~~Confidential~~

Helsinki, 03 June 2022

Addressees
Registrant(s) of [redacted] as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
28/05/2018

Registered substance subject to this decision ("the Substance")
Substance name: Reaction mass of disodium 4-amino-3-[[4-[(2,4-diaminophenyl)azo]phenyl]azo]-5-hydroxy-6-(phenylazo)naphthalene-2,7-disulphonate and disodium 4-amino-3-[[4-[(2-amino-4-hydroxyphenyl)azo]phenyl]azo]-5-hydroxy-6-(phenylazo)naphthalene-2,7-disulphonate
List number: 916-632-3

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 ***9 September 2024***.

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

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

1. Skin sensitisation (Annex VII, Section 8.3.); test method:

    i. in vitro/in chemico skin sensitisation information on molecular interactions with skin proteins (OECD TG 442C), inflammatory response in keratinocytes (OECD TG 442D) and activation of dendritic cells (EU B.71/OECD TG 442E) (Annex VII, Section 8.3.1.); and

    ii. Only if the in vitro/in chemico test methods specified under point 1.i. are not applicable for the Substance or the results obtained are not adequate for classification and risk assessment, in vivo skin sensitisation (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429).

2. In vitro cytogenicity study in mammalian cells (triggered by Annex VII, Section 8.4., column 2; test method: OECD TG 473) or In vitro micronucleus study (Annex VIII, Section 8.4.2.; test method: OECD TG 487)

3. In vivo genetic toxicity study (triggered by Annex VII, Section 8.4., Column 2) to be selected according to the following specifications:

    a. If the test results of request 2. are negative:

    Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488 from 2020) 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

b. If the test results of request 2 are positive:

In vivo mammalian alkaline comet assay (test method OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, or if justified, in mice, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

The reasons for the decision 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.

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

[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.

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Appendix 1: Reasons for the decision

Contents

Reasons related to the information under Annex VII of REACH

1. Skin sensitisation

1 Skin sensitisation is an information requirement under Annex VII to REACH (Section 8.3.). Under Section 8.3., Column 1, the registrants must submit information allowing (1) A) a conclusion whether the substance is a skin sensitiser and B) whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), and (2) risk assessment, where required.

1.1. Information provided

2 You have adapted this information requirement by using a Grouping of substances and read-across approach and provided the following information:

(i) study according to the method of [redacted] (1973) with Derma Carbon 1338 ([redacted] % (w/w) CAS 68460-07-1 (Direct Black 155), [redacted] % (w/w) CAS 68877-33-8, [redacted] % (w/w) sodium sulphate, [redacted] % (w/w) sodium chloride, [redacted] % (w/w) sodium carbonate).

3 You did not provide any reasoning for the prediction of this information requirement.

1.2. Assessment of the information provided

4 We have assessed this information and identified the following issue(s):

1.2.1. Read-across adaptation rejected

5 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.

6 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).

7 We have identified the following issue with the prediction of toxicological properties:

1.2.1.1. Absence of read-across documentation

8 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 provide a justification for the read-across including a hypothesis, explanation of the rationale for the prediction of properties and robust study summary(ies) of the study(ies) on the source substance(s) (Guidance on IRs and CSA, Section R.6.2.6.1.).

9 You have provided a robust study summary for a study conducted with another substance than the Substance in order to comply with the REACH information requirements. However, you have not provided documentation as to why this information is relevant for the Substance.

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

11 As explained above, you have not established that relevant properties of the Substance can be predicted from data on the source substance(s). Therefore, your read-across approach under Annex XI, Section 1.5. is rejected and the information requirement is not fulfilled.

12 In addition, several deficiencies are identified with the study provided.

1.2.2. Non-compliant study

13 To be considered compliant and enable concluding whether the Substance causes skin sensitisation, a study has to meet the requirements of the EU Method B.6/OECD TG 406. The following key parameter(s) of this test guideline include:

a) Dose level selection rationale;
b) The induction concentration should be the highest causing mild-to-moderate irritation to the skin and the challenge dose should be the highest non-irritation concentration (GPMT: OECD TG 406, paragraph 14);
c) Positive and negative controls to establish the sensitivity and reliability of the experimental technique (OECD TG 406, paragraph 11).

14 In the provided study:

a) No dose level selection rationale was provided;
b) The concentration used for induction did not cause mild-to-moderate irritation;
c) No information on positive and negative control group were provided.

15 Therefore, the study does not fulfil the key parameter(s) set in the EU method B.6/OECD TG 406 and does not allow to make a conclusion whether the Substance causes skin sensitisation.

1.2.3. No assessment of potency

16 To be considered compliant and enable a conclusion in cases where the substance is considered to cause skin sensitisation, the information provided must also allow a conclusion whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

17 As the currently available data does not allow to conclude whether the Substance causes skin sensitisation (see section A above), this condition cannot be assessed.

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

2. In vitro cytogencity study in mammalian cells or In vitro micronucleus study

19 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. The ECHA guidance R.7a[2] further specifies that "REACH Annex VII substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further, according to the requirements of Annex VIII." This is because an in vitro cytogenicity test under Section 8.4.2 will allow to further investigate the mutagenicity of the substance in accordance with the REACH integrated testing strategy. The obtained in vitro data will inform on the genotoxic concern(s) associated with the substance and help identify the most adequate follow-up in vivo study (requested under 3.).

2.1. Information provided

[2] ECHA Guidance R.7a, section R.7.7.6.3, p.570.

20 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria which raise the concern for gene mutation. However, no information from an in vitro cytogenicity study or an in vitro micronucleus study on the substance in mammalian cells is available in the dossier.

2.2. Assessment of the information provided

21 ECHA therefore considers that an appropriate *in vitro* cytogenicity or micronucleus study is necessary to further investigate the mutagenicity of the substance.

2.3. Test selection and study design

22 Either the *in vitro* cytogenicity study in mammalian cells (test method OECD TG 473) or the *in vitro* micronucleus study (test method OECD TG 487) are considered suitable.

3. In vivo genotoxicity study

23 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.

3.1. Triggering of in vivo mutagenicity studies

24 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD 471, 2018) which raise the concern for gene mutation.

25 The Guidance on IRs and CSA, Section R.7.7.6.3. states that following a positive result in an in vitro test, "adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo. In cases where it can be sufficiently deduced that a positive in vitro finding is not relevant for in vivo situations (e.g., due to the effect of the test substances on pH or cell viability, in vitro-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold mechanism coming into play only at high concentrations that will not be reached in vivo has been identified (e.g., damage to non-DNA targets at high concentrations), in vivo testing will not be necessary.".

3.2. Information provided

26 However, no data from an in vivo somatic cell genotoxicity study is available in the dossier. Moreover, you did not provide any considerations explaining that the genotoxic potential of the substance cannot be expressed in vivo, based e.g., on lack of relevance for in vivo situations or the existence of threshold mechanism.

3.3. Assessment of the information provided

27 ECHA considers that an appropriate in vivo follow up mutagenicity study is necessary to address the concerns identified in vitro.

3.4. Test selection

28 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.

29 As explained above, under section 2, in the dossier there is no information from an *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study. Therefore, by this decision, ECHA also requests an *in vitro* cytogenicity study or an *in vitro* micronucleus study, which may raise a concern for chromosomal aberration in case of positive results.

30 In case there is also a concern for chromosomal aberration, the comet assay can be combined with an *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) into a single study (see OECD TG 489 paragraph 33; OECD TG 474 paragraph 37c; Guidance on IRs & CSA, Section R.7.7.6.3). While the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations, the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy). A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

31 The combined study, together with the results of the in vitro mutagenicity studies, can be used to make definitive conclusions about the mechanism(s) inducing in vivo mutagenicity and lack thereof. Furthermore, the combined study can help reduce the number of tests performed and the number of animals used while addressing (structural and numerical) chromosomal aberrations as well as gene mutations.

32 Therefore, you must wait for the results of the *in vitro* test requested under section 2 and, depending on these results, to conduct either a) TGR assay or Comet assay, if the test results of request 2 are negative; or b) Comet assay combined with MN test if the test results of request 2 are positive. The deadline set in this decision allows for sequential testing.

3.5. Specification of the study design

3.5.1. TGR assay (if the test results of request 2 are negative)

33 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.

34 Based on the recent update of OECD TG 488 (2020), you are requested to follow the new 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.

35 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.

3.5.1.1. Germ cells

36 You may consider collecting the male germ cells (from the seminiferous tubules) at the same time as the other tissues, 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.

3.5.2. Comet assay (if the test results of request 2 are negative)

37 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).

38 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.

39 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.

3.5.2.1. Germ cells

40 You may consider collecting the male gonadal cells collected from the seminiferous tubules in addition to the other 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.

3.5.3. Comet assay combined with MN test (if the test results of request 2 are positive)

41 According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined study must be performed in rats, or if justified, in mice.

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

43 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.

44 The combination of the OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al*. 2011 *[1]*).

3.5.3.1. Germ cells

45 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other 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.

46 Reference:

[1] Bowen DE et al. (2011) Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. *Muta Res*.;722:7–19.

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 did not receive any comments within the commenting period.

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

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-78 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

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.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[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