EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114319627-45-01/F Helsinki, 19 February 2016

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

For 1,3-diphenylpropane-1,3-dione, EC No 204-398-9 (CAS No 120-46-7), 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 1,3-diphenylpropane-1,3-dione, EC No 204-398-9 (CAS No 120-46-7), submitted by [redacted] (Registrant).

- Subchronic toxicity study 90-day (OECD 408), rat, oral route.
- Pre-natal developmental toxicity study (OECD 414), Sprague–Dawley (SD) rat, oral gavage route.
- *In vivo* mammalian erythrocyte micronucleus test (OECD 474).

This decision is based on the registration 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 21 September 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 18 June 2014.

ECHA held a third party consultation for the testing proposals from 18 September 2014 until 3 November 2014. ECHA received information from third parties (see section III below).

On 15 July 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 21 August 2015, the Registrant did not provide any comments on the draft decision to ECHA.

On 29 October 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 4 December 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 14 December 2015 ECHA referred the draft decision to the Member State Committee.

By 4 January 2016 the Registrant did not provide any comments on the proposals for amendment.

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 as modified at the meeting 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 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:

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

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 method and the registered substance subject to the present decision:

3. *In vivo* mammalian alkaline comet assay (Annex IX Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum/jejunum

while the originally proposed test for an *in vivo* mammalian erythrocyte micronucleus test (OECD 474) proposed to be carried out using the registered substance is rejected, pursuant to Article 40(3)(d) of the REACH Regulation.

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(s) in this decision, or to fulfil otherwise the information requirement(s) 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 **26 February 2018** 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 and scientific information submitted by third parties.

1. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2)

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

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) in rats via the oral route (EU B.26/OECD 408).

ECHA considers that the proposed study via the oral route is appropriate to fulfil the information requirement of Annex IX, Section 8.6.2. of the REACH Regulation because the proposed route is the most appropriate route of administration having regard to the likely route of human exposure.

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.

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

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 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 testing in rats 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) Consideration of the information received during third party consultation

ECHA has 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 proposed "[...] a tiered testing strategy which gives priority to the proposed mammalian *in vivo* erythrocyte micronucleus test. In case of a positive result the rule for adaptation of information requirements according to Annex IX (column 2) will apply, and a standard prenatal developmental toxicity study will not be required."

ECHA notes that it is the Registrant's responsibility to consider and justify in the registration dossier any adaptation of the information requirements in accordance with Annex IX, Section 8.7., column 2, first and second indent. This adaptation specifies that in case the substance is known to be a germ cell mutagen (which correspond to a classification as germ cell mutagen category 1A or 1B) or genotoxic carcinogen (which correspond to a classification as carcinogen category 1A or 1B and germ cell mutagen category 2) and appropriate risk management measures are implemented, the prenatal developmental toxicity study does not need to be conducted.

ECHA notes that results of the *in vivo* mutagenicity test are yet not available and, therefore, this adaptation possibility cannot be applied. Furthermore, an adaptation would also need to demonstrate that the other condition of the adaptation possibility with respect to implementing appropriate risk management measures is fulfilled. However, this decision does not prevent the Registrant from adapting the requested test in accordance with the specific rules outlined in Column 2 of Annex IX, Section 8.7.2. and ECHA has set the deadline for submitting futher information to allow sequential testing, as appropriate.

Therefore, 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, 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).

3. *In vivo* mammalian alkaline comet assay (Annex IX Section 8.4., column 2)

Pursuant to Article 40(3)(c) and (d) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out other tests 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."

The technical dossier contains several reports of *in vitro* studies, performed with the registered substance, showing the following results:

- Ames test (OECD Guideline 471): negative both with and without S9, on all five relevant strains;
- *In vitro* Mammalian Cell Gene Mutation Test (OECD Guideline 476): positive both with and without S9;
- *In vitro* mammalian micronucleus test (OECD Guideline 487): positive, with the Registrant stating: "Under the test condition 24 hours [without S9,] the test substance induced chromosome breakage loss at a concentration of 7,67 g/ml, in cultured mammalian cells."

The two positive results indicate that, under the conditions of the studies, the substance may be inducing gene mutations, chromosomal aberrations, or both.

An appropriate *in vivo* genotoxicity study to follow up the concern on the possible gene mutations and/or chromosomal aberrations 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 an *in vivo* mammalian erythrocyte micronucleus test according to OECD Guideline 474.

ECHA notes that the proposed test is not the most appropriate test to investigate possible effects from both gene mutations and chromosomal 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). The proposed test is suitable to detect chromosomal aberrations but not gene mutations. Therefore, it has to be rejected as non-compliant with the information requirements of REACH in the present case pursuant to Article 40(3)(d) of the REACH Regulation.

According to the ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3 (August 2014), the *in vivo* mammalian alkaline single-cell gel electrophoresis assay ("comet assay", OECD Guideline 489) is suitable for following up positive result *in vitro* for both gene mutation and chromosomal aberrations. As the test to be performed must produce data that are tailored to real information needs, ECHA considers this test to be most appropriate for the substance subject to the decision.

According to the test method OECD TG 489, the test shall be performed in rats. 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. According to the test method (OECD TG 489), the test shall be performed by analysing tissues from liver, glandular stomach and duodenum/jejunum. As set out in the OECD TG 489, the liver is recommended as the primary site of xenobiotic metabolism, and an often highly exposed tissue. The glandular stomach and duodenum/jejunum are recommended as tissues to examine site of contact effects after oral exposure.

In view of the different pH, different tissue structure and function of the stomach and duodenum/jejunum, and known examples of differential absorption of substances between these two tissues, ECHA considers that it is necessary to sample both tissues to have a sufficient analysis of genotoxicity at the site of contact.

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 (test method: OECD Guideline 489) in rats, oral route, with analysis of the following tissues: liver, glandular stomach and duodenum/jejunum.

Notes for consideration by the Registrant

The Registrant is reminded that according to Annex IX, 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".

The Registrant may consider examining gonadal cells, as it would optimise the use of animals. 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, such positive result would indicate that 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 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

[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.