EUROPEAN CHEMICALS AGENCY

Helsinki, 19 May 2017

Addressee: [redacted]

Decision number: TPE-D-2114360326-52-01/F
Substance name: 2,2-dimethylpropane-1,3-diyl cyclohex-4-ene-1,2-dicarboxylate
EC number: 255-180-5
CAS number: 41026-17-9
Registration number: [redacted]
Submission number: [redacted]
Submission date: 22.12.2015

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA has taken the following decision.

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

1. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats using the registered substance.**
2. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rats or rabbits), oral route using the registered substance.**

You are requested to perform as additional test:

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, with the registered substance; while your originally proposed test for in vivo mammalian bone marrow chromosomal aberration test (EU B.11./OECD TG 475) is rejected.**

Your following testing proposal is rejected:

4. **Two-generation reproduction toxicity study (Annex IX, Section 8.7.3.; test method: OECD TG 416).**

You 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 and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **26 November 2019**. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. 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, shall 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, Evaluation E2

[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

0. Grouping of substances and read-across approach

In your comments to the draft decision you propose to adapt the standard information requirements for *In vivo mammalian alkaline comet assay*, sub-chronic toxicity (90-day) and pre-natal developmental toxicity by applying a read-across approach according to Annex XI, section 1.5.

In your updated registration dossier of 22 December 2015 you have provided a read-across justification document entitled "[redacted]", and have provided the following hypothesis:

"2,2-dimethylpropane-1,3-diyl cyclohex-4-ene-1,2-dicarboxylate is considered as a monoconstituent substance under REACH with a purity > 88%, and containing the following impurities: Di-neopentylglycol tri-tetrahydrophthalic acid oligoester in a range of 2 to 7%, 6-[(3- hydroxy-2,2-dimethylpropoxy)carbonyl]cyclohex-3-ene-1-carboxylic acid in a range of 0 to 3%, and higher MW oligomeric species in a range of 0 to 2%.
The hypothesis will be to read-across relevant data between the monoconstituent substance described above to the corresponding UVCB substance as described under REACH. The UVCB substance is chemically similar and just differs from the monoconstituent substance in the purity of the main constituent which is < 80% but .
still remains in the same order of magnitude. The impurities from the monoconstituent substance being also present in the same order of magnitude in the UVCB substance. The main assumption is that the minor differences in terms of percentage between the mono and UVCB substance are not significant in respect with all properties under consideration".

ECHA understands that your hypothesis is based on the chemically similar target and source substances, i.e. the UVCB substance (source) has the same main constituent as the registered (target) substance. The concentration of this main component in the source substance is < [redacted]%, and > [redacted]% in the target substance. According to you the concentrations still *"remains in the same order of magnitude"*. In addition, you state that *"impurities from the monoconstituent substance being also present in the same order of magnitude in the UVCB substance"*. ECHA understands that according to you, the impurities in the target substance and components in the source substance have negligible impact on the properties of the substances: *"main assumption is that the minor differences in terms of percentage between the mono and UVCB substance are not significant in respect with all properties under consideration"*. Structural (dis)similarity

Structural similarity is a prerequisite for applying the grouping and read-across approach.

The registered (target) substance contains [redacted]% (typically [redacted]%) of 2,2-dimethylpropane-1,3-diyl cyclohex-4-ene-1,2-dicarboxylate and as impurities [redacted]% (typical [redacted]%) neopentylglycol tri-tetrahydrophthalic acid oligoester and [redacted]% (typical [redacted]%) of 6-[(3-hydroxy-2,2-dimethylpropoxy)carbonyl]cyclohex-3-ene-1-carboxylic acid.

As stated in the hypothesis, concerning the differences between the source and the target substance, you consider that *"Based on the structural similarity of these two compounds, it is unlikely that the minor differences in terms of percentage between the mono and UVCB substance do have significant effects on the properties under consideration"*.

When making a read across hypothesis to an analogue substance, one should be able to link the structural similarities and differences with the proposed prediction and their impact on the toxicity. As noted, the main component is present at < [redacted]% in the UVCB substance and it is identical to the main component of the monoconstituent which has a typical concentration of [redacted]%.

However, ECHA notes that you have not provided any information (such as identity and concentration) on the other components or impurities in the source substance, and their possible impact on toxicity of the source substance. Based on your statement, it is not clear if these components/impurities are similar to the ones in the target substance and if their concentration is in a similar range to the ones in the target substance.

ECHA considers that although the main component is the same in the target and source substance, you have not provided information on the other components in the source substance present up to [redacted]%, and how these components may impact the toxicity of the substance and thus affect the possibility to predict the properties of the target substance from the data of the source substance. The provided explanation is therefore not sufficient to establish a scientifically credible link between the structural similarity and the prediction.

You state that the physico-chemical properties of the source substance and the target substance would be comparable in biological systems. ECHA notes that you have not explained how the differences in for example water solubility may impact the toxicokinetic behaviour and toxicological potency of the substances.

Toxicological properties

You state that "... *based on the similar structural fonctionality as well as the above mentioned experimental results and given the fact that there is no difference in functional groups between the source and target chemical, the read-across approach proposed is justified*".

ECHA notes that the main component of the target and source substance is the same thus having similar functional groups. However, since you have not provided data on the other components of the source substance, it is not possible to verify whether there are differences in structures between the target and source substance.

ECHA observes that both substances show mutagenic potential as they have both given a positive result in an *in vitro* mammalian chromosomal aberration test and an *in vitro* gene mutation study in mammalian cells. However, as explained above you have not provided a justification why the possible [redacted]% difference in composition would not affect the genotoxic potential in the *In vivo* mammalian alkaline comet assay of the substances.

ECHA notes that in addition to the genotoxicity tests mention above, there is only a 28-day study available for the target substance. For the source substance, in addition to the genotoxicity tests, acute oral and dermal, skin and eye irritation and skin sensitisation studies are available.

ECHA considers that mutagenicity data alone is not sufficient to establish the toxicological profile of a substance and support the prediction of the sub-chronic toxicity (90-day) and prenatal developmental toxicity. Therefore, ECHA concludes that based on the presented information it is not possible to confirm that the substances would have similar properties regarding these endpoints. In the absence of such information there is not an adequate basis for predicting the properties of the target substance from the data obtained with the source substance.

Conclusion

Based on the above considerations ECHA concludes that there is not a reliable basis for considering that the properties of the registered substance may be predicted from data from the source substance, and so the proposed read-across approach is not plausible for the endpoints in consideration.

ECHA therefore concludes that the criteria of Annex XI, section 1.5. are not met, and consequently the testing proposed on the read-across substance is not appropriate to fulfil the information requirements of the substance subject to the present decision.

The decision of ECHA is based on the examination of the testing proposals submitted by you.

1. Sub-chronic toxicity study (90-day) (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.

You have submitted a testing proposal for a sub-chronic toxicity study (90 day) in rats by the oral route according to EU B.26/OECD TG 408.

ECHA considers that the proposed study performed 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 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 4.0, July 2015) Chapter R.7a, section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the registered substance is a solid and there are no indications for significant inhalation exposure of humans (used in non-solid or granular forms; no spraying applications). Hence, the test shall be performed by the oral route using the test method EU B.26/OECD TG 408.

You proposed testing in rats. According to the test method EU B.26/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 your comments to the draft decision you proposed a read-across approach using 1,2,3,6-tetrahydrophthalic anhydride, oligomeric reaction products with 2,2-dimethylpropane-1,3-diol as a source substance. However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

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

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

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 EU B.31/OECD TG 414.

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 rats. According to the test method EU B.31/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 rats or rabbits 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 4.0, July 2015) R.7a, chapter 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 proposed a read-across approach using 1,2,3,6-tetrahydrophthalic anhydride, oligomeric reaction products with 2,2-dimethylpropane-1,3-diol as a source substance. However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

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: EU B.31/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 R.7a, chapter R.7.6.2.3.2 (July 2015).

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

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 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 two *in vitro* studies performed according to OECD TG 473 (*in vitro* mammalian chromosome aberration test) and to OECD TG 476 (*in vitro* mammalian cell gene mutation test) that show positive results in the presence of metabolic activation. According to the information provided, the positive result of the *in vitro* chromosome aberration test in the presence of metabolic activation indicates 'structural' chromosomal aberrations ('chromosomal breakages') which are induced by the metabolite(s) of the registered substance. Furthermore, the positive result of the *in vitro* gene mutation test in the presence of 8% (v/v) metabolic activiation shows increase in small colonies as well as large colonies which indicate clastogenic as well as mutagenic effects, respectively. Therefore, the positive results indicate that the metabolite(s) of the registered substance is (are) inducing gene mutations as well as structural chromosomal aberrations under the conditions of the tests.

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

Hence, you have submitted a testing proposal for a mammalian bone marrow chromosome aberration test according to OECD TG 475. As reason for this proposal you stated that *"an OECD 475 Mammalian Bone Marrow Chromosome aberration test is proposed to assess the ability of the test substance to induce chromosomes aberration in vivo" (see CSR, page 28).*

However, the proposed OECD TG 475 study 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 (July 2015): The proposed OECD TG 475 study "*identifies substances that induce structural chromosome aberrations in the bone-marrow cells of animals*" but not gene mutations (see Table R.7.7–3 *In vivo* test methods, somatic cells). In this respect, it should be also emphasised that, the usual ECHA practice is to request only one test which should not be limited to only one of the concerns.

According to the ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3 (July 2015), the *in vivo* mammalian alkaline single-cell gel electrophoresis assay ("comet assay", OECD TG 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, it is considered that this test is 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 as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

In your comments to the draft decision you proposed a read-across approach using 1,2,3,6-tetrahydrophthalic anhydride, oligomeric reaction products with 2,2-dimethylpropane-1,3-diol as a source substance. However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

In your comments to the Member State Competent Authority (MSCAs) proposal for amendment, you disagree to perform the test by analysing tissues from two sites of contact (glandular stomach and duodenum) because the positive results of the *in vitro* tests were only observed with metabolic activation, which indicates that it is the metabolite(s) of the registered substance that is (are) inducing genotoxic effects. In your view, '*following dosing by oral gavage mutagenic sensitivity of the tissues may increase e.g stomach < duodenum < liver with no mutagenic effects at the first site of contact (stomach) and more effects on the duodenum and liver indicating that increasing potency correlates with the stage of digestion/metabolism, and then it can be hypothesised that the mutagenic effects is likely to be a consequence of cell based detoxification or transformation process and not a direct mutagenic effect of the registered parent substance*' and '*analysis of the liver tissue as primary site of xenobiotic metabolism and one single tissue glandular stomach or duodenum/jejunum as direct site of contact are sufficient to evaluate the genotoxic potential to somatic cells*'. ECHA reminds that in the case of a comet assay after oral administration it is considered standard to request the analysis of liver, glandular stomach and duodenum. In the specific case, ECHA considers that it is not possible to identify precisely in which tissue the relevant metabolic activation may happen, and it cannot be excluded that genotoxic metabolites would be generated after interaction with stomach or duodenum cells/tissues, or with bacteria present in the duodenum.

Outcome

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vivo* mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

Notes for your consideration

You are 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".

You 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.

4. Two-generation reproduction toxicity study (Annex IX, Section 8.7.3.)

Pursuant to Article 40(3)(d) of the REACH Regulation, ECHA may reject a proposed test.

You have submitted a conditional testing proposal for a two-generation reproductive toxicity study according to OECD TG 416 with the following justification: "*A rat reproductive toxicity study may be conducted pending the outcome of the OECD test guideline no. 408: repeated dose 90-Day Oral Toxicity in Rodents as per REACH Annex IX, Column I, Section 8.7.3.*"

According to Annex IX, Section 8.7.3., as amended by Commission Regulation (EU) 2015/282 (entered into force on 13 March 2015), a two-generation reproductive toxicity study is not an information requirement any longer. However, the requirement according to Annex IX, Section 8.7.3., i.e. the extended one-generation reproductive toxicity study, is only an information requirement if adverse effects on reproductive organs or tissues have been observed in the available repeated dose toxicity studies (e.g. a 28-day or 90-day repeated dose toxicity study, OECD TG 421 or 422 screening studies) or if they reveal other concerns in relation with reproductive toxicity.

ECHA notes that there are results of a short-term repeated dose toxicity study (28 days) available in the registration dossier that did not indicate adverse effects on reproductive organs or tissues or reveals other concerns in relation with reproductive toxicity. Furthermore, ECHA notes that you have proposed to perform a sub-chronic toxicity study (90 days).

You stated that a rat reproductive toxicity study may be conducted pending the outcome of the sub-chronic toxicity study according to OECD TG 408.

ECHA considers that the proposed study is at this stage not necessary to fulfil the information requirement of Annex IX, Section 8.7.3. of the REACH Regulation because the sub-chronic toxicity study (90-day) is currently not available to evaluate if performance of such a reproductive toxicity study is required at that tonnage level and no adverse effects on reproductive organs or tissues or other concerns in relation with reproductive toxicity have been observed in the provided sub-acute toxicity study (28-day).

In your comments you acknowledge that the study proposed by you is at this stage not necessary to fulfill the information requirement of Annex IX, Section 8.7.3. of the REACH Regulation because no repeated dose toxicity study is currently available to indicate the need for a reproductive toxicity study at this tonnage level.

ECHA concludes that at this stage there is no information gap for the information requirement of Annex IX, Section 8.7.3. Therefore, pursuant to Article 40(3)(d) of the REACH Regulation, the proposed two-generation reproduction toxicity study according to OECD TG 416 is rejected.

Notes for your consideration

Once the results from the sub-chronic toxicity study (Section II, 1. above) are available, you should reconsider the information requirement of Annex IX, Section 8.7.3. If the sub-chronic toxicity study indicates adverse effects on reproductive organs or tissues, or reveals other concerns in relation with reproductive toxicity, a new testing proposal for the present endpoint would – in accordance with the REACH Regulation – have to be submitted, unless compliance with this information requirement is scientifically justified and documented by means of specific or general rules of adaptation.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposal(s) for examination pursuant to Article 40(1) on 24 May 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.

This decision does not take into account any updates after 4 January 2016, 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.

ECHA took into account your comments and did not amend the requests.

You updated your registration on 22 December 2015. ECHA took the information in the updated registration dossier into account, and did not amend the draft decision. The updated information is reflected in the Reasons (Appendix 1).

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposal for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment.

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-52 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. 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.

2. 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.

3. In carrying out the tests required by the present decision it is important to ensure that the particular sample of substance tested 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 tests must be suitable to assess these. Furthermore, there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the tests to be assessed.