~~Confidential~~

Helsinki, 19 January 2023

Addressee
Registrant of JS_EC-401-610-3 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
15/09/2021

Registered substance subject to this decision ("the Substance")
Substance name: Diamminediisocyanatozinc
EC/List number: 401-610-3

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 ***27 January 2025***.

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 vivo mammalian alkaline comet assay also requested below (triggered by Annex VIII, Section 8.4., column 2)

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

2. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method: OECD TG 489) in rats, or if justified, other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit)

4. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211)

5. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: EU C.47./OECD TG 210)

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 vivo mammalian alkaline comet assay

1 Appropriate in vivo mutagenicity studies must be considered under Annex VIII to REACH (Section 8.4., Column 2) in case of a positive result in any of the in vitro genotoxicity studies under Annex VII or VIII to REACH.

2 Your dossier contains positive results for the in vitro gene mutation study in mammalian cells (OECD TG 490, 2020) which raise the concern for gene mutations.

3 ECHA considers that an in vivo follow-up study is necessary to address the identified concern.

4 For the assessment of the testing proposal, see Section 2.

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

2. In vivo mammalian alkaline comet assay

5 An appropriate in vivo somatic cell genotoxicity is an information requirement under Annex IX to REACH (Section 8.4., Column 2) if (1) there is a positive result in any of the in vitro genotoxicity study under Annex VII or VIII to REACH and (2) there are no results available from an in vivo study.

6 Your dossier contains positive results for the in vitro gene mutation study in mammalian cells (OECD TG 490, 2020) which raise the concern for gene mutations.

7 Moreover, the in vivo study submitted in your dossier (OECD TG 474, 1988) does not address the concern on gene mutation.

2.1. Information provided to fulfil the information requirement

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

9 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.

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

2.2. Test selection

11 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 mutation.

2.3. Specification of the study design

12 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).

13 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.

14 You proposed to perform the test on the liver and the stomach. You also mentioned that "other tissues may be examined" depending on the "pre-tests and toxicokinetic considerations". ECHA notes that 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, and 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. Germ cells

15 A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required under Annex IX of REACH, in case 1) an in vivo genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

16 Therefore, you may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, in accordance with Annex IX, Section 8.4., column 2, 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.

2.4. Outcome

17 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.

3. Pre-natal developmental toxicity study

18 A pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is an information requirement under Annex IX to REACH (Section 8.7.2.).

3.1. Information provided to fulfil the information requirement

19 You have submitted a testing proposal for a PNDT study according to the OECD TG 414 by the oral route with the Substance.

20 ECHA requested your considerations for alternative methods to fulfil the information requirement for Developmental toxicity. 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.

21 ECHA agrees that a PNDT study in a first species is necessary.

22 Additionally, in your considerations you refer to Annex IX, Section 8.7., column 2, first and second indents, where the PNDT study does not need to be conducted if the substance is known to be a genotoxic carcinogen or germ cell mutagen, in specified hazard classes. Since there is a parallel testing proposal for a comet assay (request 2 above), you state that at this stage "it cannot be clearly decided whether or not the specific adaption possibilities of annexes VI to X are not adequate". You therefore propose to postpone the testing proposal for a PNDT study until the results of the comet assay become available.

23 However, ECHA notes that this decision is based on the information currently available in the dossier for the Substance. On the basis of the current data in the dossier, the Substance is not known to be a genotoxic carcinogen or germ cell mutagen.

24 ECHA therefore considers that the testing proposal for the PNDT study can be processed in parallel to the comet assay.

3.2. Specification of the study design

25 You proposed testing in the rat as a first species. You may select between the rat or the rabbit because both are preferred species under the OECD TG 414 (ECHA Guidance R.7a, Section R.7.6.2.3.2.).

26 You did not specify the route for testing. The oral route of administration is the most appropriate to investigate reproductive toxicity (ECHA Guidance R.7a, Section R.7.6.2.3.2.).

3.3. Outcome

27 Your testing proposal is accepted under Article 40(3)(a) and you are requested to conduct the test, as specified above.

4. Long-term toxicity testing on aquatic invertebrates

28 Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

4.1. Information provided to fulfil the information requirement

29 You have submitted a testing proposal for a Daphnia magna reproduction test (test method: EU C.20/OECD TG 211).

30 Your registration dossier does not include any information on long-term toxicity on aquatic invertebrates.

31 ECHA agrees that an appropriate study on long-term toxicity on aquatic invertebrates is needed.

4.2. Test selection and study specifications

32 The proposed Daphnia magna reproduction test (test method: EU C.20/OECD TG 211) is appropriate to cover the information requirement for long-term toxicity on aquatic invertebrates (Guidance on IRs and CSA, Section R.7.8.4.1.).

4.3. Outcome

33 Your testing proposal is accepted under Article 40(3)(a) and you are requested to conduct the test with the Substance, as specified above.

5. Long-term toxicity testing on fish

34 Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

5.1. Information provided to fulfil the information requirement

35 Your registration dossier does not include any information on long-term toxicity on fish.

36 Instead, you have provided the following justification to omit the study which you consider to be based on Annex XI, Section 1.2:

37 *"According to REACH Annex XI, section 1.2 of REACH Regulation (EC) No 1907/2006 further testing on vertebrate animals shall be omitted if sufficient weight of evidence is available to confirm the absence or presence of an acute or chronic hazard to the aquatic environment and to prove the absence or presence of dangerous properties for the aquatic environment. Considering all available data on short- term toxicity of the registered substance to aquatic organisms there is scientific evidence that an acute toxicity is present for all three trophic levels, leading to a classification as Aquatic Acute 1. Furthermore a chronic hazard Category 2 is recorded for algae already, taking into account that the substance is inorganic and needs to be regarded as not readily biodegradable, and a chronic hazard is highly expected for the proposed reproduction study with Daphnia magna according to OECD 211 (please refer to the respective testing proposal for further information). With a factor of about 100 compared to algae or fish Daphnia magna represents the most sensitive species based on the available short-term results, wherefore the proposed chronic reproduction study is expected to result in a chronic 1 classification even. This classification signifies the highest hazard level based on chronic toxicity data. Hence, conducting a long-term study with fish would not provide any additional relevant results or lead to a different final classification. Consequently, the presence of an acute and chronic toxicity of the registered substance is confirmed already and the worst-case classification is expected following the outstanding study on long-term toxicity to aquatic invertebrates, wherefore it is scientifically not necessary and not justifiable to perform a long-term toxicity study with fish, which is unlikely to add any further information especially considering animal welfare."*

38 ECHA has assessed this information and identified the following issue:

39 Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information enabling, through a reasoned justification, a conclusion on the information requirement, while the information from each single source alone is insufficient to fulfil the information requirement.

40 The justification must have regard to the information that would otherwise be obtained from the study that must normally be performed for this information requirement.

41 According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude on the corresponding information requirement.

42 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex IX, Section 9.1.6. includes similar information that is produced by the OECD TG 210. OECD TG 210 requires the study to investigate the following key elements:

1. Stage of embryonic development
2. Hatching and survival of embryos and larvae
3. Survival of juvenile fish
4. Abnormal appearance
5. Abnormal behaviour (e.g. hyperventilation, uncoordinated swimming, atypical quiescence and atypical feeding behaviour)
6. Weight at the end of the test

7. Length at the end of the test

43 None of the source studies cited in your justification investigate these key parameters. Therefore, they do not provide information that would contribute to the conclusion on these key parameters.

44 It is not possible to conclude, based on any source of information alone or considered together, on the information requirement for long-term toxicity in fish.

45 Therefore, your adaptation is rejected and the information requirement is not fulfilled.

46 In your comments to the draft decision, you further refer to Guidance on IRs and CSA Chapter R.7b, version 4.0 of June 2017 with a view to an adaptation based on considerations from the chemical safety assessment under Annex IX, section 9.1, Column 2. You intent to conduct the long-term toxicity testing on aquatic invertebrates (request 4) before a long-term fish toxicity test is considered. You indicate your intention to perform long-term toxicity testing in fish *"only if PEC/PNEC is >1 with assessment factor 50"*. As these considerations implicate sequential testing, you request an extension of the deadline for provision of the information.

47 Since the Board of Appeal's decision in cases A-010-2018 and A-011-2018, ECHA no longer considers REACH Annex IX, section 9.1, Column 2 as a basis for waiving of the standard information required under Column 1. In that regard, information on aquatic toxicity described in ECHA guidance on IRs and CSA related to REACH Annex IX, section 9.1, Column 2 as a waiver for the information requirement under Column 1 is no longer valid, as also highlighted on ECHA's website[2]. Both the information on long-term toxicity testing on aquatic invertebrates as well as the information on long-term toxicity testing on fish are standard information required under Annex IX, section 9.1, Column 1. Thus, the deadline of the decision is set based on standard practice for carrying out the OECD TG tests in question.

48 The information provided in your comments does not change the assessment and you remain responsible for complying with this decision for both requests 4 and 5 by the set deadline.

5.2. Test selection and study specifications

49 The Fish, Early-Life Stage Toxicity Test (test method: OECD TG 210) is appropriate to cover the information requirement for long-term toxicity on fish (Guidance on IRs and CSA, Section R.7.8.4.1.).

5.3. Outcome

50 Under Article 40(3)(c) of REACH, ECHA may require a registrant to carry out one or more additional tests in case of non-compliance of the testing proposal with Annexes IX, X or XI of the REACH Regulation. The information requirement on Aquatic toxicity at Annex IX covers both long-term toxicity on invertebrates (Section 9.1.5.) and on fish (Section 9.1.6.). However, you have provided a testing proposal for long-term testing on aquatic invertebrates only. As explained above, the information requirement for long-term toxicity on fish is not fulfilled.

51 Therefore, under Article 40(3)(c) of REACH, you are requested to carry out the additional test with the Substance, as specified above.

[2] See the information on "Adaptation of long-term aquatic toxicity testing under Annex IX to REACH" at https://echa.europa.eu/standard-information-requirements-recommendations.

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 received your testing proposal(s) on 15 September 2021 and started the testing proposal evaluation in accordance with Article 40(1).

ECHA held a third party consultation for the testing proposal(s) from 10 May 2022 until 27 June 2022. 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.

In your comments on the draft decision, you requested an extension of the deadline to provide information from 12 to 24 months from the date of adoption of the decision to allow sequential long term toxicity testing on aquatic invertebrates and fish. As explained in request 5, the deadline of the decision is set based on standard practice for carrying out OECD TG tests. However, the deadline of the decision has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.On this basis, ECHA has extended the deadline to 24 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.

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, VIII and IX to REACH, for registration at 100-1000 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[3].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, 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 must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. Test material

(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 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.

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