ECHA
EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-0000004004-88-03/F Helsinki, 28 November 2013

DECISION ON A TESTING PROPOSAL SET OUT IN A REGISTRATION PURSUANT TO ARTICLE 40(3) OF REGULATION (EC) NO 1907/2006

For 2,2`-iminodi(ethylamine), CAS No 111-40-0 (EC No 203-865-4), registration number: [redacted]

Addressee: [redacted]

The European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 51 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

I. Procedure

Pursuant to Article 40(1) of the REACH Regulation, ECHA has examined testing proposals submitted as part of the registration dossier in accordance Articles 10(a)(ix) and 12(1)(e) thereof for 2,2`-iminodi(ethylamine), CAS No 111-40-0 (EC No 203-865-4), by [redacted] (Registrant).

- Unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo* (OECD 486)
- Pre-natal developmental toxicity study in rats (OECD 414)
- Two-generation reproduction toxicity study in rats (OECD 416)
- Earthworm reproduction test (*Eisenia fetida/Eisenia andrei*) (OECD 222)

The present decision relates only to the examination of the testing proposals:

- Unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo* (OECD 486)
- Pre-natal developmental toxicity study in rats (OECD 414)
- Earthworm reproduction test (*Eisenia fetida/Eisenia andrei*) (OECD 222)

The testing proposal for fulfilling the information requirement for a reproductive toxicity study (Annex X, 8.7.3.) is addressed in a separate decision although all these were initially addressed together in the same draft decision.

The present decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 1000 tonnes or more per year. This decision does not take into account any updates after 20 June 2013, the date upon which ECHA notified its draft decision to the Competent Authorities of the Member States pursuant to Article 51(1) of the REACH Regulation.

This decision does not imply that the information provided by the Registrant in his registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

On 6 September 2010, pursuant to Article 40(1) of the REACH Regulation, ECHA initiated the examination of the testing proposals set out by the Registrant in the registration dossier for the substance mentioned above.

ECHA held a third party consultation for the testing proposals from 5 April 2011 until 20 May 2011. ECHA did receive information from third parties (see section III below).

On 5 January 2012 ECHA sent the draft decision to the Registrant and invited him to provide comments within 30 days of the receipt of the draft decision.

On 3 February 2012 ECHA received comments from the Registrant agreeing to ECHA's draft decision.

On 31 January 2013 the Registrant updated his registration dossier.

On 20 June 2013 ECHA notified the Competent Authorities of the Member States of its draft decision and invited them pursuant to Article 51(1) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, Competent Authorities of the Member States submitted proposals for amendment to the draft decision.

On 26 June 2013 ECHA notified the Registrant of proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

ECHA reviewed the proposals for amendment received and decided to amend the draft decision.

On 5 August 2013 ECHA referred the draft decision to the Member State Committee.

On 26 August 2013 the Registrant did provide comments on the proposed amendment. The Member State Committee took the comments of the Registrant into account. After discussion in the Member State Committee meeting on 25-27 September 2013, a unanimous agreement of the Member State Committee on the draft decision on the draft decision relating to Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (OECD 488), pre-natal developmental toxicity study in rats (OECD 414) and earthworm reproduction test (OECD 222) as modified at the meeting was reached on 26 September 2013. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

Pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant shall carry out the following tests using the indicated test methods with the registered substance:

1. Mutagenicity – Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (Annex X, 8.4., test method: OECD 488). The test shall be conducted in mice or rats treated for 28 days via oral route, and tissues (stomach, liver, bone marrow) shall be harvested three days after the cessation of the treatment. Mutation frequency shall be assessed in stomach, liver and bone marrow,

while pursuant to Article 40(3)(d) the original testing proposal for Unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo* (OECD 486) is rejected.

Pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant shall carry out the following proposed tests using the indicated test methods with the registered substance:

2. Pre-natal developmental toxicity study in rats or rabbits, oral route (Annex IX, 8.7.2; test method EU B.31 / OECD 414);

3. Earthworm reproduction test (*Eisenia fetida/Eisenia Andrei*) (Annex X, 9.4.4; test method OECD 222).

The Registrant shall determine the appropriate order of the studies taking into account the possible outcome and considering the possibilities for adaptations of the standard information requirements according to the column 2 provisions of the respective Annex and those contained in Annex XI of the REACH Regulation.

Pursuant to Articles 40(4) and 22 of the REACH Regulation, the Registrant shall submit to ECHA by **28 May 2015** an update of the registration dossier containing the information required by this decision.

Data from a second pre-natal developmental toxicity study on another species is a standard information requirement according to Annex X, 8.7.2. of the REACH Regulation. The Registrant should firstly take into account the outcome of the pre-natal developmental toxicity on a first species and all other relevant available data to determine if the conditions are met for adaptations according to Annex X, 8.7. column 2, or according to Annex XI. If the Registrant considers that testing is necessary to fulfill this information requirement, he should include in the update of his dossier a testing proposal for a pre-natal developmental toxicity study on a second species.

At any time, the Registrant shall take into account that there may be an obligation to make every effort to agree on sharing of information and costs with other Registrants.

III. Statement of reasons

The decision of ECHA is based on the examination of the testing proposals submitted by the Registrant for the registered substance and scientific information submitted by third parties.

1. Mutagenicity, *in vivo* (Annex X, 8.4., OECD Guideline 488)

An *in vitro* gene mutation study in bacteria is required under both Annex VII, 8.4.1 of the REACH Regulation and Annex VIII, Level 1 of Directive 67/548/EEC. According to Article 13(3) of the REACH Regulation, tests that are required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the Agency as being appropriate.

According to Annex X, section 8.4., of the REACH Regulation, in case any *in vitro* test required at Annex VII or VIII revealed positive results and no appropriate results are available, a second *in vivo* somatic cell test may be necessary, depending on the quality and relevance of all the available data.

ECHA notes that information on seven *in vitro* gene mutation studies in bacteria have been submitted, three of which were positive. No information from an appropriate follow-up *in vivo* assay for gene mutations has been submitted, while an *in vivo* assay for structural and numerical chromosome damage was negative.

The Registrant has proposed the conduct of the Unscheduled DNA Synthesis (UDS) *in vivo* assay but after considering the scientific reasoning for a proposal for amendment ECHA rejects the proposal for a UDS assay and replaces it by a requirement for the conduct of the Transgenic Rodent Somatic and Germ Cell Assay (TGR) because of substance specific scientific reasons. There is a concern for a direct action of the substance as a mutagen at initial sites of contact with the body, as evidenced by the ability of the substance to cause mutations in an Ames test without activation, and consistent with the ability of the substance to act as a sensitiser. The UDS assay in liver is not capable of detecting genotoxicity in the initial site of contact with the body, and therefore, there is substance specific information available that an UDS test is not an appropriate *in vivo* follow-up test concerning the indicated *in vitro* gene mutagenicity. The appropriate test to follow-up *in vivo* gene mutation is the TGR assay.

The Registrant is requested to perform the TGR assay in stomach, liver and bone marrow. The reasons for tissue selection, as outlined in the test guideline (OECD 488 paragraphs 37 and 38), are that the stomach was chosen due to oral administration and to evaluate mutation at the initial site of contact with the body, and also because it is a rapidly dividing tissue. Liver was chosen to study an effect on a tissue that is also exposed to systematically available substances and as it is a main site of metabolism. Finally, the bone marrow was selected since there are indications from the repeat dose studies that this is a target organ (i.e. changes in various blood cell counts), and also because it is a rapidly dividing cell population.

The Registrant is reminded that according to the column 2 of section 8.4 of Annex X of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, "the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered". The TGR test method can detect some gene mutations in germ cells in spermatozoa from the vas deferens and in developing germ cells from the seminiferous tubules collected three days after a 28-day exposure (OECD 488, paragraph 33). Thus, the Registrant may consider collecting and storing e.g. male germ cells for potential further analysis of germ cell mutagenicity in case positive results are obtained from the somatic cells.

Pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant is required to carry out the following test: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (test method: OECD 488) using the registered substance. The originally proposed test for an *in vivo* UDS test with mammalian liver cells is rejected in accordance with the Article 40(3)(d). The test shall be conducted in mice or rats treated for 28 days via oral route, and tissues (stomach, liver, bone marrow) shall be harvested three days after the cessation of treatment. Mutation frequency shall be assessed in the collected in stomach, liver and bone marrow.

The Registrant provided comments on the proposal for amendments (i.e. proposal to perform a TGR assay instead of the original proposed *in vivo* UDS test) submitted by the Member State Competent Authority. In his comments the Registrant indicated that further analysis of the mutagenicity data has been performed and based on their weight of evidence evaluation specifically for the Ames studies and other *in vitro* and *in vivo* studies on genetic toxicity. The Registrant considers that the data does not suggest a consistent mutagenic response of the registered substance. Based on this the Registrant considers that no further testing is warranted to evaluate the genotoxic potential of this chemical.

ECHA acknowledges the Registrant's comments. Based on the Registrant's comments ECHA understands that the Registrant intends to withdraw its testing proposal for *in vivo* mutagenicity. However the current draft decision is based on the information provided in the dossier at the time the draft decision was submitted to the Member State Competent Authorities and the decision does not take into account any updates after ECHA has notified its draft decision to the Competent Authorities of the Member States pursuant to Article 51(1) of the REACH Regulation i.e. 20 June 2013.

2. Pre-natal developmental toxicity study (Annex IX, 8.7.2; test method EU B.31 / OECD 414)

A pre-natal developmental toxicity study for a first species is a standard information requirement as laid down in Annex IX, section 8.7.2., of the REACH Regulation. According to section 8.7.2. of Annex X subject to the Annex IX, 8.7.2. column 2 requirements of the REACH Regulation, a further pre-natal developmental toxicity study performed in a second species is required to fulfil the standard information requirements. The information available on this endpoint for the registered substance in the technical dossier does not meet these information requirements. Consequently there is an information gap and it is necessary to generate the data for this endpoint.

The Registrant proposes to perform a pre-natal developmental toxicity study in rats. In his technical dossier the Registrant additionally proposes that depending on the outcome of the pre-natal developmental toxicity study in rats and two-generation reproductive toxicity study in rats, it will be decided if a second pre-natal developmental toxicity study in rabbits is necessary.

According to the test method EU B.31/OECD 414, the rat is the preferred rodent species, the rabbit the preferred non-rodent species and the test substance is usually administered orally. ECHA considers these default parameters appropriate and testing should be performed by the oral route with the rat or the rabbit as a first species to be used.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed study: Pre-natal developmental toxicity study in rats or rabbits, oral route (Annexes IX and X , 8.7.2; test method EU B.31 / OECD 414) using the registered substance.

3. Earthworm reproduction test (*Eisenia fetida/Eisenia Andrei*) (Annex X, 9.4.4; OECD 222)

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed earthworm reproduction test (*Eisenia fetida/Eisenia Andrei*) (Annex X, 9.4.4; OECD 222).

An earthworm reproduction test is a standard information requirement as laid down in Annex X, section 9.4.4., of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to generate the data for this endpoint.

The Registrant has justified the reason for performing the proposed test as follows: "*Since invertebrates were the most sensitive group as indicated from aquatic toxicity data, a chronic earthworm study (limit test) is planned for this endpoint in accordance with REACh guidance for compounds in soil hazard category 3 (Table R.7.11 -2). Based on the outcome of this test and the screening level risk assessment, a decision will be rendered as to whether additional terrestrial toxicity testing is necessary for diethylene triamine*".

The establishment of invertebrates as the most sensitive group is based on the following values: "*Since invertebrates were the most sensitive group as indicated from aquatic toxicity data, a chronic earthworm study (limit test) is planned for this endpoint in accordance with REACh guidance for compounds in soil hazard category 3 (Table R.7.11 -2)*".

"*For long-term toxicity to aquatic invertebrates, the 21-day NOEC is 5.6 mg/L based on survival and reproduction" while the long-term fish toxicity 28-day NOEC based on an early life stage test is 10 mg/L and "in a growth inhibition test the 72-hour ErC50 is 1164 mg/L and the 72-hour NOEC is 10 mg/L".*

ECHA considers this justification appropriate for testing of the registered substance due to its intrinsic properties. The Registrant indicates that "*the substance is a liquid, acid dissociation constants (pKa) of approximately 4.9, 9.4, and 10.1; very high water solubility (miscible), estimated octanol-water partition coefficient (log Kow) of -1.58 in its completely non-ionized state, estimated octanol-water distribution coefficient (log D) of -5.58 at pH 7, and an average Koc value was determined which was 19,111 ± 12,496 at 25 °C (mean ± 1 std. dev., n=6) (range log Koc: >= 3.4 — <= 4.6). The substance exhibits a high affinity for adsorption to soil and sediment, despite these hydrophilic properties, due to participation in cation exchange interactions with soil minerals (i. e., clay). Collectively, these properties result in low potential for inter-media transport in the environment. Atmospheric emissions will be readily deposited to soil and surface waters by both wet-and dry deposition processes. Conversely, emissions to soil or water will neither result in volatilization to air, nor significant exchange between soil and water, due to high water solubility and high affinity for soil/sediment*".

The Registrant has indicated "*a chronic earthworm study (limit test) is planned for this endpoint in accordance with REACh guidance for compounds in soil hazard category 3 (Table R.7.11 -2)".* In the ECHA guidance R7 C table R7.11-2, it states "conduct a confirmatory long term soil toxicity Testing (e.g. one limit test with the most sensitive organism group as indicated from aquatic toxicity data)." Thus, the guidance indicates for example a "limit test". ECHA advises the Registrant to perform the test in accordance with the OECD 222 guideline which states, "If no effects are observed at the highest concentration in the range-finding test (i.e. 1000 mg/kg), the reproduction test would be performed as a limit test, using a test concentration of 1000 mg/kg. A limit test will provide the opportunity to demonstrate that the NOEC for reproduction is greater than the limit concentration whilst minimising the number of worms used in the test. Eight replicates should be used for both the treated soil and the control". Thus, a range-finding test will determine the concentrations selected for the definitive testing.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed study: Earthworm reproduction test (*Eisenia fetida/Eisenia Andrei*) (Annex X, 9.4.4; OECD 222) using the registered substance.

Notes for consideration of the Registrant:

ECHA notes that the proposed test only addresses invertebrates (i.e. the information requirement in Annex X, section 9.4.4.) and does not address the other two trophic levels required for this tonnage band (effects on soil micro-organisms (Annex IX, section 9.4.2.) and long-term toxicity testing on plants (Annex X, section 9.4.6.). ECHA acknowledges the Registrant's assessment regarding the particular characteristics of this substance, justified by the reported physical-chemical and environmental fate properties as well as the low toxicity observed in the available studies. The Registrant has indicated that further testing will be considered depending on the outcome of the current testing proposal.

Once results of the requested toxicity test on terrestrial invertebrates are available, in accordance with Annex I of the Reach Regulation, the Registrant shall revise the chemical safety assessment as necessary. He shall furthermore consider whether there is a need to investigate further the effects on terrestrial organisms in order to fulfil the information requirements of section 9.4. of Annexes IX and X and if necessary, submit testing proposals for additional terrestrial toxicity tests. If the Registrant concludes that no further investigation of effects on terrestrial organisms is required, he shall update his technical dossier by clearly stating the reasons for adapting any information requirement of Annex IX, section 9.4. and Annex X, section 9.4. of the REACH Regulation.

4. Response to third party information

In response to the testing proposals involving vertebrate animals (points 1 and 2 of Section II), a third party has proposed evaluation based on existing data and an alternative testing strategy in a weight-of-evidence approach.

ECHA concludes the following:

The third party has proposed a strategy for ECHA to consider before further vertebrate tests are requested. However, third parties were invited, as specified by Article 40(2) of the REACH Regulation to submit "scientifically valid information and studies that address the relevant substance and hazard end-point, addressed by the testing proposal". As the proposal for a strategy as such cannot be regarded information or studies, ECHA concludes that this is not a sufficient basis to fulfil the data/information requirement.

Another third party has proposed a prediction using a non-linear classification ANN QSAR model for pre-natal developmental toxicity.

ECHA concludes the following:

The result from the QSAR classification model (i.e. "toxic" or "non-toxic") is not suitable for the purposes of classification and labelling for the endpoint for which testing has been proposed to meet the information requirement (Annexes IX or X, 8.7.). Compliance with the Annex XI, section 1.3 requirements could not be established as the required information concerning the validity, adequacy for classification, labelling and/or risk assessment and documentation of the model was not provided. In addition, the submitted information suggests that the registered substance might be outside the applicability domain of the model. The model does not provide sufficient information to deduce whether the training set was constructed from studies that cover the information requirements of the OECD TG 414 guideline, or important study aspects, such as the species, dose selection and number of animals used.

ECHA concludes that on this occasion, the information submitted does not meet the conditions for the adaptation on the basis of QSAR models set out in Annex XI, Section 1.3. Therefore, it cannot constitute an acceptable adaptation to standard information requirements.

5. Deadline for submitting the information

In the draft decision communicated to the Registrant the time indicated to provide the requested information was 30 months from the date of adoption of the decision. This period of time took into account the fact that the draft decision also requested one other study. As the study is not addressed in the present decision, ECHA considers that a reasonable time period for providing the required information in the form of an updated IUCLID5 dossier is 18 months from the date of the adoption of the decision. The decision was therefore modified accordingly.

IV. Adequate identification of the composition of the tested material

The process of evaluation of testing proposals set out in Article 40 of the REACH Regulation aims at ensuring that the generation of information is tailored to real information needs in order to prevent unnecessary testing. The information submitted in the registration dossier was sufficient to confirm the identity of the substance for the purpose of assessing the testing proposal. It is noted, however, that this information, or the information submitted by other registrants of the same substance, has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In relation to the proposed tests, the sample of substance used for the new studies must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is within the specifications of the substance composition that are given by the joint registrants. It is the responsibility of all the joint registrants of the same substance to agree with the tests proposed in the testing proposal (as applicable to their tonnage level) and to document the necessary information on its composition. The substance identity information of the registered substance and of the sample tested must enable ECHA to confirm the relevance of the testing for the substance actually registered by each joint registrant. Finally, the studies must be shared by the joint registrants concerned.

V. General requirements for the generation of information and Good Laboratory Practice

ECHA always reminds registrants of the requirements of Article 13(4) of the REACH Regulation that ecotoxicological and toxicological tests and analyses shall be carried out in compliance with the principles of good laboratory practice (GLP). National authorities monitoring GLP maintain lists of test facilities indicating the relevant areas of expertise of each facility.

According to Article 13(3) of the REACH Regulation, tests that are required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the European Chemicals Agency as being appropriate. Thus, the Registrant shall refer to Commission Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 as adapted to technical progress or to other international test methods recognised as being appropriate and use the applicable test methods to generate the information on the endpoints indicated above.

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VI. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Article 51(8) of the REACH Regulation. Such appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/web/guest/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

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