EUROPEAN CHEMICALS AGENCY

Helsinki, 26 October 2017

Addressee: [redacted]

Decision number: CCH-D-2114375731-46-01/F
Substance name: 1,4-DIOXACYCLOHEXADECANE-5,16-DIONE
EC number: 259-423-6
CAS number: 54982-83-1
Registration number: [redacted]
Submission number: [redacted]
Submission date: 25/01/2017
Registered tonnage band: 100-1000

**DECISION ON A COMPLIANCE CHECK**

Based on Article 41 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA requests you to submit information on:

1. **Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: OECD [421/422]) in rats, oral route with 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 (rat or rabbit), oral route with the registered substance;**

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 to the REACH Regulation. 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 adequate and reliable documentation.

You have to submit the requested information in an updated registration dossier by **3 May 2019**. You also have to 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 and 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, has to 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 Kevin Pollard, 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.

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**Appendix 1: Reasons**

**TOXICOLOGICAL INFORMATION**

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

**1. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)**

"Screening for reproductive/developmental toxicity" (test method OECD TG 421 or 422) is a standard information requirement as laid down in Annex VIII, Section 8.7.1. of the REACH Regulation if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from *in vitro* methods that the substance may be a developmental toxicant. No such evidence is presented in the dossier. Therefore, adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have not provided any study record of a screening for reproductive/developmental toxicity in the dossier that would meet the information requirement of Annex VIII, Section 8.7.1.

The technical dossier does not contain an adaptation in accordance with column 2 of Annex VIII, Section 8.7.1. or with the general rules of Annex XI for this standard information requirement.

You have provided a study for this endpoint. You indicated that this study is equivalent or similar to a study performed according to OECD TG 422. However, the study provided was performed according to OECD TG 407 which addresses parameters for sub-acute toxicity and does not provide any information on reproductive performance or on peri- and post-nata developmental toxicity as required acording to OECD TG 422. Hence, this study is not appropriate for addressing fertility as the animals were not mated. You have furthermore sought to adapt this information requirement according to Annex VIII, Section 8.7.1., column 2. indicating that a pre-natal developmental toxicity study according to OECD TG 414 on a read-across substance is available. However, as explained below in Section 2 below your read-across adaptation is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to the test methods OECD TG 421/422, the test is designed for use with rats. On the basis of this default assumption ECHA considers testing should be performed with rats.

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 6.0, July 2016) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision according to Article 50(1) of the REACH Regulation you explain that you considered an adaptation justification for this endpoint superfluous in view of the available 90-day repeated dose toxicity information and the developmental toxicity information (OECD TG 414) available for analogue substances. You also argue that you consider the repeated dose toxicity part of the OECD TG 422 similar to the OECD 407 and that, according to your understanding, the fertility parameters are sufficiently covered in the 407-test when the male and female reproductive organs are assessed and no effects are seen. You also propose to include an adaptation justification in the updated dossier indicating that the OECD TG 421/422 is not considered necessary because the fertility aspect of the OECD TG 421/422 is sufficiently addressed in the repeated dose toxicity information presented: absence of fertility in Zenolide OECD TG 407 and for EG in the 90-day study, as can be read from the REACH text 8.6.2.

As explained above, information from repeated dose toxicity studies (OECD TGs 407, 408) cannot fulfil the information requirements for this endpoint, since there was no mating of animals and production of offspring. According to Annex VIII, Section 8.7.1., Column II of the REACH regulation, a screening study according to OECD TG 421 or 422 does not need to be conducted if a pre-natal developmental toxicity study (Annex IX, 8.7.2) or, either an Extended One-Generation Reproductive Toxicity Study (B.56, OECD TG 443) (Annex IX, section 8.7.3) or a two-generation study (B.35, OECD TG 416), is available. However, as explained in Section 2 of this decision, the study record for a pre-natal developmental toxicity study (OECD TG 414) you provided is with the analogue substance Habanolide (CAS 111879-80-2), for which ECHA has rejected the read-across. Hence, you need to provide an acceptable pre-natal developmental toxicity study according to OECD TG 414 or a screening study according to OECD TG 421 or 422, with the registered substance, to fulfil the information requirements for this endpoint (Annex VIII, Section 8.7.1.).

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:
- Reproductive/developmental toxicity screening test (test method: OECD TG 421) *or* Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (test method: OECD TG 422) in rats by the oral route.

*Notes for your considerations*

For the selection of the appropriate test, please consult ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.5 and 7.6 (version 6.0, July 2016).

You should also carefully consider the order of testing especially the requested screening (OECD TG 421/211) and the developmental toxicity studies (OECD TG 414) to ensure unnecessary animal testing is avoided, paying particular attention to the end point specific guidance, which you can find on ECHA's webpage (https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf)."

Your comments according to article 50(1) of the REACH Regulation contain detailed questions on the order of testing and the requests contained in this decision. ECHA cannot give you substance specific advice. ECHA therefore specifically refers to pp 468, 477, and 486 in the abovementioned guidance. It is your responsibility to consider the most appropriate testing strategy.

ECHA reminds that the general and specific adaptation rules of Annex IX section 8.7.1 and of Annex XI shall be considered for fulfilling information requirements in order to avoid unnecessary animal testing. If results from a study screening for reproductive/ developmental toxicity wourld corroborate your hypothesis, this would strengthen the read-across adaptation.

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

A "pre-natal developmental toxicity study" (test method EU B.31./OECD TG 414) for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study records for a pre-natal developmental toxicity study (OECD TG 414) with the analogue substance Habanolide (CAS 111879-80-2). However, the proposed read-across adaptation is inadequate, as explained below.

Description of the grouping and read-across approach

In the technical dossier, you provide a read-across hypothesis in the CSR:

*"Zenolide is a cyclic aliphatic double ester for which developmental toxicity information is missing. In accordance with Article 13 of REACH, lacking information should be generated whenever possible by means other than vertebrate animal tests, i.e. e. applying alternative methods such as in vitro tests, QSARs, grouping and read-across. For assessing the developmental toxicity of Zenolide the read across approach is applied. For the structural related analogue Habanolide developmental toxicity is available. There is also supporting information from Zenolides metabolites Ethylene Glycol and DoDecaneDiocAcid (DDDA).*

*Hypothesis: No developmental toxicity of Zenolide is anticipated based on absence of effects (NOAEL >=1000 mg/kg bw) in an OECD TG 414 study with Habanolide.*

*Available information: For Habanolide an OECDTG 414 is present. The low order of toxicity of Zenolide can be retrieved from a 28 day GLP repeated dose study was undertaken (OECD TG 407), resulting in a NOAEL of >=1000 mg/kg/day ([redacted], 2000). Supporting developmental toxicity information is present from Zenolides key metabolites. For the metabolite EG developmental toxicity is reviewed in an ASTDR review in 2007 showing minimal embryotoxicity at 1000 mg/kg bw. For the other metabolite DDDA an OECD TG 422 is available presenting absence of toxicity >=1000 mg/kg bw (ECHA dissemination site)."*

Support of the grouping and read-across approach and ECHA's analysis in light of the requirements of Annex XI, 1.5.

In order to meet the provisions in Annex XI, Section 1.5. to predict human health effects from data for a reference substance within the group by interpolation to other substances in the group, ECHA considers that structural similarity alone is not sufficient. It has to be justified why such prediction is possible in view of the identified structural differences and the provided evidence has to support such explanation. In particular, the structural similarities must be linked to a scientific explanation of how and why a prediction is possible.

*Structural similarities and differences*

You indicate the following: *"Zenolide is a cyclic aliphatic di-ester. Habanolide is also a cyclic aliphatic ester but with only a single ester bond. It has a double bond in the cyclic alkyl-chain not present in Zenolide (Table 1). The presence of an additional ester group is not expected to result in additional toxicity as is explained below.*

ECHA notes the stuctural differences between source and target substance which will result in different metabolites as described below. You conclude that the presence of an additional ester group is not expected to result in additional toxicity. However, you did not provide any factual evidence (appropriate study) to support your assumption that the structural differences will not have an impact on the development of the offspring (see below).

*Toxicokinetics and metabolism*

You provided the following arguments:

*"Absorption: Based on the similarities in molecular weight, physical appearance and the physico-chemical properties of Zenolide and Habanolide absorption via oral, dermal and inhalation route are expected to be similar (see Table 1). Though Zenolide has a somewhat lower log Kow compared to Habanolide which is not anticipated to result in differences in absorption because both substances will be metabolised via all routes. The Log Kow values calculated with EpiSuite show similar log Kow values 4.22 and 4.39, respectively, indicating some experimental variability.*

*Their moderate and low water solubilities are in the range of good oral absorption (see toxico-kinetic section). The vapour pressures of bot substances are low. Because of these similar physico-chemical properties it is anticipated that the toxico-kinetic behaviour of Zenolide and Habanolide are alike (e.g. oral, dermal and inhalation absorption)."*

*Metabolisation: Zenolide will be metabolised in the gut and liver into EG and DDDA, the latter being a saturated fatty acid. Habanolide will be metabolised too but its key metabolite will be C-15-hydroxypentadec-14-enoic acid with and acid group on one side and an alcohol at the other side of the alkyl chain, being an unsaturated fatty acid. Thereafter the substances will be conjugated and finally will be metabolised into CO2 and H2O. At high dosed excretion via the urine will occur.*

ECHA observes that you have not provided any information on the rate and kinetics of metabolism for the target (registered) and the source substances. In the absence of information on instantaneous hydrolysis/metabolism of the parent compound, supporting information on the developmental toxicity of the parent compound, for example by providing a screening study according to OECD TG 421 or 422, would be required to appropriately address the impact of the parent compound on the overall developmental effect of the target substance and its metabolites (see below).

ECHA notes that – as you indicated above - the potential metabolites formed by the source and target substances are different (see below).

*Toxico-dynamics similarities and differences*

You conclude the following: *Habanolide has one double bond in the ring which is absent in Zenolide. This double bond is not expected to cause a difference in reactivity, because no additional electronegative groups are present to this double bond. Zenolides key metabolites are EG and DDDA, the latter being a saturated fatty acid. Habanolide after metabolisation becomes an unsaturated fatty acid with a slightly longer chain. Both acids will be metabolized into CO2 and H2O and no reactivity is anticipated."*

As indicated above, the provided information on the metabolites of the registered (target) substance does not address the contribution of potential developmental toxicity of the parent compound (Zenolide). Furthermore, the metabolites for the source and the target substance are different and one metabolite of the registered substance (ethylene glycol) is known to be developmentally toxic at high doses. Moreover, the similarity you indicate for the other metabolites (fatty acids) of target and source substance is the endogenous metabolism of fatty acids to $CO_2$ and $H_2O$ with the difference that the metabolite of the target substance is saturated, whereas the metabolite of the source substance is unsaturated and could for that reason have a different toxicological profile. Hence, the proposed read-across to the source substance has several short-comings due to the differences in the metabolic pattern of the target and the source substance.

In your comments to the draft decision according to Article 50(1) of the REACH Regulation you state the following:

- In view of the similar backbone and ester as a functional group, the rate and kinetics are expected to be the same between Zenolide and its source Habanolide.
- The fatty acid metabolites (saturated and unsaturated) of both Zenolide and Habanolide, respectively, will not present developmental toxicity being normal constituents of the feed.
- The additional reactivity/toxicity or 3-D configuration of the double ester compared to the single ester can be derived from the similarity in all other human health toxicity endpoints. The key and the source materials are not irritants or sensitisers and not genotoxic.
- You provide information on the target and the source substance such as molar volume, rotational bonds and H-donors and acceptors to underpin the likely similarity related to receptor fit.
- You conclude that the key difference between Zenolide and Habanolide is the Ethylene Glycol (EG) metabolite that can be formed from Zenolide and not from Habanolide.
- You propose to add a record on the developmental toxicity of EG to the updated dossier.

ECHA observes that the read across assessment framework (RAAF, https://www.echa.europa.eu/documents/10162/13628/raaf_en.pdf) specifically states that "...structural similarity alone is not sufficient to justify the possibility to predict property(ies) of the target substance by read-across.", specifying "There may be several lines of supporting evidence used to justify the read-across hypothesis,
with the aim of strengthening the case." and "Supporting evidence may range from theoretical considerations or expert systems, to results from *in vivo* or *in vitro* studies. For many cases, toxicokinetic data constitute valuable supporting evidence. Often quantitative information is needed."

ECHA stresses that the metabolic rate and pattern of the target and source substances are not underpinned by experimental data and hence, they are solely built on theoretical considerations. ECHA is of the view that on the basis of the available studies with the registered substance, none of which resulted in any offspring, theoretical considerations are insufficient to demonstrate that reliable predictions can be made for this endpoint.

Furthermore, you provided information related to receptor fit, differences in H-bond acceptors, and polar surface area. This information is regarded as sufficient to demonstrate relevant *differences* in receptor binding. This is particularly relevant for the multitude of quickly changing targets in a developing mammal (PNDT-specific argument, screening-study specific for the offspring (survival)). In the absence of experimental information on reproductive toxicity on the target substance, the basis to predict properties from Habanolide to Zenolide remains insufficient.

*Conclusion*

ECHA finds the read-across justification currently not convincing to allow the prediction from studies conducted with the analogue source substance Hababolide (CAS 111879-80-2) of the properties for the target substance Zenolide (CAS 54982-83-1) with regard to pre-natal developmental toxicity.

ECHA notes that factual evidence, *e.g.* an OECD TG 421 or 422 screening study performed with the registered substance, is required to support the proposed read-across. In case the screening study does not support the read-across hypothesis, a definitive pre-natal developmental toxicity study will be required.
Thus, the adaptation does not comply with the general rules of adaptation as set out in Annex XI, 1.5. Therefore, your adaptation of the information requirement is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently, there is an information gap and it is necessary to provide information for this endpoint.

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 assumption ECHA considers testing should be performed with rats or rabbits as a first species.

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 6.0, July 2016) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

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: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a first species (rat or rabbit) by the oral route.

## Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 15 February 2017.

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.

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

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

As no amendments were proposed, ECHA took the decision according to Article 51(3) of the REACH Regulation.

**Appendix 3: Further information, observations and technical guidance**

1. This compliance check decision does not prevent ECHA from initiating further compliance checks on the present registration at a later stage.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests 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 or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.