EUROPEAN CHEMICALS AGENCY

Helsinki, 26 April 2019

Addressee: [redacted]

Decision number: TPE-D-2114465991-37-01/F
Substance name: 2,2'-azobis[2-methylpropionamidine] dihydrochloride
EC number: 221-070-0
CAS number: 2997-92-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 07/05/2018
Registered tonnage band: 100-1000

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation ((EC) No 1907/2006) (the REACH Regulation), ECHA examined your testing proposal(s) and decided as follows.

Your testing proposal is accepted and you are requested to carry out:

1. **Dissociation constant (Annex IX, Section 7.16.; test method: OECD TG 112) using the registered substance.**

Your testing proposal is modified and you are requested to carry out:

2. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: OECD TG 408) in rats using the registered substance modified to include urinalysis and a full histopathological examination which is to include immunohistochemical investigation of renal pathology to determine if the pathology is mediated by alpha-2u globulin nephropathy.**

Your testing proposals are accepted and you are requested to carry out:

3. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) in a first species (rat or rabbit), oral route using the registered substance.**
4. **Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: Daphnia magna reproduction test, EU C.20./OECD TG 211) using the registered substance.**
5. **Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.; test method: Aerobic mineralisation in surface water – simulation biodegradation test (test method: EU C.25/OECD TG 309) at a temperature of 12°C including the identification of the degradation products (Annex IX, Section 9.2.3.) with the registered substance.**

You have to submit the requested information in an updated registration dossier by **3 May 2021**. You shall also update the chemical safety report, where relevant.

The reasons for this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and advice and further observations are provided in Appendix 3.

Appeal

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

Authorised[1] by **Claudio Carlon**, Head of Unit, Hazard Assessment

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

The decision of ECHA is based on the examination of the testing proposals submitted by you and scientific information submitted by third parties.

1. Dissociation constant (Annex IX, Section 7.16.)

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Dissociation constant" is a standard information requirement as laid down in Annex IX, Section 7.16 of the REACH Regulation. The information on this endpoint is not available for the registered substance subject to the present decision but needs to be present in the technical dossier to meet the information requirements. Consequently, there is an information gap and you need to provide information for this endpoint.

You have submitted a testing proposal for a dissociation constant in water study (test method: OECD TG 112).

ECHA considers the proposed test appropriate and testing should be performed with the registered substance.

In your comments to the draft decision you agreed to perform the test.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed test using the registered substance: Dissociation constants in water (test method: OECD TG 112).

2. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.)

a) Examination of the testing proposal

Pursuant to Article 40(3)(b) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test under modified conditions.

A sub-chronic toxicity study (90 day) is a standard information requirement as laid down in Annex IX, Section 8.6.2. 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 provide information for this endpoint.

You have submitted a testing proposal for a sub-chronic toxicity study (90 day) in rats by the oral route according to OECD TG 408.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Sub-chronic toxicity (90-day): oral. ECHA notes that 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.

You proposed testing by the oral route. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA agrees that the oral route - which is the

preferred one as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017) Chapter R.7a, Section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the registered substance is a solid used in granular form and there are no indications for significant inhalation exposure of humans (e.g., spray application). Hence, the test shall be performed by the oral route using the test method OECD TG 408.

Therefore, ECHA considers that the proposed study performed by the oral route with the registered substance is appropriate to fulfil the information requirement of Annex IX, Section 8.6.2. of the REACH Regulation.

You proposed testing in rats. According to the test method OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

In the "Repeated dose 28-day oral toxicity study", conducted according to OECD TG 407, present in your registration dossier, adverse effects such as an increase in the kidney weight at the highest dose level of 75 mg/kg bw/day were observed in male rats. No adverse kidney findings were observed in female rats at any of the dose levels studied. ECHA notes that you considered the finding in the kidneys of male rats as an adverse effect as it was taken into account in NOAEL setting among other findings at the highest dose level. Although there were no histopathological findings in the kidneys, the fact that these effects were only observed in male rats may indicate that the registered substance may induce alpha-2u-globulin-mediated nephropathy. ECHA accordingly considers that the kidney is a target organ of the registered substance. Since humans do not excrete alpha-2u-globulin and this mode of action is not relevant to humans, the involvement of alpha-2u-globulin in the kidney effects is a key parameter for establishing the relevance of the kidney effects for risk assessment. For these reasons, ECHA considers that urinalysis is required to investigate kidney function (which is optional in paragraphs 3 and 37 of OECD TG 408). Additionally, a full histopathological examination (paragraphs 3, 45 and 47 of OECD TG 408), which is to include immunohistochemical investigation of renal pathology to determine if the pathology is indeed mediated by alpha-2u globulin.

In your comments to the draft decision you agreed to perform the test.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

The third party has indicated that "*The published Registration Dossier includes a OECD Guideline 407 (Klimisch 1), a daily oral administration of the test item, 2,2'-azobis(2-amidinopropane)-dihydrochloride, to Wistar rats at a dose level of 75, 25 and 8 mg/kg body weight over a time period of 28 days resulted in minor systemic effects in the male high dose group. Concerning the findings in kidney weight, adrenal-gland size and potassium levels in the male high dose group, the NOAEL in this study was 25 mg/kg body weight over a 28 day application period. Based on the findings in this study a sub-chronic study for the oral route in rats is proposed as no other study is available using other administration routes. If the proposed studies are confirmed by ECHA, they should be performed in a step-wise manner to avoid unnecessary testing (e.g. if clearly adverse effects sufficient for classification are seen in a study in the initial species).*"

ECHA notes that the third party did not provide any scientific data which would fulfil the information requirement for subchronic 90-day toxicity study (Annex IX, Section 8.6.2.). ECHA also considers that there is no rationale to consider sequential testing in terms of classification and conduct subchronic toxicity study (90-day) and pre-natal developmental study in a step-wise manner. ECHA notes that subchronic toxicity study cannot provide any classification which could be relevant to justify an adaptation of the standard information requirement for pre-natal developmental toxicity study, or vice versa.

c) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the modified study with the registered substance subject to the present decision: Sub-chronic toxicity study (90-day) in rats, oral route (test method: OECD TG 408), modified to include urinalysis and a full histopathological examination which is to include immunohistochemical investigation of renal pathology to determine if the pathology is mediated by alpha-2u globulin nephropathy.

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

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. 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 provide information for this endpoint.

You have submitted a testing proposal for a pre-natal developmental toxicity study in rats according to OECD TG 414.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Reproductive toxicity (pre-natal developmental toxicity). ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA considers that the proposed study performed with the registered substance is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

You proposed testing with the rat as a first species. According to the test method OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default consideration, ECHA considers testing should be performed with the rat or rabbit as a first species.

You did not specify the route for testing.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision you agreed to perform the test.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

A third party has indicated that "*The published Registration Dossier includes a combined OECD 421 study. In this study a daily oral administration of the test item to male rats at dose level of 6,7 mg, 20 mg and 60 mg/kg body weight over a time period of 43 to 50 days did not produce any pathological evidence to toxic effects on the reproduction performance of male rats. However, effects of the spermatogenesis may not have had an adequate time to become evident (such as reduced sperm counts affecting the fertility) as chemical exposure does not cover a complete cycle of spermatogenesis in male test animals.*"

ECHA notes that the third party did not provide any scientific data which would fulfil the information requirement for pre-natal developmental toxicity study (Annex IX, Section 8.7.2.).

ECHA notes that an OECD 421 screening study is not a test method that corresponds to the standard information requirement of Annex IX, Section 8.7.2 for a pre-natal developmental toxicity study because it does not provide equivalent information. The screening study does not cover the key parameters of a pre-natal developmental toxicity study which are, for example, examinations of the foetuses for skeletal and visceral malformations. Consequently, the information provided by third parties is not sufficient to adapt this information requirement.

c) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed study with the registered substance subject to the present decision: Pre-natal developmental toxicity study in a first species (rats or rabbits), oral route (test method: OECD TG 414).

Notes for your consideration

For the selection of the appropriate species you are advised to consult ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017), Chapter R.7a, Section R.7.6.2.3.2.

4. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex IX, Section 9.1.5. 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 provide information for this endpoint.

You have submitted a testing proposal for testing the registered substance for long-term toxicity testing on aquatic invertebrates *Daphnia magna* reproduction test, EU C.20/OECD TG 211 with the following justification: *"Based on the available short-term testing exposing all three trophic levels it is concluded that invertebrates are the most sensitive level. Therefore a long-term study with invertebrates is proposed to evaluate the potential for long-term effects of the test item towards aquatic organisms."*

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 9.1.5 of the REACH Regulation.

According to ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), Chapter R7b (Section R.7.8.5 including Figure R.7.8-4), if based on acute aquatic toxicity fish or invertebrates is shown to be substantially more sensitive than the other, a long-term study on the more sensitive species is required.

You have indicated that aquatic invertebrates are substantially more sensitive than fish. ECHA agrees with your assessment.

In your comments to the draft decision you agreed to perform the test.

Therefore, pursuant to Article 40(3)(a)of the REACH Regulation, you are requested to carry out the proposed test using the registered substance subject to the present decision: *Daphnia magna* reproduction test (test method: EU C.20/OECD TG 211).

Notes for your consideration

Once results of the proposed test on long-term toxicity to aquatic invertebrates are available, you shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation. If the revised chemical safety assessment indicates the need to investigate further the effects on aquatic organisms, you shall submit a testing proposal for a long-term toxicity test on fish in order to fulfil the standard information requirement of Annex IX, 9.1.6. If you come to the conclusion that no further investigation of effects on aquatic organisms is required, you shall update your technical dossier by clearly stating the reasons for adapting the standard information requirement of Annex IX, 9.1.6. taking into account the new data generated by the *Daphnia* study requested by the present decision.

Due to the fact that the substance is hydrolytically unstable you should consult OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO (2000)6/REV1 (6 July 2018) and ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), Chapter R7b, table R. 7.8-3 summarising aquatic toxicity testing of difficult substances for choosing the design of the requested long-term ecotoxicity tests and for calculation and expression of the result of this test.

5. Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.)

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Simulation testing on ultimate degradation in surface water" is a standard information requirement as laid down in Annex IX, Section 9.2.1.2. 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 provide information for this endpoint.

You have submitted a testing proposal for testing the registered substance in a Simulation biodegradation study in surface water (OECD TG 309 / EU C.25) with the following justification:

"*The substance itself or its transformation products identified based in the findings in the publication of Werber can be considered as soluble in water. Additionally, findings in the screening tests for biodegradation showed limited degradation under test conditions only, and therefore neither the substance nor (all) transformation products can be considered as readily biodegradable. These conclusions are supported by estimations on biodegradation using the OECD QSAR Toolbox. The study should be performed at 12 °C in line with the guidance documents. Based on the findings and estimations regarding the partition coefficient of the substance and its transformation products as well as the identified use pattern a relevant exposure of the sediment and/or soil is considered to be unlikely. Therefore, only simulation testing in surface water is proposed.* ".

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 9.2.1.2. of the REACH Regulation.

You propose to test at the temperature of 12°C. ECHA notes that performing the test at the temperature of 12°C is within the applicable test conditions of the Test Guideline OECD TG 309. Therefore, the test should be performed at the temperature of 12°C.

The information currently available in the technical dossier and the Chemical Safety Assessment (CSA) is not sufficient to conclude on the biodegradation potential and consequently the persistence of the registered substance or its degradation products in water and thus, it is necessary to generate additional information for this endpoint.
In the OECD TG 309 Guideline two test options, the "pelagic test" and the "suspended sediment test", are described. ECHA considers that the pelagic test option should be followed as that is the recommended option for P assessment. The amount of suspended solids in the pelagic test should be representative of the level of suspended solids in EU surface water. The concentration of suspended solids in the surface water sample used should therefore be approximately 15 mg dw/L. Testing natural surface water containing between 10 and 20 mg SPM dw/L is considered acceptable. Quantification of non-extractable residues (NER) is also recommended in surface water simulation degradation studies. Furthermore, when reporting NER in your test results you should explain and scientifically justify the extraction procedure and solvent used obtaining a quantitative measure of NER as described in Guidance on information requirements and chemical safety assessment R.7b (version 4.0, June 2017) and R.11 (version 3.0, June 2017).

According to Section 9.2.3 in Annex IX of the REACH Regulation identification of degradation products is a standard information requirement. You have not justified an adaptation of this requirement. Consequently there is an information gap and it is necessary to provide information for this information requirement. The identification of degradation products should therefore be included in the requested degradation simulation test. It is also noted that the OECD TG 309 Test Guideline features the formation and identification of the degradation products.

In your comments to the draft decision you agreed to perform the test.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed study using the registered substance subject to the present decision: Aerobic mineralisation in surface water – simulation biodegradation test (test method: EU C.25/OECD TG 309) at a temperature of 12°C including the identification of the degradation products (Annex IX, Section 9.2.3.).

Notes for your consideration

In accordance with Annex I, Section 4, of the REACH Regulation you should revise the PBT assessment when results of the test(s) detailed above is available. The Registrant is also advised to consult the ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, June 2017), Chapter R.11.4.1.1. and Figure R. 11-3 on PBT assessment for the integrated testing strategy for persistency assessment in particular taking into account the degradation products of the registered substance.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 7 May 2018.

ECHA held a third party consultation for the testing proposals from 05 July 2018 until 20 August 2018. ECHA received information from third parties (see Appendix 1).

This decision does not take into account any updates after **7 January 2019**, 30 calendar days after the end of the commenting period.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

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: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your 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.

2. Failure to comply with the requests in this decision will result in a notification to the enforcement authorities of the Member States.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.