other substances than the Substance in order to comply with the REACH information requirements.

152 In the absence of such documentation, the properties of the Substance cannot be reliably predicted from the data on the source substances.

12.3.1.1.3. Conclusion on the read-across approach

153 As explained above, the sources of information (i), (ii) and (iii) cannot be considered as reliable sources of information that could contribute to the conclusion on the key parameter investigated by the required study.

12.3.1.2. The provided (Q)SAR adaptation is rejected for sources of information (v) and (vi).

154 Under Annex XI, Section 1.3., the following conditions must be fulfilled whenever a (Q)SAR approach is used:

i. the prediction needs to be derived from a scientifically valid model,
ii. the substance must fall within the applicability domain of the model,
iii. results need to be adequate for the purpose of risk assessment or classification and labelling, and
iv. adequate and reliable documentation of the method must be provided.

155 With regard to these conditions, we have identified the following issues which are common to both sources of information (v) and (vi):

12.3.1.2.1. Selection of the representative structure.

156 Under ECHA Guidance R.6.1.7.3. a prediction is adequate for the purpose of classification and labelling and/or risk assessment if the following condition is met:

- representative structures for the assessment are selected.

157 Your registration dossier provides the following information:

- In Section 1.1 of your technical dossier, you define the Substance as mono-constituent substance.
- In Section 1.2, you indicate the following impurities in the composition of your Substance:
  i. disodium (E)-4-amino-5-hydroxy-3-((4-nitrophenyl)diazenyl)naphthalene-2,7-disulfonate
  ii. disodium (E)-4,5-dihydroxy-3-((4-nitrophenyl)diazenyl)naphthalene-2,7-disulfonate
  iii. disodium 4,5-dihydroxy-3-((E)-(4-hydroxyphenyl)diazenyl)-6-((E)-(4-nitrophenyl)diazenyl)naphthalene-2,7-disulfonate
  iv. (Z)-4-amino-N-(4-((2,4-diaminophenyl)diazenyl)phenyl)benzenesulfonamide
  v. oligomeric reaction products
  vi. isomer of disodium 4-Amino-6-{4-[4-(2,4-diamino-phenylazo)-phenylsulfamoyl]- phenylazo}-5-hydroxy-3-(4-nitro-phenylazo)-naphthalene-2,7-disulfonate
  vii. disodium 4-amino-6-((E)-(4-(N-(4-((E)-(2-amino-4-(4-nitrophenylamino)phenyl)diazenyl)phenyl)sulfamoyl)phenyl)diazenyl)-5-hydroxy-3-((E)-(4-nitrophenyl)diazenyl)naphthalene-2,7-disulfonate
- For the assessment, you provided predictions for the following constituent:

viii. 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.

158 You have considered the constituent (viii) as representative structure for the whole Substance. While (viii) is the main constituent of the Substance the impurities present in the composition, as reported in the section 1.2 of the registration dossier, are not addressed.

159 Therefore, you have not demonstrated that the prediction is adequate for the purpose of classification and labelling and/or risk assessment.

12.3.1.2.2. The selected structure is outside the applicability domain of the models.

160 Under ECHA Guidance R.6.1.5.3., a prediction is within the applicability domain of the model, when, among others, the substance and the structures selected for the prediction falls within descriptor, structural, mechanistic and metabolic domain.

161 However, the selected structures used as input for the QSAR predictions you have provided are outside the mechanistic domain of the model as the model uses log Kow as an input parameter. However, as already explained above, the Substance is surface active and ionisable at environmentally relevant pH. Hence logKow is not a suitable descriptor to predict bioaccumulation because it does not take into account other potential mechanisms of bioaccumulation than lipid storage.

12.3.1.2.3. The predictions are not adequate due to low reliability.

162 Under ECHA Guidance R.6.1.3.4 a prediction is adequate for the purpose of classification and labelling and/or risk assessment when the model is applicable to the chemical of interest with the necessary level of reliability. ECHA Guidance R.6.1.5.3. specifies that, among others, the following condition must be met:

- the model predicts well substances that are similar to the substance of interest

163 The predictions for the selected structure used as input are not reliable because no similar substances to the Substance are included in training set of the model in study.

164 Therefore, you have not demonstrated that the prediction for the Substance is adequate for the purpose of classification and labelling and/or risk assessment.

12.3.1.2.4. Conclusion on the (Q)SAR adaptation

165 In conclusion, the provided predictions cannot be considered as reliable source of information that could contribute to the conclusion on the key parameter investigated by the required study.

12.3.1.3. Conclusion on the Weight of Evidence

166 In summary, the sources of information (i), (ii), (iii), (v) and (vi) provide relevant information on the key elements of this information requirement. However, these sources of information have significant reliability issues as described above and cannot contribute to the conclusion on the information requirement for bioaccumulation in aquatic species.

167 As it is not possible to conclude, based on any source of information alone or considered together, on the information requirement for bioaccumulation in aquatic species. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

12.3.2. Assessment of the adaptation under Annex IX, Section 9.3.2., Column 2

12.3.2.1. The log Dow is not a valid descriptor of the bioaccumulation potential of the Substance (source of information viii.)

168 Under Section 9.3.2., Column 2, first indent of Annex IX to REACH, the study may be omitted if the substance has a low potential for bioaccumulation and/or a low potential to cross biological membranes. A low log Kow (i.e., log Kow < 3) may only be used to support low potential for bioaccumulation if the partitioning to lipids is the sole mechanism driving the bioaccumulation potential of a substance. For some groups of substances (e.g., organometals, ionisable substances, surfactants) other partitioning mechanisms may drive bioaccumulation (e.g., binding to protein/cell membranes). For this reason, log Kow is not considered a valid descriptor of the bioaccumulation potential for such substances (Guidance on IRs and CSA, Appendix R.7.10-3). Similarly, the log Dow would only address the potential for bioaccumulation for substances for which the bioaccumulation is solely driven by lipophilicity. This excludes, for example, situations where the substance is surface active or ionisable at environmental pH (pH 4 – 9).

169 In your comments to the draft decision you provided the source of information (viii) based on which you conclude that the Substances has low likelihood to cross biological membranes based on a calculated Log Dow with [redacted] Chemicalize platform and a comparison with BCF data from [redacted]. You report the log Dow ranging from 3.96 to 1.03 at pH values of 1.7 and 8 respectively. You then conclude that *"as the logD is < 2.5 at pH (7), therefore no Bioaccumulation is expected."*

170 The Substance is ionisable and it may interact with cell membranes based on chemical structure. Therefore, log Dow is not a valid descriptor of the bioaccumulation potential of the Substance.

12.3.2.2. Low likelihood to cross biological membranes is not demonstrated (source of information ix.)

171 Under Section 9.3.2., Column 2, first indent, Annex IX to REACH, the study may be omitted if the Substance is unlikely to cross biological membranes. Guidance on IRs and CSA, Section R.7.8.5. explains that there is no scientific basis to define molecular characteristics that would render a substance unlikely to cross biological membranes. In this context, the indicators used for low likelihood of a high bioaccumulation potential (Guidance on IRs and CSA, Section R.11, Figure R.11-4) must be considered, including:

- physico-chemical indicators of hindered uptake due to large molecular size (*e.g.* D_{max} > 17.4 Å and MW > 1100 or MML > 4.3 nm) or high octanol-water partition coefficient (log K_{ow} > 10) or low potential for mass storage (octanol solubility (mg/L) < 0.002 x MW), and
- supporting experimental evidence of hindered uptake (no chronic toxicity for mammals and birds, no chronic ecotoxicity, no uptake in mammalian toxicokinetic studies, very low uptake after chronic exposure).

172 In your comments to the draft decision you provided the source of information (ix) on which you based your conclusion of low likelihood to cross biological membranes based on hindered uptake of the Substance and substantiated with the following physico-chemical indicators:

- the molecular weight of the substance, 505.5 g/mol
- the measured octanol solubility is 0.92±0.21 mg/l
- the $D_{maximum}$ of 34.06 Å as calculated by [redacted] Chemicalize platform.

173 The predicted $D_{maximum}$ and the measured octanol solubility alone are not sufficient to demonstrate low likelihood to cross biological membranes. The available information on the

Substance do not support that the Substance is unlikely to cross biological membranes. In particular in the registration dossier you report that during a 28 day oral administration study (OECD 407), symptoms of toxicity were evident at 1000 mg/kg/day. You also report a NOEC=2.54 mg/l for aquatic invertebrates (OECD 211). This information is indicative of systemic exposure to the substance. Therefore, you have not demonstrated that the Substance has low likelihood to cross biological membranes. Therefore the adaptation is rejected.

174 On this basis, the information requirement is not fulfilled.

12.4. Study design and test specifications

175 Bioaccumulation in fish: aqueous and dietary exposure (Method EU C.13 / OECD TG 305) is the preferred test to investigate bioaccumulation (Guidance on IRs and CSA, Section R.7.10.3.1.). Exposure via the aqueous route (OECD TG 305-I) must be conducted unless it can be demonstrated that:

- a stable and fully dissolved concentration of the test material in water cannot be maintained within ± 20% of the mean measured value, and/or
- the highest achievable concentration is less than an order of magnitude above the limit of quantification (LoQ) of a sensitive analytical method.

176 This test set-up is preferred as it allows for a direct comparison with the B and vB criteria of Annex XIII of REACH.

177 You may only conduct the study using the dietary exposure route (OECD 305-III) if you justify and document that testing through aquatic exposure is not technically possible as indicated above. You must then estimate the corresponding BCF value from the dietary test data according to Annex 8 of the OECD 305 TG and OECD Guidance Document on Aspects of OECD TG 305 on Fish Bioaccumulation (ENV/JM/MONO(2017)16).

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; (ECHA 2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).

All Guidance on REACH is available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

RAAF, 2017 Read-across assessment framework (RAAF), ECHA (2017)
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs), ECHA (2017).

The RAAF and related documents are available online:
https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: Procedure

The information requirement for an Extended one-generation reproductive toxicity study (EOGRTS; Annexes IX or X, Section 8.7.3.) is not addressed in this decision. This may be addressed in a separate decision once the information from the Sub-chronic toxicity study (90-day) requested in the present decision is provided; due to the fact that the results from the 90-day study is needed for the design of the EOGRTS. Similarly the information requirement for a Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.) is not addressed in this decision; as the EOGRTS will cover the same parameters.

This decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 21 April 2021.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and did not amend the requests.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

Appendix 3: Addressees of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
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[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. Test methods, GLP requirements and reporting

(1) Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

(2) Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

(3) Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[3].

1.2. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

(1) Selection of the Test material(s)
The Test Material used to generate the new data must be selected taking into account the following:
- the variation in compositions reported by all members of the joint submission,
- the boundary composition(s) of the Substance,
- the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

(2) Information on the Test Material needed in the updated dossier
- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
- The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[4].

[3] https://echa.europa.eu/practical-guides
[4] https://echa.europa.eu/manuals

2. General recommendations for conducting and reporting new tests

2.1. Strategy for the PBT/vPvB assessment

Under Annex XIII, the information must be based on data obtained under conditions relevant for the PBT/vPvB assessment. You must assess the PBT properties of each relevant constituent of the Substance present in concentrations at or above 0.1% (w/w) and of all relevant transformation/degradation products. Alternatively, you would have to justify why you consider these not relevant for the PBT/vPvB assessment.

You are advised to consult Guidance on IRs & CSA, Sections R.7.9, R.7.10 and R.11 on PBT assessment to determine the sequence of the tests needed to reach the conclusion on PBT/vPvB. The guidance provides advice on 1) integrated testing strategies (ITS) for the P, B and T assessments and 2) the interpretation of results in concluding whether the Substance fulfils the PBT/vPvB criteria of Annex XIII.

In particular, you are advised to first conclude whether the Substance fulfils the Annex XIII criteria for P and vP, and then continue with the assessment for bioaccumulation. When determining the sequence of simulation degradation testing you are advised to consider the intrinsic properties of the Substance, its identified uses and release patterns as these could significantly influence the environmental fate of the Substance. You must revise your PBT assessment when the new information is available.