~~Confidential~~

Helsinki, 17 November 2022

Addressees
Registrants of JS_DBk168_Na as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
14/01/2019

Registered substance subject to this decision ("the Substance")
Substance name: 2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-, coupled with 3-aminophenol, diazotized 5-amino-2-[(4-aminophenyl)amino]benzenesulfonic acid and diazotized benzenamine, sodium salts
EC number: 297-025-4

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 genetic toxicity study (triggered by Annex VIII, 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; 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.

3. Simulation testing on ultimate degradation in surface water (triggered by Annex VIII, Section 9.2.; 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.

4. Identification of degradation products (triggered by Annex VIII, Section 9.2; test method: OECD TG 309).

5. Bioaccumulation in aquatic species (triggered by Annex I, sections 0.6.1. and 4.; Annex XIII, Section 2.1.; test method: EU C.13./OECD TG 305, aqueous exposure)

The reasons for the requests 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.

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 for the in vitro gene mutation study in bacteria which raise the concern for gene mutation that must be investigated.

3 In the comments to the draft decision, you argue that there is a data gap for Annex VIII, Section 8.4. because the available studies have deficiencies which makes them inadequate to cover the endpoint, i.e. no Prival modification in the study with positive results and missing the fifth strain (*E. coli* WP2 *uvr*A, or *E. coli* WP2 *uvr*A (pKM101), or *S. typhimurium* TA102) in both studies. You consider that the datagap should be fulfilled by conducting a "*confirmatory AMES test OECD TG471 (2020) on the registered substance EC 297-025-4, on 5 Salmonella strains + Escherichia coli, with the Prival modification*." and that an *in vivo* study is not necessary. You argue that for a similar substance, Direct Black 168 Lithium salt EC 411-890-9, which differs from the Substance only by Li instead of Na, three Ames studies are available with negative results. One of them, performed with Prival modification shows negative result in the strain TA 1538 which was positive in the study provided in the dossier.

4 You also inform that the "*dossier will be updated with the result of the newly performed Ames test and the tests on the Lithium derivative*."

5 However, several considerations can be made related to your arguments:

6 Firstly, the purpose of Prival modification is to enhance the reductive metabolic activation system, which, as explained in the OECD 471, may be more appropriate for azo-dyes. Thus, a positive result obtained in a less optimal conditions remains a demonstration of a mutagenic effect of this substance in the Ames test. This effect is not undermined by the lack of the fifth strain.

7 Secondly, the studies which you consider in your comments "inadequate" were given by you a reliability score of 1 ([redacted], 1990 with positive results in TA 1535 with the Substance), and 2 ([redacted], 1993 with negative results in TA 1535 with the Substance). Thus, you considered the provided information as reliable to cover this endpoint.

8 Finally, the negative Ames studies (robust study summaries not provided) performed with the analogues substance Lithium salt (EC 411-890-9, CAS 108936-08-9) do not invalidate the positive results in TA 1538 obtained with the Substance. Furthermore, currently no appropriate read-across justification was provided.

9 Therefore, as positive results for the *in vitro* gene mutation study in bacteria are already available for your substance, you remain responsible for complying with this decision by the set deadline.

10 The examination of the information provided, as well as the selection of the requested test and the test design are addressed under Appendix 1.2.

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

11 Appropriate in vivo mutagenicity studies must be considered under Annex VIII to REACH (Section 8.4., Column 2) in case of a positive result in any of the in vitro genotoxicity studies under Annex VII or VIII to REACH.

2.1. Trigering of in vivo mutagenicity studies

12 As already explained under Appendix 1.1., 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.

2.2. Information provided

13 Your dossier contains the following other in vivo studies with the Substance:

(i) a study according to OECD 474 (1990)

(ii) a study equivalent or similar to OECD 486 (1991)

2.3. Assessment of the information provided on in vivo gene mutation

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

2.3.1. The provided in vivo studies are not appropriate

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

16 However, the in vivo studies (i.) and (ii.) above are not addressing the gene mutation concern raised by the in vitro data.

17 Therefore, the provided in vivo tests are not appropriate to investigate the gene mutation concern identified in vitro.

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

19 Your comments on the draft decision described under Request 1 equally apply to this information requirement.

2.4. Test selection

20 According to the Guidance on IRs and CSA, 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.

2.5. Specification of the study design

2.5.1. TGR Assay

21 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.

22 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.

23 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.

2.5.2. Comet Assay

24 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).

25 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.

26 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.

2.5.3. Germ cells

2.5.3.1. TGR Assay

27 You may 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.

2.5.3.2. Comet Assay

28 You may consider to collect 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. 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.

3. Simulation testing on ultimate degradation in surface water

29 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. Trigering of further degradation testing

30 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.* <70% degradation in an OECD 301A), and
- 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.

31 Your registration dossier provides the following:

- The Substance is not readily biodegradable (0% degradation after 28 days in OECD TG 301A);
- The Substance is an ionisable substance and therefore high potential for bioaccumulation cannot be excluded based on available information.

32 Furthermore,

- for the reasons explained in request 5 of this decision, it is not possible to conclude on the bioaccumulation potential of the Substance, and
- for the reasons explained in request 2 of this decision, it is not possible to conclude on the toxicity of the Substance.

33 Under section 5.3.1 of your IUCLID dossier and section 8 of your CSR ('PBT assessment'), you conclude that the Substance is not B/vB. In support of your conclusion you provide the following additional information:

- The log Kow of the substance is below the B/vB screening critierion of log Kow ≤ 4.5;
- The molecular weight of the substance is >800g/mol and you state that '*many substances with a molecular mass greater than 700 are not readily taken up by fish, because of possible steric hindrance at passage of gill membranes or cell membranes of respiratory organs*';
- A QSAR prediction of BCF of 3.162 for the substance.

34 However,

- Log Kow is not a valid descriptor of the bioaccumulation potential because the substance is ionised under environmentally relevant pH.
- Hindered uptake due to large molecular size is not a valid justication on its own for concluding that a substance is not vB/B (Guidance on IRs and CSA, Section R.11,

Figure R.11-4). In addition, the aforementionned guidance document specifies that large molecular size may be considered an indication of hindered uptake when the molecular weight (MW) of the substance is > 1100 g/mol, whereas your substance has a MW of 899.77 g/mol.

- The QSAR prediction of BCF cannot be assessed because you provide no documentation.

35 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.

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

3.2. Information provided on further degradation

37 Your dossier contains no information on further degradation.

38 In the comments to the draft decision, you state that "*the substance is persistent, but not bioaccumulative, therefore not considered either PBT or vPvB*". As explained under Request 5, 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.

39 Therefore, the requirements for further degradation are not met and the information requirement is not fulfilled.

3.3. Study design and test specifications

40 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.

41 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.).

42 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.

43 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.

44 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.).

4. Identification of degradation products

45 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. Trigering of identification of degradation products

46 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.).

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

48 In the comments to the draft decision, you state that "*the substance is persistent, but not bioaccumulative, therefore not considered either PBT or vPvB*". As explained under Request 5, 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.

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

4.2. Information provided on identification of degradation products

50 Your dossier contains no information on identification of degradation products.

51 Therefore, the requirements for identification of degradation products are not met and the information requirement is not fulfilled.

4.3. Study design and test specifications

52 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 3 or by some other measure.

53 To determine the degradation rate of the Substance, the requested study according to OECD TG 309 (Request 3) 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).

5. Bioaccumulation in aquatic species

54 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. Trigering of bioaccumulation in aquatic species

55 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.).

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

57 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

58 Your dossier contains no information on bioaccumulation in aquatic species.

59 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. a bioconcentration test on fish (2019) according to Bioconcentration test of substances in fish or shellfish Testing Guideline (MITI), [redacted], with the Substance.
ii. QSAR predictions of BCF with BCF model (Meylan) 1.0.3 for the Substance.
iii. QSAR predictions of BCF with BCFBAF v.3.01 model of the potential main degradation products for the Substance.
iv. a bioconcentration test on fish (2019) with an analogue substance (Direct Black 163, EC) according to Bioconcentration test of substances in fish or shellfish Testing Guideline (MITI). [redacted]
v. LogBCF values on analogues substances derived from studies performed under the Japanese Regularoty framework
vi. a scientific publication entitled "[redacted] (1981);
vii. a survey report entitled "[redacted] (1998);
viii. the test guideline for "[redacted] (2021);
ix. a presentation entitled "[redacted] (2016);
x. Log Dow predictions with [redacted] Chemicalize model for the Substance.
xi. Information on the topological general characteristics of the Substance

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

5.3.1. Assessment of the weight of evidence approach

60 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.

61 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.

62 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.

63 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).

64 The source of information (viii) and (ix) do not provide relevant information on any of the key elements listed above. Source of information (viii) 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 (ix) 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.

65 The sources of information (x) and (xi) that 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.

66 The sources of information (i) to (vii) 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:

5.3.1.1. The source of information (i) does not meet the information requirement

67 To fulfil the information requirement, a study must comply with the OECD TG 305 (Article 13(3) of REACH). Therefore, the following specifications must be met:

68 Reporting of the methodology and results

a) test procedure used (e.g. flow-through or semi-static); regular study or minimised design (including rationale and justification)
b) test design (e.g. number and size of test chambers, water volume replacement rate, loading rate, number of replicates, number of fish per replicate, number of test concentrations, length of uptake and depuration phases, sampling frequency for fish and water samples);
c) method of preparation of stock solutions and frequency of renewal (the solvent, its concentration and its contribution to the organic carbon content of test water must be given, when used) or description of alternative dosing system;

d) the analytical method used for the quantification of the test material in the [test solutions/feed] and in fish tissues is described. The recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range are reported;
e) the lipid content measured at least before the beginning and at the end of the uptake phase and the method used for its determination are reported;
f) individual fish wet weights and total lengths for all sampling intervals are provided, and be linked to the analysed chemical concentration for that individual. The data are used to correct the BCF for growth dilution;
g) tabulated test material concentration data in individual fish and water (including mean values for test group and control, standard deviation and range, if appropriate) for all sampling times are provided;
h) the steady-state bioconcentration factor, (BCFSS), if steady-state is (almost) achieved;
i) kinetic bioconcentration factor (BCFK) and derived uptake and depuration rate constants k1 and k2, together with the variances in k2 (slope and intercept) if sequential fitting is used;
j) confidence limits, standard deviation (as available) and methods of computation/data analysis for each parameter for each concentration of test substance used;
k) any information concerning radiolabelled test chemical metabolites and their accumulation;
l) growth rate constant(s) (including 95% confidence interval(s)) and calculated growth-corrected depuration rate constant (k2g), half-life and BCF (BCFKg) values;

69 The study (i) does not include any of above mentioned information .

70 Based on the above, the reporting of this study is not sufficient to conduct an independent assessment of their reliability.

5.3.1.2. Read-across adaptation rejected for the sources of information (iv) to (vii)

71 ECHA understands that the sources of information (iv), (v), (vi) and (vii) included in your weight of evidence approach rely on grouping and read-across approach under Annex XI, Section 1.5. Regarding the source of information (iv) ECHA understands that you consider an analogue approach and read-across from a single source substance to the Substance. Regarding the sources of information (v), (vi) and (vii) 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.

72 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.

73 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).

74 You do not provide a read-across justification document in your comments to cover either the analogues o the category approach proposed in your comments.

75 Regarding the 'category' approach you define the the structural basis for the grouping as "azo-dyes". ECHA understands that this is the applicability domain of the grouping and will assess your predictions on this basis. ECHA understands that this is the applicability domain of the grouping and your predictions are assessed on this basis. You predict the properties of the Substance from information obtained from the source substances listed in the respective information source (vi) and (vii). ECHA further noted that the source substances in the information source (v) are not reported.

76 Your reasoning for the prediction of bioaccumulation in aquatic species for the analogue approach was based on the claim that the source and target substance "are comparable in adsorption, distribution and reactivity towards biological membranes". Respectively for the category approach the prediction was based on the claimed applicability of logKow as a predictor of bioaccumulation for the azo dyes groupd of substances.

77 ECHA understands that your read-across hypothesis for both the analogue and the category approach assumes that different compounds have the same type of effects. You predict the properties of your Substance to be quantitatively equal to those of the source substance for the analogue approach. For the category approach, You predict the properties of your Substance based on an identified trend within the group.

78 We have identified the following issue(s) with the proposed scope of the grouping for your category approach:.

5.3.1.2.1. *Incomplete description of the applicability domain of the category for the sources of information (v), (vi) and (vii)*

79 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.

80 You describe the applicability domain of the substances covered by the grouping as: "azo-dyes".

81 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.

5.3.1.2.2. *Absence of read-across documentation for the sources of information (iv) to (vii)*

82 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).

83 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.

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

5.3.1.2.3. *Conclusion on the read-across approach*

85 As explained above, the sources of information (iv) to (vi) cannot be considered as reliable sources of information that could contribute to the conclusion on the key parameter investigated by the required study.

5.3.1.3. The provided (Q)SAR adaptation is rejected for sources of information (ii) and (iii).

86 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.

87 With regard to these conditions, we have identified the following issues which are common to both sources of information (ii) and (iii):

5.3.1.3.1. *The selected structure is outside the applicability domain of the models.*

88 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.

89 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.

5.3.1.3.2. *The predictions are not adequate due to low reliability.*

90 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

91 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.

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

5.3.1.3.3. *Conclusion on the (Q)SAR adaptation*

93 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.

5.3.1.4. Conclusion on the Weight of Evidence

94 In summary, the sources of information (i) to (vii) 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.

95 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.

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

5.3.2.1.1. *The log Dow is not a valid descriptor of the bioaccumulation potential of the Substance (source of information x.)*

96 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).

97 In your comments to the draft decision you provided the source of information (x) based on which you conclude that the Substances has low potential for bioaccumulation based on a calculated log Dow with [redacted] Chemicalize platform and a comparison with BCF data from National Institute of Technology and Evaluation (Japan). You report the log Dow ranging from 0.63 to -0.46 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."

98 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.

5.3.2.2. Low likelihood to cross biological membranes is not demonstrated (source of information xi.)

99 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. Dmax > 17.4 Å and MW > 1100 or MML > 4.3 nm) or high octanol-water partition coefficient (log Kow > 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).

100 In your comments to the draft decision you the source of information (xi) 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, 900 g/mol
- the measured octanol solubility is $<3.10^{-8}$ mol/l
- the Dmaximum of 26.03 Å as calculated by ChemAxon's Chemicalize platform.

101 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 centrilobular vacuolation in the hepatocytes at 350 mg/kg bw/day in male animals (OECD 422). 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.

102 Therefore, the requirements on bioaccumulation in aquatic species are not met and the information requirement is not fulfilled.

5.4. Study design and test specification

103 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.

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

105 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

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.

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]

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[2].

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[3].

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

2.2. Environmental testing for substances containing multiple constituents

Your Substance contains multiple constituents and, as indicated in Guidance on IRs & CSA, Section R.11.4.2.2, you are advised to consider the following approaches for persistency, bioaccumulation and aquatic toxicity testing:

- the "known constituents approach" (by assessing specific constituents), or
- the "fraction/block approach, (performed on the basis of fractions/blocks of constituents), or
- the "whole substance approach", or
- various combinations of the approaches described above

Selection of the appropriate approach must take into account the possibility to characterise the Substance (i.e. knowledge of its constituents and/or fractions and any differences in their properties) and the possibility to isolate or synthesize its relevant constituents and/or fractions.

References to Guidance on REACH and other supporting documents can be found in Appendix 1.