EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114322607-52-01/F Helsinki, 07 June 2016

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

For 3,5-bis(2,4-dimethylcyclohex-3-en-1-yl)polyheterocycle, EC No 700-437-3 (CAS RN 1196069-20-1), 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 proposal submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for 3,5-bis(2,4-dimethylcyclohex-3-en-1-yl)polyheterocycle, EC No 700-437-3 (CAS RN 1196069-20-1), submitted by [redacted] (Registrant).

- *In vivo* alkaline single-cell gel electrophoresis assay for DNA strand breaks (comet assay) (Annex VIII, Section 8.4., column 2; test method: OECD 489) combined with *in vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method EU B.12./OECD 474), on rats, by the intraperitoneal route

This decision is based on the registration as submitted with submission number [redacted], for the tonnage band of 10 to 100 tonnes per year.

This decision does not take into account any updates after 29 July 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.

The examination of the testing proposal was initiated upon the date when receipt of the complete registration dossier was confirmed on 25 November 2014.

ECHA held a third party consultation for the testing proposal from 16 March 2015 until 30 April 2015. ECHA did not receive information from third parties.

On 22 May 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.

By 29 June 2015 the Registrant did not provide any comments on the draft decision to ECHA.

On 3 September 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 9 October 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 19 October 2015 ECHA referred the draft decision to the Member State Committee.

By 9 November 2015, in accordance to Article 51(5), the Registrant provided comments on the proposals for amendment. The Member State Committee took the comments of the Registrant on the proposals for amendment into account.

After discussion in the Member State Committee meeting on 7–11 December 2015, a unanimous agreement of the Member State Committee on the draft decision was reached on 9 December 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 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:

- *In vivo* mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD 489) combined with *in vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method EU B.12./OECD 474); on rats; oral route (gavage). For the comet assay, two tissues shall be analysed: liver, and glandular stomach or duodenum/jejunum. For the micronucleus test, the bone marrow shall be analysed.

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 request in this decision, or to fulfil otherwise the information requirement 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 **14 June 2017** 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 proposal submitted by the Registrant for the registered substance.

A. Tests required pursuant to Article 40(3)

- *In vivo* mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD 489) combined with *in vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method EU B.12./OECD 474);

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.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex VIII, Section 8.4. provides that "Appropriate in vivo mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII.

The technical dossier contains an *in vitro* mammalian cell gene mutation test performed according to OECD Guideline 476 with the registered substance that shows positive results. Furthermore, the dossier contains an *in vitro* mammalian chromosome aberration test performed according to OECD Guideline 473 with the registered substance that demonstrates negative results, but also shows an increase in the number of polyploid cells. The positive result in the gene mutation test indicates that the substance is inducing gene mutations under the conditions of the test, while the increase in polyploid cells may indicate the potential to disturb mitosis.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance but shall be considered. Consequently, there is an information gap and the Registrant considered it necessary to generate information for this endpoint.

Hence, the Registrant has submitted a testing proposal for an *in vivo* alkaline single-cell gel electrophoresis assay for DNA strand breaks (comet assay) (OECD 489) combined with *in vivo* mammalian erythrocyte micronucleus test (OECD 474) to be performed with the registered substance.The Registrant proposed to combine these two studies taking account of combination aspects such as dosing and sampling based on the principles outlined in the scientific literature (e.g. Bowen *et al.*, 2011, Mutation Research, 722:7-19). The Registrant also proposed to perform the study on rats, by the intraperitoneal route, using the following tissues: the bone marrow for micronucleus test; blood and liver tissue for the comet assay. The Registrant proposed the intraperitoneal route in order to maximise exposure to the substance, as the substance is expected to hydrolyse under acidic conditions.

ECHA notes that the proposed test is an appropriate test to investigate effects on gene mutations/chromosome aberrations *in vivo* as described in the ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.1. and figure R.7.7-1 (August 2014).

According to the ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3 (August 2014), the transgenic rodent somatic and germ cell gene mutation assays ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD 489) are suitable to follow up a positive *in vitro* result on gene mutation.

Furthermore, according to this ECHA Guidance document, the mammalian erythrocyte micronucleus test ("MN test", OECD TG 474), the mammalian bone marrow chromosomal aberration test ("CA test", OECD TG 475) or the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow-up a positive *in vitro* result on chromosomal aberration.

Hence, ECHA considers that the comet assay is a suitable test to follow up the concern on gene mutation for the substance subject to the decision. Although in this case there were no positive results in the *in vitro* mammalian chromosomal aberration test available in the registration dossier, the micronucleus test is suitable to follow up on the Registrant's concern regarding potential effect of the substance on mitosis, as evidenced by the increase in polyploidal cells observed in the *in vitro* chromosome aberration test.

The comet assay shall be performed in rats because rats are routinely used for this test and also for other (acute or repeated) toxicity studies. Rats are also routinely used for the micronucleus test. Moreover, acute toxicity by oral route data are available on rats in the dossier.

ECHA notes that the Registrant proposed to perform the study using the intraperitoneal route and explained that *"[the registered substance] is known to hydrolyze already under slight acidic conditions as can be found on the skin of humans and also of rodents. Hydrolysis must be even more expected after oral application as soon as the material reaches the strong acidic environment in the stomach. Therefore, an i.p. application is necessary in order to ensure the highest possible systemic availability and thus investigating the inherent genotoxic potential of [the registered substance] in vivo"*. However, ECHA notes the OECD test guideline 489 statement: "intraperitoneal injection is generally not recommended since it is not a typical relevant route of human exposure, and should only be used with specific justification (e.g. some positive control substances, for investigative purposes, or for some drugs that are administered by the intraperitoneal route)". Similarly the OECD test guideline 474 does not recommend the intraperitoneal route. Moreover, the ECHA Guidance R.7a, chapter R.7.7.6.3 (August 2014) states that 'The route of exposure should be selected that best allows assessment of the hazard posed to humans'.
ECHA notes that there is no information in the dossier indicating that the intraperitoneal route of exposure would be a relevant route of human exposure for this substance. In the summary of the genetic toxicology endpoint, the registrant states 'The site of contact in case of the intended consumer products would be the skin'. However ECHA considers that dermal administration would not ensure optimal systemic availability.
In relation to the hydrolysis of the registered substance, ECHA notes that the substance(s) causing gene mutations in the in vitro mammalian cell gene mutation test (OECD 476) has(have) not been identified. The substance(s) inducing genotoxicity in vitro may thus be either the parent compound or a by-product(s) of the parent compound (for instance the hydrolysis product(s)).
In light of the physicochemical properties of the substance (liquid with a low vapour pressure, i.e. <0.18 Pa at 20 °C), ECHA considers that the oral route of administration is appropriate. In conclusion on the route, ECHA considers that the test shall be performed by the oral route to obtain in vivo data that are relevant for human exposure and to ensure adequate exposure of the target tissues.

As regards the tissues to be studied, the bone marrow is the tissue normally examined for the micronucleus test. For the comet assay, the Registrant proposed to use the liver as the primary site of xenobiotic metabolism, and the blood as the site of first contact. However, the blood as the site of first contact is not appropriate in the case of oral exposure. Paragraph 42 of the draft OECD test guideline 489 states that "[t]he liver has been the tissue most frequently studied and for which there are the most data. Therefore, in the absence of any background information, and if no specific tissues of interest are identified, sampling the liver would be justified as this is a primary site of xenobiotic metabolism and is often highly exposed to both parent substance(s) and metabolite(s). In some cases examination of a site of direct contact (for example, for orally-administered substances the glandular stomach or duodenum/jejunum, or for inhaled substances the lungs) may be most relevant." Therefore ECHA considers that the comet assay via oral route should be performed in liver (as the primary site of xenobiotic metabolism) and either glandular stomach or duodenum/jejunum (as the site of first contact).
ECHA considers that it is suitable to combine the micronucleus test and the comet assay and agrees with Registrant, as proposed in the testing proposal, that combination aspects such as dosing and sampling should be taken into account following principles described in the literature (see e.g. Bowen et al. 2011).

b) Outcome

Therefore, pursuant to Article 40(3)(b) 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 (test method: OECD 489) combined with i*n vivo* mammalian erythrocyte micronucleus test (test method EU B.12./OECD 474); in rats, oral route (gavage). For the comet assay, two tissues shall be analysed: liver, and glandular stomach or duodenum/jejunum. For the micronucleus test, the bone marrow shall be analysed.

Note for consideration by the Registrant:

Regarding follow up testing for germ cell mutagenicity, the Registrant is reminded that according to Annex IX/X, Section 8.4., column 2 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".

Regarding the comet assay, the Registrant may consider examining gonads for analysis of germ cell mutagenicity. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, the positive result could indicate if the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

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 study meets 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, 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 test, the sample of substance used for the new study 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 joint registrants of the same substance to agree to the test proposed (as applicable to their tonnage level) and to document the necessary information on their substance composition.

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

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