Decision number: TPE-D-2114319631-56-01/F Helsinki, 18 February 2016

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

For 2-imidazolidone, EC No 204-436-4 (CAS No 120-93-4), 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 the following testing proposals submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for 2-imidazolidone, CAS No 120-93-4 (EC No 204-436-4), submitted by [redacted] (Registrant).

- 90-day toxicity study (OECD 408), oral route.
- Developmental toxicity / teratogenicity study (OECD 414), oral route.

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 27 May 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 3 May 2013.

ECHA held a third party consultation for the testing proposals from 15 April 2014 until 30 May 2014. ECHA did not receive information from third parties.

On 20 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 24 April 2015 ECHA received comments from the Registrant on the draft decision.

The ECHA Secretariat considered the Registrant's comments.

On basis of this information, Section II was amended. The Statement of Reasons (Section III) was changed accordingly.

In the draft decision communicated to the Registrant the time indicated to provide the requested information was 24 months from the date of adoption of the decision. In his comments on the draft decision of 24 April 2015, the Registrant requested an extension of the timeline of 3 months. He sought to justify this request by the claim the additional investigations requested by ECHA will considerably increase the experimental effort and limit the number of testing facilities. Therefore, ECHA has granted the request and set the deadline to 27 months.

On 29 October 2015, ECHA notified the competent authorities of the Member States of its draft decision and invited them to propose amendment to the draft decision under Article 51 of the REACH Regulation.

Subsequently, amendment to the draft decision was proposed.

The ECHA Secretariat reviewed the proposed amendment and amended the draft decision. On 14 December 2015 ECHA referred the draft decision to the Member State Committee.

By 4 January 2016, in accordance to Article 51(5), the Registrant did not provide comments on the proposal for amendment.

A unanimous agreement of the Member State Committee on the draft decision was not reached on 18 January 2016 in a written procedure launched on 8 January 2016.

After discussion in the Member State Committee meeting on 2–4 February 2016, a unanimous agreement of the Member State Committee on the draft decision was reached on 3 February 2016.

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 modified and additional tests pursuant to Article 40(3)(b) and Art 40(3)(c) and Article 13(4) of the REACH Regulation using the indicated test methods and the registered substance subject to the present decision:

1. Sub-chronic toxicity study (90-days) in rats, oral route (Annex IX, Section 8.6.2.; test method: EU B.26/OECD 408), including additional investigations as follows:

The study protocol shall be modified to include the following additional parameters: additional examinations of male and female reproductive parameters (oestrous cycle, sperm parameters, and reproductive and other certain organs and tissues) that produce respective information as outlined for P parental animals in EU test method B.35, sections 1.5.3., 1.5.4. and 1.5.6. to 1.5.8.

In addition, in order to further investigate the mode of action of the observed thyroid toxicity, one of the following shall be provided, either:

a. The study protocol shall be modified as described in Section III.A.1a. to further investigate potential effects on reproductive organs. The study shall include satellite animal groups (which may start with older animals, and which may be of 14 days duration) to conduct mechanistic investigations with additional thyroid measurements to further investigate the mode of action of the observed thyroid toxicity and the protocol shall be modified accordingly; or

b. an *in vivo* mechanistic study with additional thyroid investigations to further investigate the mode of action of the observed thyroid toxicity, as described in Section III.A.1b.

The Registrant shall carry out the following proposed test pursuant to Article 40(3)(a) and 13(4) of the REACH Regulation using the indicated test method 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.

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 **25 May 2018** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report.

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

Examination of the testing proposal

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

A sub-chronic toxicity study (90-days) 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-days) in rats via the oral route (EU B.26/OECD 408).

ECHA considers that the proposed study type 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 solid with a low vapour pressure and is classified for serious eye irritation/corrosion. The dossier also includes industrial and professional uses with spray and brush applications where the substance is used up to [redacted]% in the mixture, and suitable eye protection is required. However, consumer uses indicate that the exposure to the substance via inhalation is negligible. Furthermore, reported adverse effects of the substance found after repeated oral administration in the sub-acute (OECD 407) and screening for reproduction/developmental toxicity (OECD 422) studies require further investigations. 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 the most appropriate route of administration.

The Registrant proposed testing in rats. 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 to the testing proposal, the technical dossier also contains the two aforementioned repeated exposure toxicity studies which raise concerns for toxicities which may be relevant to human safety and should be further investigated in the proposed sub-chronic toxicity study.

In the Repeated dose 28-day oral toxicity study in rodents (OECD 407, 2002), the registered substance was administered to rats at 0, 1000, 4000 and 12000 ppm in drinking water. The Registrant reported toxic effects at all dose levels and that target organs included the thyroid, the testis and epididymidis, among others. The lowest-observed-adverse-effect level (LOAEL) of 1000 ppm (76 and 92 mg/kg bw/day in males and females, respectively) was based on '*hypertrophy of the follicular epithelium of the thyroid gland*' in both male and female rats. At 4000 ppm, the hypertrophy was accompanied by hyperplasia of the follicular epithelium of the thyroid (in 2/5 males and 4/5 females) and a '*decrease in food and water consumption and impairment of body weight*' was also observed in both male and female rats. Furthermore, '*testicular tubular giant cells and tubular hypoplasia*' (in 1/5 males) and '*cellular debris in the epididymides*' (in 1/5 males) were observed. At 12000 ppm, '*hypertrophy and hyperplasia of the follicular epithelium of the thyroid gland*' and a '*decrease in food and water consumption and impairment of body weight and food efficiency*' were observed in all animals. A '*testicular tubular giant cell and tubular hypoplasia*' and '*cellular debris in the epididymides*' were observed (in 2/5 and 5/5 males, respectively).

In the Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422, 2013), the registered substance was administered to rats at 0, 100, 500 and 2000 ppm in drinking water. Males were exposed for 30 days and females for 51 days. With regard to systemic toxicity, the Registrant reported a no-observed-adverse-effect level (NOAEL) of 500 ppm (37 and 57 mg/kg bw/day in males and females, respectively) based on toxicologically relevant decreases in body weight and food consumption seen in the high dose group. At 2000 ppm, '*a diffuse hypertrophy/ hyperplasia of follicular epithelium was observed in all males and in 5 out of 10 females*'; the effects were considered to be treatment-related and not adverse by the Registrant. No effects were reported in the testis or epididymis. With regard to toxicity for reproduction, this study reports a NOAEL for reproductive performance of the parental animals at 2000 ppm (155 and 214 mg/kg bw/day in males and females, respectively). For developmental toxicity, a NOAEL of 500 ppm (37 mg kg/bw day for males and 57 mg/kg bw/day for females, respectively) is reported based on reduced pup viability.

Investigation of reproductive parameters

In its draft decision, ECHA noted that the Registrant reported in the OECD 407 study that the testis was a target organ for treatment-related effects. Such effects were not observed in the OECD 422 study. ECHA therefore considered that the potential for testicular effects shall be further investigated in the proposed sub-chronic toxicity study (90-days). In doing so, a chemically-related effect could thereby be clarified and either excluded or confirmed. As set out in paragraphs 35 and 36 of OECD 408, target organs should be subject to full histopathology, and this is in accordance with the aims of the 90-day study set out in paragraphs 3 and 4. Therefore additional examinations on reproductive parameters that are normally performed in the two-generation toxicity to reproduction study (test method: EU B.35/OECD 416) shall be included into the proposed sub-chronic repeated dose toxicity study (90-days) to investigate this effect. In it's draft decision, ECHA therefore has requested the Registrant to include the following additional parameters:

> additional examinations of male and female reproductive parameters (oestrous cycle, sperm parameters, and reproductive and other certain organs and tissues) that produce the respective information as outlined for P parental animals in EU test method B.35, sections 1.5.3., 1.5.4. and 1.5.6. to 1.5.8.

The Registrant has submitted comments on the ECHA decision agreeing to perform additional examinations of male and female reproductive parameters (oestrous cycle, sperm parameters, and reproductive and other certain organs and tissues) in the sub-chronic repeated dose toxicity study.

Further investigation of the mode of action of thyroid effects

In ECHA's original draft decision, ECHA noted that the thyroid was a target organ and that treatment-related effects on the thyroid have been consistently observed in both of the aforementioned repeated exposure toxicity studies. ECHA therefore considered that the thyroid effects shall be further investigated in order to assessthe possible mode-of-action of the thyroid toxicity. Furthermore, ECHA considered that adverse effects observed in the 28-days study should be further investigated. Therefore, in the original draft decision, ECHA has requested the Registrant to include the following additional measurements in order to establish the mode of action into the proposed sub-chronic toxicity study (90-days; EU B.26/OECD 408), without compromising the integrity of the study design:

i. Venous blood should be sampled in all animals after fasting (at least 12 hours) on the day: -3; 3; 7; 14; 21 and 90 of treatment for determination of thyroid hormones (total and free T_3 and T_4) and thyroid stimulating hormone (TSH);

ii. Liver microsomes should be isolated for assessment of the following enzyme amounts/activities using well-established and internationally recognised analytical methods:
   - Cytochrome P450 (Cyt P450) total amount;
   - Ethoxyresorufin-O-deethylase (EROD);
   - Pentoxyresorufin-O-depentylase (PROD);
   - Benzoxyresorufin-O-debenzylase (BROD);
   - T4-specific UDP-glucuronosyltransferase (T4-UDP-GT);
   - 4-Methylumbeliferone-glucuronosyltransferase (MUF-GT); and
   - Hydroxybiphenyl- glucuronosyltransferase (HOBI-GT).

The Registrant has submitted comments on the draft decision addressing the requests for modification of sub-chronic study design including additional investigations of blood parameters and hepatic enzymes.

The Registrant agreed with ECHA's rationale for the request to follow up the possible thyroid effects. However, the Registrant has adequately identified the reasons that might have an impact on the integrity of the 90-days study, if the investigation of thyroid effects and hepatic enzymes are included as required by ECHA.

Firstly, the Registrant indicated that "*repeated fasting and blood sampling causes stress especially in young animals, i.e. at the beginning of an OECD 408 study, and is likely to compromise the integrity of the 90-day study (e.g. influence on body weight development or generation of secondary toxicological effects). It is known that stress has an influence on thyroid hormone levels in rats and might therefore have an impact on the investigation of thyroid hormones*".

Secondly, the Registrant stressed that "*It is common standard to measure liver enzyme induction in the course of a 14-day study; the measurement of hepatic enzymatic activity after 90 days is too late (possible adaptation) and not appropriate for the investigation if hepatic enzyme activity is involved in thyroid hormone dysregulation. Early time points (e.g. days 3, 7, and 14) after test substance application are most relevant for the determination of thyroid hormone dysregulation in the blood; measurements at later time points do not provide useful information due to possible adaptation to thyroid hormone dysregulation*". The Registrant also referred to the importance of having relevant historical control data which are reported to be available for the short term studies mentioned above. The Registrant also has control data for tests conducted on older animals but not in the course of a 90-day study.

Thirdly, the Registrant proposed to perform only the determination of the total T3 and T4 levels and claimed that "*there is sufficient evidence available that measurement of total T4 levels in rat blood serum leads to the same conclusion as measurement of free T4 levels* ([redacted])." The Registrant also pointed out that, according to OECD test guideline 407, only the determination of total T3 and T4 levels is required.

Finally, the Registrant proposed, as one alternative, to conduct a separate mechanistic study addressing only effects on thyroid parameters and hepatic enzymes, and as the other alternative, including the satellite groups within the 90-day study. These alternative options would both ensure the integrity of the 90-day study.

ECHA has analysed the comments and scientific explanations provided by the Registrant. ECHA concludes that, for the reasons as outlined by the Registrant above, the investigations as proposed in the original draft decision might have an impact on the integrity of the 90-days study if included in the 90-day study. ECHA considers that both alternatives are acceptable. Therefore ECHA concluded that, in addition, in order to further investigate the mode of action the observed thyroid toxicity, one of the following alternatives in III.A.1.a or III.A.1.b (as per below) shall be provided.

a) The study protocol for sub-chronic toxicity study (90-days) shall be modified to include satellite animal groups (which may start with older animals, and which may be of 14 day duration) to conduct mechanistic investigations with additional thyroid measurements to further investigate the mode of action of the observed thyroid toxicity and the protocol shall be modified accordingly

The Registrant proposed, as one alternative, to include satellite groups within the 90-day study, so that the integrity of the 90-days study remains uncompromised.

ECHA considers that, as set out in paragraphs 35 and 36 of OECD 408, target organs should be subject to full histopathology, and that according to paragraph 12, interim kills may be included in the study. ECHA considers that, in line with an aim of the 90-day study to provide information on the major toxic effects, it is entirely appropriate to use approximately 70-day old animals at the start of dosing for specific interim kill groups, in order to ensure compatibility with appropriate historical control experiments. This is in accordance with the aims of the 90-day study set out in paragraphs 3 and 4. ECHA therefore considers that the proposed additional interim kill groups and specific investigations are appropriately covered under the terms of OECD 408. Specifically, ECHA notes that the interim kill groups would start using older animals (i.e. about 70 days of age), and would typically involve approximately 14 days of treatment. The following parameters would be investigated:

i. determination of thyroid hormones (total T3 and T4) and thyroid stimulating hormone (TSH) in venous blood sampled after fasting on days -3; 3; 7; 14 of treatment;

ii. isolation of liver microsomes on day 14 of treatment for the assessment of the following enzyme amounts/activities using well-established and internationally recognized analytical methods:

- Cytochrome P450 (Cyt P450) total amount;
- Ethoxyresorufin-O-deethylase (EROD);
- Pentoxyresorufin-O-depentylase (PROD);
- Benzoxyresorufin-O-debenzylase (BROD);
- T4-specific UDP-glucuronosyltransferase (T4-UDP-GT);
- 4-Methylumbeliferone-glucuronosyltransferase (MUF-GT); and
- Hydroxybiphenyl- glucuronosyltransferase (HOBI-GT).

b) *In vivo* mechanistic study with additional thyroid investigations to further investigate the mode of action of the observed thyroid toxicity.

Pursuant to third intent of Column 2 of Annex IX, Section 8.6.2 of the REACH Regulation, further studies shall be proposed by the Registrant or may be required by Agency in accordance with Articles 40 and 41 in case of indications of an effect for which the available evidence is inadequate for toxicological and/or risk characterisation. In such cases it may also be more appropriate to perform specific toxicological studies that are designed to investigate these effects (e.g. immunotoxicity, neurotoxicity).

The Registrant has proposed, as one alternative, the conduct of a separate mechanistic study. ECHA considers that the effects seen on thyroid (see Section III.A.1 above) are indications of an effect for which the available evidence is inadequate for toxicological and/or risk characterization. ECHA additionally considers that it is appropriate to perform a specific toxicological study that is designed to investigate this concern. ECHA notes that the study would start using older animals (i.e. about 70 days of age), and would typically involve approximately 14 days of treatment. The following parameters would be investigated:

i. determination of thyroid hormones (total T3 and T4) and thyroid stimulating hormone (TSH) in venous blood sampled after fasting on days -3; 3; 7; 14 of treatment;

ii. isolation of liver microsomes on day 14 of treatment for the assessment of the following enzyme amounts/activities using well-established and internationally recognized analytical methods:

- Cytochrome P450 (Cyt P450) total amount;
- Ethoxyresorufin-O-deethylase (EROD);
- Pentoxyresorufin-O-depentylase (PROD);
- Benzoxyresorufin-O-debenzylase (BROD);
- T4-specific UDP-glucuronosyltransferase (T4-UDP-GT);
- 4-Methylumbeliferone-glucuronosyltransferase (MUF-GT); and
- Hydroxybiphenyl- glucuronosyltransferase (HOBI-GT).

Outcome

Therefore, pursuant to Article 40(3)(b) and Art 40(3)(c) and Article 13(4) of the REACH Regulation shall carry out the following modified and additional tests using the indicated test methods and the registered substance subject to the present decision:

1. Sub-chronic toxicity study (90-days) in rats, oral route (Annex IX, Section 8.6.2.; test method: EU B.26/OECD 408), including additional investigations as follows:

   The study protocol shall be modified to include the following additional parameters: additional examinations of male and female reproductive parameters (oestrous cycle, sperm parameters, and reproductive and other certain organs and tissues) that produce respective information as outlined for P parental animals in EU test method B.35, sections 1.5.3., 1.5.4. and 1.5.6. to 1.5.8.

   In addition, in order to further investigate the mode of action of the observed thyroid toxicity, one of the following shall be provided, either :

   a. The study protocol shall be modified as described in Section III.A.1a. to further investigate potential effects on reproductive organs. The study shall include satellite animal groups (which may start with older animals, and which may be of 14 day duration) to conduct mechanistic investigations with additional thyroid measurements to further investigate the mode of action of the observed thyroid toxicity and the protocol shall be modified accordingly; *or*

   b. an *in vivo* mechanistic study with additional thyroid investigations to further investigate the mode of action of the observed thyroid toxicity, as described in Section III.A.1b.

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

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 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 suggested 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 (test method: EU B.31/OECD 414).

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 Ofelia Bercaru, Head of Unit, Evaluation E3

[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.