~~Confidential~~

Helsinki, 9 September 2022

Addressees
Registrant of JS_CAS_3468-11-9 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
08/02/2021

Registered substance subject to this decision ("the Substance")
Substance name: 1-imino-1H-isoindol-3-amine
EC number: 222-426-8

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 ***17 March 2025***.

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

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

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

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.

~~Confidential~~

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.

~~Confidential~~

Appendix 1: Reasons for the decision

Contents

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

1. In vivo mammalian alkaline comet assay

1 Further mutagenicity studies must be considered under Annex VII to REACH in case of a positive result (Section 8.4., Column 2).

2 Your dossier contains positive results for the *in vitro* mammalian cell gene mutation study (OECD TG 490; 2018) which raise the concern for gene mutations.

3 Additionally, the OECD TG 490 study shows an increase in the number of large colonies as well as an increase in number (and percentage) of small colonies, which also raise a concern for chromosomal aberrations (clastogenicity). The study report states that '*Due to the increased number of small colonies and corresponding mutagenicity in the two highest dose groups, these concentrations of the test items were considered as clastogenic*'.

4 Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

1.1. Information provided to fulfil the information requirement

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

6 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.

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

1.2. Test selection

8 The positive *in vitro* results available in the dossier indicate a concern for both chromosomal aberration and gene mutation.

9 According to the Guidance on IRs & CSA, Section R.7.7.6.3, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is suitable to follow up a positive *in vitro* result on gene mutations and chromosomal aberrations.

10 However, you may also combine the comet assay with an *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) in a single study (see OECD TG 489 para. 33; OECD TG 474 para. 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.

11 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.

12 In your comments to the draft decision, though you indicate that you will consider the option to perform the combined assay, you also state that according to the ECHA Guidance, the comet assay is considered appropriate. You question the added value of combining the MN test to the comet assay.

13 We acknowledge that the ECHA guidance in the current version does not yet reflect on the combination study (comet assay and MN test). However, we note that at the 70th and 74th meeting of the Member States Committee (MSC-70[2] and MSC-74[3], respectively), it was agreed in other cases that the combined study of the comet assay and the MN test would be most suitable when both concerns for chromosomal aberration and gene mutation exist, and no *in vivo* genotoxicity data are available in the dossier. This practice has also been communicated via the ECHA website, under the recommendations to registrants concerning the mutagenicity information requirement[4].

14 As explained above and based on the data available in your dossier, there are both concerns for gene mutation and chromosomal aberration and there are no *in vivo* genotoxicity studies available in the dossier; therefore, the combined study would be the most appropriate study to consider for your Substance.

1.3. Specification of the study design

15 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. If you decide to perform the comet assay combined with the MN test, the combined study can be performed in rats, or if justified, in mice.

16 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.

17 You proposed to perform the comet assay in the liver and in the small intestine (duodenum or jejunum). In the testing proposal and in your comments to the draft decision, you further indicate that the glandular stomach should not be used as a target organ due to the expected cytotoxicity, which may lead to equivocal or false positive reactions.

18 However, in line with the test method OECD TG 489, the test must be performed by analysing tissues from the liver, as the primary site of xenobiotic metabolism, and the 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.

19 ECHA agrees that irritation of the glandular stomach may occur with the Substance, based on its effects on the skin and the eye. However, the cytotoxicity expected in the glandular stomach does not prevent testing of this target organ. According to OECD TG 489 (see paragraphs 54 to 56), target tissue toxicity may result in increases in DNA migration, but examination of one or more indicators of cytotoxicity, including cytotoxicity markers and

[2] Minutes of the of the 70th Meeting of the Member State Committee (MSC-70), 10-12 June 2020, web conference: https://echa.europa.eu/documents/10162/28685870/MinutesofMSC-70_adopted-1.pdf/2972d2e5-6a5b-67ce-efc8-1a67a8e025a9

[3] Minutes of the of the 74th Meeting of the Member State Committee (MSC-74), 14-17 June 2021, web conference: https://echa.europa.eu/documents/10162/2200440/minutes_msc-74_en.pdf/3d901d5e-2325-227e-6fd2-c78432bdcf0a?t=1631697313501

[4] ECHA website, Support, Recommendations to registrants, Standard information requirements, Mutagenicity: https://echa.europa.eu/standard-information-requirements-recommendations

histopathological changes, can be performed to aid in the interpretation of the findings. OECD TG 489 further highlights that information on cytotoxicity at the target tissue is required to assess the biological relevance of a positive or equivocal result.

20 Based on the above, the three default tissues (liver, glandular stomach and duodenum), as agreed at the 46th Member's State Committee (MSC-46) meeting[5], must be therefore analysed, according to OECD TG 489.

21 Finally, you also indicated that care should be taken to ensure that site-of-contact tissues *'are not exposed to excessively high test substance concentrations, but rather to expected exposure concentrations to avoid false positive reactions'*, and you referred to a publication from Donovan & Burlinson (2013). ECHA reminds you that OECD TG 489 indicates that the study should aim to identify the maximum tolerated dose (MTD) or doses not inducing too high toxicity in the target organs of the comet assay (see paragraphs 36 to 38).

22 If you decide to combine OECD TGs 489 and 474 you 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]).

1.3.1. Germ cells

23 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.

24 *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.

1.4. Outcome

25 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.

[5] MSC-46 meeting, 2-4 February 2016, adopted minutes: https://echa.europa.eu/documents/10162/22837890/msc-46_minutes_en.pdf/25f1f8ff-7e8e-40d5-8910-152bf060f6a6

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 18 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 request but amended the deadline.

> In your comments on the draft decision, you requested an extension of the deadline to provide information from 18 to 24 months from the date of adoption of the decision.
>
> You justify the request for the deadline extension as you would need to perform additional investigations (1) to perform additional steps if the combined study is performed, including method development/validation of toxicokinetics and dose range finding, and (2) to differentiate between stomach cytotoxicity and genotoxicity in the comet assay.
>
> As regards (1) we note that the deadline of 18 months is the standard timeline indicated by ECHA when a combined study is requested. As for (2) we acknowledge that additional time might be required considering the properties of the Substance.
>
> On this basis, ECHA has extended the deadline to 21 months.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

The deadline of the decision has been exceptionally extended by additional 9 months from the deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

Appendix 3: Addressees of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa.

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[6].

(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 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[7].

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