Decision number: TPE-D-2114309029-55-01/F Helsinki, 30 September 2015

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

For Reaction mass of N-[2-(2-oxoimidazolidin-1-yl)ethyl]methacrylamide and methacrylic acid, EC No 934-058-1, 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 the following testing proposals submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for Reaction mass of N-[2-(2-oxoimidazolidin-1-yl)ethyl]methacrylamide and methacrylic acid, EC No 934-058-1, submitted by [redacted] (Registrant).

- *In vivo* genotoxicity: micronucleus assay (chromosome aberration) (OECD 474);
- 90-days oral toxicity study (OECD 408);
- Developmental toxicity / teratogenicity study (OECD 414) in rats, oral route;
- Long-term toxicity testing on fish (OECD 210).

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 100 to 1000 tonnes per year. This decision does not take into account any updates after 7 June 2015, i.e. 30 calendar days after the end of the commenting period.

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.

ECHA received the registration dossier containing the above-mentioned testing proposals for further examination pursuant to Article 40(1) on 11 October 2013.

ECHA held a third party consultation for the testing proposals from 16 May 2014 until 30 June 2014. ECHA did not receive information from third parties.

On 31 March 2015 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 16 April 2015 ECHA received comments from the Registrant agreeing to ECHA's draft decision.

The ECHA Secretariat considered the Registrant's comments and did not amend the draft decision.

On 11 June 2015 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 for amendment of the draft decision within 30 days of the receipt of the notification. Subsequently, proposals for amendment to the draft decision were submitted.

On 17 July 2015 ECHA notified the Registrant of the proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposals for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposals for amendment received and amended the draft decision.

On 27 July 2015 ECHA referred the draft decision to the Member State Committee.

By 17 August 2015 the Registrant did not provide any comments on the proposal for amendment.

A unanimous agreement of the Member State Committee on the draft decision was reached on 31 August 2015 in a written procedure launched on 20 August 2015.

ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

A. Tests required pursuant to Article 40(3)

The Registrant shall carry out the following additional test pursuant to Article 40(3)(c) and 13(4) of the REACH Regulation using the indicated test methods and the registered substance subject to the present decision:

1. *In vivo* mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method OECD 489); under conditions as described in the Section III.1;

while the originally proposed *in vivo* mammalian erythrocyte micronucleus test, oral route (test method OECD 474) is rejected pursuant to Article 40(3)(d) of the REACH Regulation.

The Registrant shall carry out the following proposed tests pursuant to Article 40(3)(a) and 13(4) of the REACH Regulation using the indicated test methods and the registered substance subject to the present decision:

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

3. Fish, early-life stage (FELS) toxicity test (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage toxicity test, OECD 210),

The Registrant shall carry out the following modified test pursuant to Article 40(3)(b) and 13(4) of the REACH Regulation using the indicated test methods and the registered substance subject to the present decision:

4. Sub-chronic toxicity study (90-day) in rats, oral route (Annex IX, Section 8.6.2.; test method: EU B.26/OECD 408). The study protocol shall be modified to include additional reproduction parameters (sperm parameters), as described in the Section III. 4.

Note for consideration by the Registrant:

The Registrant may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring to and conforming with the appropriate rules in the respective Annex, and an adequate and reliable documentation.

Failure to comply with the requests in this decision, or to fulfil otherwise the information requirements with a valid and documented adaptation, will result in a notification to the Enforcement Authorities of the Member States.

B. Deadline for submitting the required information

Pursuant to Articles 40(4) and 22(2) of the REACH Regulation, the Registrant shall submit to ECHA by **9 October 2017** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report. The timeline has been set to allow for sequential testing as appropriate.

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.

A. Tests required pursuant to Article 40(3)

1. *In vivo* mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method OECD 489).

a) Examination of the testing proposal

Pursuant to Article 40(3)(d) and (c) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out other test(s) in cases of non-compliance of the testing proposal with Annexes IX, X or XI.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "If there is a positive result in any of the in vitro genotoxicity studies in Annex VII or VIII and there are no results available from an in vivo study already, an appropriate in vivo somatic cell genotoxicity study shall be proposed by the Registrant."

An appropriate *in vivo* genotoxicity study to follow up the concern on chromosomal aberrations identified *in vitro* is not available for the registered substance but shall be

proposed by the Registrant. Consequently, there is an information gap and the Registrant proposed to generate information for this endpoint.

Hence, the Registrant has submitted a testing proposal for a *in vivo* mammalian erythrocyte micronucleus test with the following justification: *"In accordance with Regulation (EC) No. 1907/2006 (REACH), Annex IX 8.4, since positive results were obtained in the in vitro chromosome aberration test in the absence of metabolic activation, an in vivo micronucleus test is proposed by the Registrant to be conducted according to the OECD No. 474 test guideline".*

ECHA notes that this test is mentioned as one of the appropriate tests to investigate further effects on chromosomal aberrations *in vivo* as described in the ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3. and figure R.7.7-1 (August 2014).

ECHA considers that the registered substance is foreseen to be reactive. ECHA also notes that the registered substance is classified for eye damage 1 and that adverse effects in repeated dose study after oral administration were observed mainly in the digestive and respiratory tract. Moreover, ECHA observes that the strong positive result observed in the absence of S9 in the *in vitro* mammalian chromosome aberration test completely disappeared when the assay was performed in the presence of metabolic activation. In the absence of further toxicokinetics data, the elements mentioned above tend to corroborate the probable reactivity of the registered substance, and raises a concern on potential deactivation of the registered substance after oral administration and first pass (liver) metabolism.
Furthermore, ECHA draws attention on the fact that, according to paragraph 10 of the updated OECD 474 guideline (adopted on 26 September 2014), *"If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test"*.

Taking into account the above considerations, in particular the *in vitro* clastogenic effect shown for the parent compound (and not for its metabolites), ECHA is of the opinion that the test proposed by the Registrant is not the most appropriate test to investigate further effects of the registered substance on chromosomal aberrations *in vivo.*

The comet assay (OECD 489 guideline, adopted on 26 September 2014) is also mentioned by ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3. and figure R.7.7-1 (August 2014) as appropriate to investigate further effects on chromosomal aberrations *in vivo*. ECHA is of the opinion that the comet assay would be the most suitable method to conduct such investigation on the registered substance, as it would enable the study of both the liver and first site of contact tissues, and would avoid the uncertainty related to the target tissue exposure. 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. ECHA considers that the most appropriate tissues to be investigated in case of comet assay via the oral route are either the glandular stomach or the duodenum/jejunum, together with the liver.

ECHA reminds the Registrant that, as outlined in the paragraph 7 of the OECD 489 guideline, "to fulfil animal welfare requirements, <...> the endpoint can be combined with other genotoxicity endpoints such as *in vivo* mammalian erythrocyte micronucleus assay".

b) Outcome

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, the Registrant is requested to carry out the following study with the registered substance subject to the present decision:

In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method OECD 489, oral route, 2 tissues to be analysed: liver and either glandular stomach or duodenum/jejunum,

while the originally proposed *in vivo* mammalian erythrocyte micronucleus test, oral route (test method OECD 474) is rejected pursuant to Article 40(3)(d) of the REACH Regulation.

Note for consideration by the Registrant:

The Registrant is reminded that according to the column 2 of section 8.4 of Annex IX 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". ECHA notes that the examination of gonadal cells would optimize the use of animals. Positive results in whole gonad that contains a mixture of somatic and germ cells are not necessarily reflective of germ cell damage, but they indicate that tested substance(s) and/or its metabolites have reached the gonad. This type of evidence may still be relevant for the overall assessment of possible germ cell mutagenicity including classfication and labelling according to the CLP Regulation (EC) No 1272/2008.

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

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.

The Registrant has submitted a testing proposal for a pre-natal developmental toxicity study in rats according to EU B.31/OECD 414.

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

The Registrant proposed rat to be used for testing. He proposed testing by the oral route. 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.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: Pre-natal developmental toxicity study in rats or rabbits, oral route (Annex IX, Section 8.7.2.; test method: EU B.31/OECD 414).

3. Fish, early-life stage (FELS) toxicity test (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage toxicity test, OECD 210)

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.

"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. "Long-term toxicity testing on fish" is a standard information requirement as laid down in Annex IX, Section 9.1.6. of the REACH Regulation. The information on these endpoints 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 these endpoints.

The Registrant has submitted a testing proposal for testing Reaction mass of N-[2-(2-oxoimidazolidin-1-yl)ethyl]methacrylamide and methacrylic acid for long-term toxicity testing on fish: Fish, early-life stage toxicity test, OECD 210 with the following justification: "*Long-term toxicity testing on fish is proposed in order to improve knowledge on chronic toxicity of the substance on Fish*". ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 9.1.6 of the REACH regulation.

According to ECHA *Guidance on information requirements and chemical safety assessment* (version 2.0, November 2014), Chapter R7b, (Section R.7.8.5 including Figure R.7.8-4), if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both; according to the integrated testing strategy (ITS), the Registrant should be requested to perform the long-term toxicity testing on invertebrates first.

However, ECHA notes that the short-term toxicity studies on aquatic species, available in the dossier submitted by the Registrant, did not demonstrate that fish (based on a 48 hour (h) exposure duration period, only) would be substantially more sensitive (at least by a factor of ten) than aquatic invertebrates. The Registrant reported that "*An acute toxicity study was performed on the reaction mass of N-[2-(2-oxo-1-imidazolidinyl)ethyl] methacrylamide and methacrylic acid in accordance with the Japan guideline Acute testing on fish (JIS K 0102 -1986, 71). Himedaka (Oriza latipes) were exposed to the reaction mass of N-[2-(2-oxo-1-imidazolidinyl)ethyl] methacrylamide and methacrylic acid at unknown concentrations (test material including water under semi-static conditions). The 48 -h LC50 is calculated to 22.6 mg/L*". A short-term toxicity test on fish is a standard information requirement under Annex VIII, 9.1.3. of the REACH Regulation. In the registration dossier, the Registrant has provided a non valid short-term toxicity test on fish as a Key study. ECHA considers that the test is invalid for the following reason:

The existing short-term toxicity test on fish is not valid as the test duration is only 48h. A standard test duration for a short-term toxicity test on fish is 96h. This is a principle of the

test method from which all the validation criteria are based on according to the Commission Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 adapted to the technical progress by Commission Regulation (EC) No 761/2009, C.1. Acute toxicity of Fish.

In addition, ECHA notes that the Registrant did not report any details on analytical methods, information on positive control, monitoring or test organisms, and provided conflicting information about GLP in the submitted robust study summary. Furthermore, the composition and the water content in the test material used in testing varied between the aquatic toxicity endpoints.

ECHA therefore concludes that the short-term toxicity test on fish is not valid due to the test duration used and, consequently, there is a data gap for this endpoint.

ECHA notes that based on the available 48h LC50 of 22.6 mg/L, fish appear to be eight times more sensitive than daphnia, where the test duration was only half as long as the standard duration. However, the information on time-effect a relationship is not present in the current registration dossier, thus an extrapolation of the fish lethality to 96h contains too much uncertainty. The relative sensitivity of fish and that of algae and daphnia cannot therefore be predicted with sufficient certainty.

ECHA therefore concludes that there is no valid evidence presented in the dossier to establish relative species sensitivity and that as a consequence, ITS cannot be applied. Therefore, both "Long-term toxicity testing on aquatic invertebrates" and "Long-term toxicity testing on fish" are standard information requirements as laid down in Annex IX of the REACH Regulation. The Registrant proposed to conduct long term study on fish. As the ITS cannot be applied in this particular case, ECHA agrees with the Registrant that this test is required. ECHA notes that this data will cover the information gaps in "Short-term toxicity testing on fish" (Annex VIII, 9.1.3.) and "Long-term toxicity testing on fish" (Annex IX, 9.1.6.).

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed study using the registered substance subject to the present decision: Fish, early-life stage (FELS) toxicity test (Annex IX, 9.1.6.1.; test method: Fish, early-life stage toxicity test, OECD 210).

4. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26/OECD 408) in rats)

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.

The Registrant has submitted a testing proposal for a sub-chronic toxicity study (90 day) via the oral route (EU B.26/OECD 408).
ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.6.2. of the REACH Regulation.

ECHA notes that the registered substance is a liquid with a low vapour pressure, classified as corrosive to eye, although no uses for spray applications are reported in the dossier. In light of the physico-chemical properties of the substance, and the information provided on the uses and human exposure, ECHA considers that testing by the oral route is most appropriate.

The Registrant did not specify the species to be used for proposed testing. According to the test method EU B.26/OECD 408, the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

In addition, the available oral sub-acute study indicates that the digestive tract and testes may be target organs, which require further information on repeated dose toxicity via the oral route. More specifically, ECHA refers to the 28-day oral gavage study performed on rat with registered substance (OECD 407, 1994; doses 40; 200 and 1000 mg/kg bw/day). The Registrant reported that "*mortality was observed in the highest tested dose groups where 2/10 males died on day 8 and 1/10 died on day 14. During an autopsy to determine the cause of death, the lungs were either darkened in a reddish color or exhibited a slight darkening, and the stomach and/or the intestines were filled with gas. The histopathological examination revealed hyperkeratinosis of the forestomach, decrease in the size of the spleen, and a congestion and edema in the lung, thus, related to alterations in the respiratory system and digestive system leading to death. In the surviving animals, the test item induced an increased thickness of the mucous membrane of the forestomach, correlated histopathologically with hyperkeratosis of the forestomach, in both males and females given 1000 mg/kg bw/day. One male of the 1000 mg/kg bw/d group exhibited whitening of the left testis associated by microscopic observation of decrease in sperm formation, and sperm granuloma. Althought believed to be related to the test substance by the authors, these changes in testis in only one animal is likely incidental since it was not observed in any animals in the reproduction/developmental toxicity screening test ([redacted]). An increase in eosinophilic bodies in the kidneys of the 1000 mg/kg bw/d males was also noted in the OECD 407 study and was believed to be related to the test substance.*"

ECHA notes that the sperm parameters were not analysed and testicular effects were not confirmed in the screening study (OECD 421). ECHA therefore considers that the potential testicular effects and sperm parameters shall be further investigated in the proposed sub-chronic toxicity study, in accordance with this provision of test method B.26. In doing so, a chemically-related effect could thereby be clarified and either excluded or confirmed.

The measurement of sperm parameters is not a default requirement of the B.26 (sub-chronic toxicity study, 90-days) test method. However, paragraph 1.5.2.2. of the test method B.26 states that "*Overall, there is a need for a flexible approach, depending on the species and the observed and/or expected effect from a given substance*". In addition, according to paragraph 1.5.2.3 of the test method B.26, "*Also any organs considered likely to be target organs based on the known properties of the test substance should be preserved*". ECHA considers that the testis is a likely target organ, and hence it is necessary to evaluate testis, and sperm parameters as a measure of testicular function. Suitable methods on how to investigate the effects on male reproductive tract and sperm can be found in OECD test guideline 416, paragraphs 29-32, 39, 41-44.

b) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, the Registrant is requested to carry out the proposed study under modified conditions with the registered substance subject to the present decision: Sub-chronic toxicity study (90-day) in rats, oral route (Annex IX, Section 8.6.2.; test method: EU B.26/OECD 408). The study protocol shall be modified to include additional reproduction parameters (sperm parameters), as described in the Section III. 4.

IV. Adequate identification of the composition of the tested material

The process of examination of testing proposals set out in Article 40 of the REACH Regulation aims at ensuring that the new studies meet real information needs. Within this context, the Registrant's dossier was sufficient to confirm the identity of the substance to the extent necessary for examination of the testing proposal. The Registrant must note, however, that this information has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In addition, it is important to ensure that the particular sample of substance tested in the new studies 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. If the registration of the substance covers different grades, the sample used for the new studies must be suitable to assess these.

Finally, there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the studies to be assessed.

V. 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/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[1] by Guilhem de Seze, Head of Unit, Evaluation E1

[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.