~~Confidential~~

Helsinki, 4 May 2022

Addressees
Registrant of JS_R YE 039 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
18/11/2019

Registered substance subject to this decision ("the Substance")
Substance name: Disodium 4-[4-[[5-[(2-bromo-1-oxoallyl)amino]-2-sulphonatophenyl]azo]-4,5-dihydro-3-methyl-5-oxo-1H-pyrazol-1-yl]-2,5-dichlorobenzenesulphonate
EC number: 274-499-0

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 ***13 May 2024***.

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

Information required from all the Registrants subject to Annex VIII 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);

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 section 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.

   OR

   Transgenic rodent somatic and germ cell gene mutation assay (test method: 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.

   b. If the results of the *in vitro* test requested under section 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.

Your originally proposed test using the Substance is rejected, according to Article 40(3)(d):

> *In vivo Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells (OECD TG 486)*

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

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

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

1 An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is an information requirement under Annex VIII to REACH (Section 8.4.2.).

2 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.

1.1. Information provided to fulfil the information requirement

3 You have submitted a testing proposal to follow up the positive results from an *in vitro* gene mutation study in mammalian cells raising the concern for gene mutations.

4 However, the information from the *in vitro* cytogenicity study in mammalian cells (OECD TG 473, 1999) with the Substance, available in the dossier, did not include:

- one of the three experimental conditions required to conclude a negative outcome, i.e. short-term treatment without metabolic activation; and
- the scoring of at least 300 metaphases per concentration, since only 100 metaphases were scored.

5 Therefore, the information provided does not cover key conditions required by OECD TG 473 and is considered unreliable.

6 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.

1.2. Test design

7 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

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

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

2. *In vivo genetic toxicity study*

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

11 Your dossier contains positive results for the *in vitro* gene mutation study in mammalian cells which raise the concern for gene mutations.

2.1. Information provided to fulfil the information requirement

12 You have submitted a testing proposal for an *in vivo* Unscheduled DNA Synthesis (UDS) test to be performed with the Substance.

13 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.

14 ECHA received third party information concerning the testing proposal during the third party consultation, which is addressed by the reasons set in section 2.2. below. The third party information indicated that the transgenic rodent somatic and germ cell gene mutation assay or the *in vivo* mammalian alkaline comet assay would be more suitable studies for the Substance, as there are concerns with the sensitivity of the UDS assay in detecting gene mutagens.

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

2.2. Test selection

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

17 You proposed to perform an Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* (OECD TG 486).

18 The UDS test provides an indication of induced damage to DNA, followed by DNA repair (measured as unscheduled DNA synthesis in liver cells). However, as reminded in the Guidance on IRs & CSA, R.7a, Section R.7.7.6.3 (page 571-572), the UDS test is sensitive to some (but not all) DNA repair mechanisms and not all gene mutagens are positive in the UDS test. The sensitivity of the UDS test has been questioned (Kirkland and Speit, 2008[2]) and its lower predictive value towards rodent carcinogens and/or *in vivo* genotoxicants has been confirmed in comparison with the TGR assay and comet assay (EFSA, 2017[3]). Therefore, a negative result in a UDS assay alone is not a proof that a substance does not induce gene mutation. Moreover, though a positive result in the UDS assay can indicate exposure of the liver DNA and induction of DNA damage by the substance under investigation, it is not sufficient information to conclude on the induction of gene mutation by the substance.

19 Therefore, as also explained in the third party comments, the comet assay or TGR assay, and not the UDS test, are the most appropriate follow-up test(s) for the Substance. Additionally, as explained in Guidance on IRs & CSA, R.7a, Section R.7.7.6.3 (page 573), both the comet assay and the TGR assay '*offer greater flexibility than the UDS test, most notably with regard to the possibility of selecting a range of tissues for study on the basis of what is known of the toxicokinetics and toxicodynamics of the substance*.'

[2] Kirkland D and Speit G (2008) Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens III. Appropriate follow-up testing *in vivo*. Mutat Res 654:114-32.

[3] EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Younes M, Aquilina G, Crebelli R, Gurtler R, Hirsch-Ernst KI, Mosesso P, Nielsen E, van Benthem J, Carfì M, Georgiadis N, Maurici D, Parra Morte J and Schlatter J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113.

20 Independent of the above, as explained in section 1, the *in vitro* chromosomal aberration study in your dossier does not meet the requirements of Section 8.4.2., Annex VIII to REACH. Therefore, by this decision, ECHA also requests an *in vitro* chromosomal aberration test (for the reasons see section 1 above), which may raise a concern for chromosomal aberration in case of positive results.

21 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 474 para. 37c; OECD TG 489 para. 33; Guidance on IRs & CSA, R.7a, Section R.7.7.6.3).

22 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 the identified concerns for chromosomal aberration as well as gene mutation.

23 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 or lack thereof. Furthermore, the combined study can help reduce the number of tests performed and the number of animals used while investigating (structural and numerical) chromosomal aberrations as well as gene mutations.

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

2.3. Specification of the study design

2.3.1. *Comet or TGR assay (if the test results of section 1 request are **negative**)*

2.3.1.1. Comet assay

25 You did not specify the species to be used for testing. 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).

26 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.

27 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.

2.3.1.1.1. Germ cells

28 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.

29 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.

2.3.1.2. TGR assay

30 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.

31 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.

32 According to the test method OECD TG 488, the test must be performed by analysing tissues from the 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, the duodenum must be stored (at or below −70 ºC) until the analysis of the 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.

2.3.1.2.1. Germ cells

33 You may consider to collect the male germ cells 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). Following the generation and analysis of data on somatic cells you should consider analysing the collected germ cells.

34 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.

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

35 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.

36 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.

37 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.

38 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[4]).

2.3.2.1. Germ cells

39 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.

40 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.

2.4. Outcome

41 Under Article 40(3)(d) and (c) your testing proposal is rejected and you are requested to carry out additional test(s) with the Substance, as specified above.

42 In the comments to the draft decision, you agree to perform the requested study. However, you specify that the performance of the TGR assay "*as an alternate test*" to the comet assay under option 2.a. is "*rejected*".

[4] Bowen D.E. *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. Mutation Research 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

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 25 November 2020.

ECHA held a third party consultation for the testing proposal(s) from 18 February 2021 until 5 April 2021. ECHA received information from third parties (see corresponding Appendix 1).

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.

In your comments you agreed to the draft decision. ECHA took your comments into account 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 took the decision according to Article 51(3) 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 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]

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[5].

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[6].

[5] https://echa.europa.eu/practical-guides
[6] https://echa.europa.eu/manuals