~~Confidential~~

Helsinki, 15 June 2022

Addressees
Registrant(s) of JS_Acid_Brown_126 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
23/09/2020

Registered substance subject to this decision ("the Substance")
Substance name: Reaction products of diazotised 3-amino-2-hydroxy-5-nitrobenzenesulphonic acid, coupled with 1,3-diaminobenzene and diazotised sodium 4-aminobenzenesulfonate, metallised with Basic Chromium (III) Sulphate
List number: 947-395-4

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

DECISION ON TESTING PROPOSAL(S)

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***20 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. *In vitro* cytogenicity study in mammalian cells (test method: OECD TG 473) or *In vitro* micronucleus study (test method: OECD TG 487); and subsequently

2. *In vivo* genetic toxicity study to be selected according to the following specifications:

    a. If the results of the *in vitro* test requested under 1 are **negative**:

    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 results of the *in vitro* test requested under 1 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(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.

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

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

Appendix 1: Reasons for the decision 3

Reasons for the decision(s) related to the information under Annex VII of REACH 4

1. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study 4

2. In vivo genetic toxicity study 6

References 9

Reasons for the decision(s) related to the information under Annex VII of REACH

1. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study

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. ECHA guidance R.7a, section R.7.7.6.3 (p.570), 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.*" It is necessary to request an *in vitro* cytogenicity test as an additional test 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.

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

1.1. Information provided to fulfil the information requirement

3 You have submitted a testing proposal for an *In vivo* mammalian alkaline comet assay to be performed with the Substance to further investigate the mutagenicity of the substance.

4 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.

5 ECHA therefore considers that an appropriate *in vitro* cytogenicity or micronucleus study is necessary to further investigate the mutagenicity of the Substance and to help identify the most adequate follow-up *in vivo* study.

6 For the following reasons ECHA further considers that the data provided in your dossier and in your comments do not meet the conditions for adaptation of the information on an *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study.

1.1.1. Information in your dossier

7 In the justification provided in your dossier you indicate that the Substance, being an azo-dye, will "*not be expected to give positive results in mammalian cells, where reductive metabolic conditions are not applied*". Moreover you indicate that that the specific metabolic pathway explaining the positive Ames findings "*cannot be simulated adequately using mammalian cells in vitro*".

8 Under Section 8.4.2., Column 2, Annex VIII to REACH, the study may be omitted 1) if adequate data from an *in vivo* cytogenicity test are available or 2) if the Substance is known to be carcinogenic category 1A or 1B or germ cell mutagenic category 1A, 1B or 2.

9 We note the following:

1) You have not provided an *in vivo* chromosomal aberration test; and
2) The Substance is not known to be Carc. 1A/1B or germ cell muta 1A, 1B, or 2.

10 Therefore, the requirements of Section 8.4.2., Column 2, Annex VIII to REACH for an adaptation of the information on an *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study are not met.

1.1.2. Information in your comments on draft decision

11 In your comments to the draft decision, you have submitted information to adapt this information by means of grouping and read-across according to Annex XI, Section 1.5 of the REACH Regulation.

12 You propose to predict the genotoxicity properties of the Substance from studies conducted on various source substances, which belong to different sub-groups of azo-dyes, namely the azo-dyes with chromium complex, with pyrazole complex or with benzene derivatives.

13 You provide the following reasoning for the prediction of (eco)toxicological properties:

14 You claim that the Substance and the source substances have similar structural and physicochemical characteristics, as all these substances have:

15 [redacted]In your comments you also included the results of the mutagenicity studies performed with the source substances; you report negative results for the *in vitro* cytogenicity test(s) or the micronucleus test(s) conducted with some of the source substances listed in your comments.

16 Based on the above you indicate that "*there is no concern for cytogenicity in mammalian cells for any of the substances analogue*" to the Substance, therefore you conclude that "*the cytogenicity does not need to be further investigated*".

17 We have assessed this information and identified the following issues:

18 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.

19 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).

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

1.1.2.1. Missing supporting information

21 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 supporting information to scientifically justify the read-across explanation for prediction of properties. The set of supporting information should strengthen the rationale for the read-across in allowing to verify the crucial aspects of the read-across hypothesis and establishing that the properties of the Substance can be predicted from the data on the source substance(s) (Guidance on IRs and CSA R.6, Section R.6.2.2.1.f.).

22 Supporting information must include toxicokinetic information on the formation of the common compounds.

23 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, information characterising the rate and extent of the (bio)transformation of the Substance and of the source substance(s) is necessary to confirm the formation of the proposed common (bio)transformation product and to assess the impact of the exposure to the parent compounds.

24 However, you have not provided any experimental information, about the (bio)transformation of the Substance nor the source substances to support your claims regarding formation of a common compound(s).

25 In the absence of such information, you have not established that the Substance and the source substances are likely to have similar properties. Therefore, you have not provided sufficient supporting information to scientifically justify the read-across.

1.1.2.2. Missing robust study summaries

26 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 robust study summary for each source study used in the adaptation.

27 In your justification document you refer to the source studies: "*In vitro cytogenicity study in mammalian cells: chromosome aberration test and / or In vitro cytogenicity study in mammalian cells: micronucleus test*"; however, you only report the outcome of these studies.

28 You have not provided detailed information on the methods, results and conclusions, allowing for an independent assessment of the studies. Therefore, you have failed to provide a robust study summary for each source study used in the adaptation as required by Annex XI, Section 1.5.

29 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.

1.2. Test design

30 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.

1.3. Outcome

31 Under Article 40(3)(c) of REACH, you are requested to carry out the additional test, as indicated above.

2. In vivo genetic toxicity study

32 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.

33 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471; 2018), which raise the concern for gene mutations.

2.1 Information provided to fulfil the information requirement

34 You have submitted a testing proposal for an *in vivo* mammalian alkaline comet assay to be performed with the Substance.

35 ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. You provided your considerations concluding that there were no alternative methods which could be used to adapt the information

requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

36 ECHA agrees that an appropriate *in vivo* follow up genotoxicity study is necessary to address the concerns identified *in vitro*.

2.2 Test selection

37 ECHA notes that the proposed *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is suitable to follow up a positive *in vitro* result on gene mutation.

38 However, as explained above under section 1, ECHA also requests an *in vitro* cytogenicity study or an *in vitro* micronucleus study (for the reasons see above Appendix A.1.) which may additionally raise a concern for chromosomal aberration in case of positive results.

39 In case there is also a concern for chromosomal aberration, you must combine the comet assay and the *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) into a single study (see OECD TG 489 para. 33; OECD TG 474 para. 37c; Guidance on IRs & CSA, Chapter R.7a, 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 concerns for chromosomal aberration as well as gene mutation.

40 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.

41 In your comments to the draft decision, you indicated that you would need to perform "*just*" the comet assay, because you disagree with the study requested under section 1. However, as explained under section 1 above, the *in vitro* test is required to inform on the genotoxic concern(s) associated with the Substance and thus to identify the most adequate follow-up *in vivo* study.

42 Therefore, you must wait for the results of the *in vitro* test requested and, depending on these results, conduct either a) Comet assay if the test results of request 1 are negative; or b) Comet assay combined with MN test if the test results of request 1 are positive. The deadline set in this decision allows for sequential testing. Specification of the study design

*2.2.1 Comet assay (if the test results of request 1 are **negative**)*

43 You proposed testing in the rat. 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).

44 You did not specify the route for testing. 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.

45 In line with the test method OECD TG 489, the test must be performed by analysing tissues from the 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.

*2.2.2 Comet assay combined with MN test (if the test results of request 1 are **positive**)*

46 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.

47 For the choice of route and tissues to be analysed refer to section 2.2.1. above.

48 The combination of 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]).

2.2.3 Germ cells

49 You may consider to collect the male gonadal cells 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.

50 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.

51 *Reference:*

52 [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.

2.3 Outcome

53 Under Article 40(3)(b) your testing proposal is accepted under modified conditions and you are requested to conduct the test with the Substance, as specified above.

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

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 12 February 2021.

ECHA held a third party consultation for the testing proposal(s) from 18 March 2021 until 3 May 2021. ECHA did not receive information from third parties.

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

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.

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]
[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].

(4) Where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design shall ensure that the data generated are adequate for hazard identification and risk assessment.

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 the careful identification and description of the characteristics of the Tests Materials in accordance with OECD GLP (ENV/MC/CHEM(98)16) and EU Test Methods Regulation (EU) 440/2008 (Note, Annex), namely all the constituents must be identified as far as possible as well as their concentration. Also any constituents that have harmonised classification and labelling according to the CLP Regulation must be identified and quantified using the appropriate analytical methods.

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

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].

[3] https://echa.europa.eu/manuals