~~Confidential~~

Helsinki, 4 October 2021

Addressees
Registrants of JS_ReactiveRed066 listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
30/03/2020

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Disodium 5-[[4-[(2-bromo-1-oxoallyl)amino]-2-sulphonatophenyl]azo]-4-hydroxy-6-(methylamino)naphthalene-2-sulphonate
EC number: 274-436-7
CAS number: 70210-39-8

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 ***10 July 2023***.

The requested information must be generated using the Substance unless otherwise specified.

A. Information required from the Registrants subject to Annex VII of REACH

1. *In vivo* mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

Reasons for the request(s) are explained in the appendix entitled "Reasons to request information required under Annex VII of REACH".

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH, 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.

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 testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Approved[1] under the authority of Christel Schilliger-Musset, 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.

Appendix A: Reasons to request information required under Annex VII of REACH

This decision is based on the examination of the testing proposal you submitted.

1. **In vivo mammalian alkaline comet assay combined with *in vivo* mammalian erythrocyte micronucleus test**

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.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria. Even though the study showed a "*weak mutagenic effect*" in three of the *S. typhimurium* strains tested (TA 98, TA 1535 without metabolic activation and TA 100 with metabolic activation), the positive results raise the concern for gene mutations.

In the dossier there are also positive results for the *in vitro* mammalian chromosomal aberration test that indicate also a concern for chromosomal aberration.

In your comments on the proposal for amendment (PfA), you indicate the following:

(1) The Substance '*may have the potential to induce mutations in bacterial cells' however*, it *did not induce mutations in the mammalian cells, and hence considered as not mutagenic*';
(2) Ames Data with other azo-dyes gave negative results, therefore Ames test with the Substance '*seems to be not reliable*'; and
(3) *In vivo* data with other dyes gave a negative outcome, confirming the differences in the *in vitro* and *in vivo* testing conditions.

However, ECHA notes the following:

(1) As explained above, we understand that the results obtained in the OECD 471 study ([redacted], 1993) are '*positive*', based on what is reported in the dossier and in your comments on the PfA. We also acknowledge the negative result obtained in the OECD TG 476 study ([redacted], 1996). However, a study in mammalian cells is complementary to a study in bacteria, which it is not intended to supersede, so we note that this negative result does not remove the concern for gene mutation. Therefore, it cannot be concluded that the Substance is '*not mutagenic*'.

    As explained above, due to the positive result obtained in OECD TG 471 study ([redacted], 1993) there is still a concern for gene mutation that must be further investigated by means of an *in vivo* study. This is in line with REACH Annex VII Section 8.4., column 2, where further mutagenicity studies must be considered in case of a positive result in an *in vitro* gene mutation study in bacteria.

(2) & (3)
    In your comments you refer to the Ames data of other azo-dyes, i.e. '*similar substances with a high structural relationship*'.

    You also refer to study results, in general, obtained in various *in vitro* and *in vivo* genetic toxicity data with '*more than 100 dyes*' with '*all kind of structures*'.

    You have, however, not provided any justification nor documentation to explain how and why this information can be used to predict the outcome on mutagenicity for the Substance.

In the absence of such documentation, ECHA cannot assess the relevance of such comments in respect to the mutagenic properties of the Substance.

1.1. Information provided to fulfil the information requirement

You have submitted a testing proposal for an *In vivo* mammalian erythrocyte micronucleus test to be performed with the Substance.

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.

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

1.2. Test selection

You have proposed to perform an *In vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474). However, the positive *in vitro* results available in the dossier indicate a concern for both chromosomal aberration and gene mutation. The proposed MN test addresses only chromosomal aberrations and does not investigate gene mutations.

Under OECD TG 474 the MN test can be combined with an *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) in a single study (see OECD TG 474, paragraph 37c; ECHA Guidance R.7a, Section R.7.7.6.3). While the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations. A combined study will thus address both identified concerns, chromosomal aberration as well as gene mutation.

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.

Therefore, the comet assay combined with the MN test is the most appropriate study for the Substance.

ECHA notes that in the initial draft decision the MN test was only recommended to be performed in combination with the comet assay. In your comments on the draft decision, you agreed to perform the comet assay. You indicated that you do not intend to perform the MN test in parallel.

However, in a PfA, submitted by a Member State Competent Authority, it was noted that at the 74th meeting of the Member States Committee (MSC-74) (June 2021), MSC agreed that the combined study of the comet assay and the MN test is the most suitable at Annex VII when both concerns for chromosomal aberration and gene mutation exist and no other adequate and appropriate *in vivo* genotoxicity data are available in the dossier.

As explained above, in the dossier there are positive results for OECD TG 471 and 473 studies and there are no available *in vivo* genotoxicity studies. Therefore, the criteria to request the

comet assay combined with the MN test are met, and the approach agreed at MSC-74 must be followed. ECHA has therefore amended the request and the reasons, as specified above and in section 1.3. below.

In your comments on the PfA, you indicate that the performance of the OECD TG 474 study will '*not add any value*' for this Substance and that the OECD TG 489 is '*sufficient*'. The main arguments raised in your comments have already been addressed above. Moreover, you provide the following additional arguments:

(1) '*It is not possible to perform both tests [Comet assay and MN test] in parallel*' as '*our new service provider has only validated the test on OECD TG 489 in rats while having validated the test according to OECD TG 474 in mice*' therefore '*additional animals have to be dosed for the OECD TG 474 test*'; and
(2) The comet assay, as also recommended in the ECHA Guidance, is a suitable follow-up test to investigate *in vitro* chromosomal aberration / gene mutation concern(s).

However, ECHA notes the following:

(1) Your comment is based on the input from a single CRO, which does not demonstrate that the combined test cannot be performed. We thus do not agree that the combined comet assay and MN test cannot be performed in rats.

(2) Firstly, the legal text applies, taking into account the objective of protection of human health of the REACH Regulation. In this case, this means that a combined study is needed.

    We acknowledge that the ECHA guidance still needs to be updated and aligned with the current practice agreed at MSC-74. This information concerning the need to perform a combination study (comet assay and MN test) has been communicated in the ECHA website, following the MSC-74 agreement (June 2021). Moreover, the advice and recommendations in the ECHA website[2], concerning the mutagenicity standard information requirement, have also been updated accordingly to reflect this agreement.

    Additionally, as explained above, we note that, in contrast with the comet assay, only the MN test can detect both structural and numerical chromosomal aberrations.

    In your comments you refer to the positive result obtained in the OECD TG 473 study ([redacted], 2014), where you conclude that the Substance was '*found to be clastogenic*'. ECHA notes that OECD TG 473 is not designed to measure aneuploidy. Therefore, only the structural chromosomal aberrations were investigated in the *in vitro* study (OECD TG 473), which is also only a preliminary study.

    Therefore, the MN test is required and will add 'value' when combined with the comet assay, as it will provide further investigation of genotoxicity detected by an *in vitro* system and will detect *in vivo* (if any), not only structural chromosomal aberrations but also numerical chromosomal aberrations, as explained above.

1.3. Specification of the study design

You did not specify the species to be used for testing. According to the test method OECD TG 489, the test must be performed in rats. Therefore, the combination test must be performed in rats.

[2] https://echa.europa.eu/standard-information-requirements-recommendations

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.

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.

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]*).

Germ cells
You may consider collecting 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. 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.

References

[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.

1.4. Outcome

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.

Appendix B: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. 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].

B. 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

Appendix C: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 10 September 2020.

ECHA held a third party consultation for the testing proposal(s) from 19 October 2020 until 3 December 2020. 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 request.

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 amendments and referred the modified draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee unanimously agreed on the draft decision in its MSC-75 written procedure. ECHA adopted the decision under Article 51(6) of REACH.

Appendix D: List of references - ECHA Guidance[5] and other supporting documents

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)[6]

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)[5]

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

OECD Guidance documents[7]

[5] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[6] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[7] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Guidance Document on aqueous–phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix E: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

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.