Helsinki, 23 November 2018

Addressee: [redacted]

Decision number: TPE-D-2114449799-25-01/F
Substance name: (1s,5s)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
EC number: 232-077-3
CAS number: 7785-26-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 19 September 2017
Registered tonnage band: 100-1000

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation ((EC) No 1907/2006) (the REACH Regulation), ECHA examined your testing proposal(s) and decided as follows.

While your originally proposed test for a Pre-natal developmental toxicity study (OECD TG 414) using the analogue substance reaction mass of (1R,5R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene and (1S,5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene is rejected, you are requested to perform:

1. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) in a first species (rats or rabbits), oral route using 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 an adequate and reliable documentation.

You have to submit the requested information in an updated registration dossier by **31 May 2021**. You also have to update the chemical safety report, where relevant.

The reasons for 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 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.

Appendix 1: Reasons

The decision of ECHA is based on the examination of the testing proposal submitted by you for the registered substance (1s,5s)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (EC 232-077-3), hereafter the 'target substance' or 'registered substance', common name '(-)-alpha-pinene'.

In relation to the testing proposal subject to the present decision, you propose a testing strategy intending to fulfil the standard information requirement for a pre-natal developmental toxicity study (Annex IX, Section 8.7.2). You propose to test the analogue substance 'reaction mass of (1R,5R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene and (1S,5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene' (EC No 201-291-9) (hereafter the 'source substance', common name 'alpha-pinene multi-constituent'). You propose to use the results to adapt this standard information requirement for your registered substance by using a grouping and read-across approach following Annex XI, Section 1.5. of the REACH Regulation.

ECHA has considered first the scientific and regulatory validity of your grouping and read-across approach in general before assessing the individual properties in section 1 of this appendix.

Grouping of substances and read-across approach

You have sought to adapt the information requirement listed above by applying a read-across approach in accordance with Annex XI, Section 1.5. According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach). ECHA considers that the generation of information by such alternative means should offer equivalence to prescribed tests or test methods.

Based on the above, a read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological or ecotoxicological property is reliable and should be based on recognition of the structural similarities and differences between the source and registered substances. This hypothesis explains why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.

Due to the different nature of each endpoint and consequent difference in scientific considerations (e.g. key parameters, biological targets), a read-across must be specific to the endpoint or property under consideration.

The ECHA 'Read-across assessment framework (RAAF)' foresees that there are two options, which may form the basis of the read-across hypothesis:

- (Bio)transformation to common compound(s)- the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed, and
- Different compounds have the same type of effect(s)- the read-across hypothesis is that

the organism is exposed to different compounds which have similar (eco)toxicological and fate properties as a result of structural similarity (and not as a result of exposure to common compounds).

Finally, Annex XI, Section 1.5. lists several additional requirements, which deal with the quality of the studies which are to be read-across.

i. Description of the grouping and read-across approach proposed by you

You have provided a read-across documentation as a separate attachment in the registration ('[redacted]').

You state that "*This document is based on RAAF Scenario 1: "analogue approach for which the read-across hypothesis is based on (Bio)transformation to common compound(s)"* and that the RAAF was used to assess the read-across used in the registration dossier of the registered substance.

You use the following arguments to support the prediction of properties of the registered substance from data for source substances within the group:

"*This read-across is based on the hypothesis that source and target substances have similar physicochemical, toxicological, ecotoxicological and environmental fate properties because of their structural, physicochemical and pharmacokinetic similarities (enantiomers).*"

You explain that the target substance is a bicyclic monounsaturated monoterpene and a mono-constituent substance. The composition is:

- (-)-alpha-pinene / (1S,5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (EC 232-077-3), range [redacted] %, typical [redacted] %;
- (+)-alpha-pinene / (1R,5R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (EC 232-087-8), range [redacted] %, typical [redacted] %;
- (-)-camphene / (1S,4R)-2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane (EC 227-337-8), range [redacted] %, typical [redacted] %;
- (+)-camphene / (1R,4S)-2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane (EC 227-336-2), range [redacted] %, typical [redacted] %;
- (-)-beta-pinene / (1S,5S)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (EC 242-060-2), range [redacted] %, typical [redacted] %;
- further identified impurities at or below [redacted] % typical concentration.

ECHA notes that the boundary composition indicates for the composition on the one hand up to [redacted] % of (-)-alpha pinene and on the other hand up to [redacted] % of non-identified impurities.

The source substance is a multi-constituent substance. The composition according to your testing proposal is:

- (+)-alpha-pinene / (1R,5R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (EC 232-087-8), typical concentration ca. [redacted] %;
- (-)-alpha-pinene / (1S,5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (EC 232-077-3), ca. [redacted] %;
- (-)-beta-pinene / (1S,5S)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (EC 242-060-2), ca [redacted] %;

- (+)-camphene / (1R,4S)-2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane (EC 227-336-2), ca. [redacted] %;
- (-)-camphene / (1S,4R)-2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane (EC 227-337-8), ca. [redacted] %;
- Tricyclene / 1,7,7-trimethyltricyclo[2.2.1.0~2,6~]heptane (EC 208-083-7); ca. [redacted] %
- (+)-beta-pinene / (1R,5R)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (CAS 19902-08-0), ca [redacted] %;
- Other identified impurities below [redacted] %.

You point out that the target substance is composed mainly of two enantiomers. The main constituent is the (-) alpha-pinene [redacted] %. The (+)-alpha-pinene is present at up to 12 % w/w (typical concentration = 9.9% w/w) as an impurity in the target substance. The main constituent of the target substance (-)alpha-pinene is also a constituent in the source substance at ca. [redacted] according to the information in the testing proposal and at typical [redacted] % according to the information in your read-across justification. You claim that the impurities are the same and in the same concentration range and are expected to have low impact on toxicological endpoints.

Your main justification is based on structural similarity:
"The main constituents of source and target substances belong to the bicyclic terpene hydrocarbons. More precisely, they are enantiomers from each other (see Table 1) therefore they have very similar chemical structures."

You provide a data matrix to compare the physicochemical properties of the source and target substance and conclude that they have very similar physicochemical properties. For environmental properties, the short-term toxicity in fish was conducted in both, source and target substance.

For toxicological properties you provide information obtained with the source substance, but no information obtained with the target substance.

You report that there are comparative data on the toxicokinetic behaviour of the two entantiomers in rabbits and humans. You state that *"it was experimentally shown, mostly in human volunteers exposed by inhalation, that (+)-alpha-pinene and (-)-alpha-pinene are absorbed, distributed, metabolised and eliminated in a similar way. Therefore, (-)-alpha-pinene and alpha-pinene multiconstituent are absorbed, distributed, metabolised and eliminated in a similar way".*

Overall you conclude: *"As it was shown that enantiomers show similar structural, physicochemical and ecotoxicological properties, it is concluded that they share similar toxicological properties. Therefore, it is not deemed necessary to test target substance as a monoconstituent because it can be considered that its toxicological properties have already been assessed by testing alpha-pinene multiconstituent".*

As an integral part of this prediction, you propose that the source and registered substances have similar properties for the above-mentioned information requirement. ECHA considers that this information is your read-across hypothesis.

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

a) Explanation on why and how the structural similarities allow predictions

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.

ECHA notes the following observations:

1. You claim that RAAF scenario 1 applies to your read-across approach: *analogue approach for which the read-across hypothesis is based on (Bio)transformation to common compound(s).* But you do not specify these common compounds. Therefore, ECHA considers your claim as not supported. Your arguments on toxicokinetics in this regard are addressed under point d. below.
2. Furthermore, ECHA understands that you assume that enantiomers are structurally similar and therefore have the same toxicological properties. You do not support this claim by any data, neither in general nor specifically for the substances under consideration. ECHA points out that enantiomers are stereoisomers with the same molecular formula and similar physical chemical properties except for the rotation of polarized light (optical isomers). This feature indicates the relevant difference between enantiomers: they differ in the three dimensional orientation in space, i.e. they are mirror images of each other. Therefore, they interact differently with other optical isomers, such as occurring for many biological molecules. Consequently, enantiomers may have quite different biological or toxicological effects.[2,3] Prominent examples are ibuprofen and thalidomide. Ibuprofen consists of the racemic mixture of the pharmacologic active S(+)-ibuprofen (inhibitor of cyclooxygenase) and the inactive R(-)-ibuprofen. Thalidomide consists of the racemic mixture of R(+)- and S(-)-form. The sedative effect is attributed to the R-form, the S-form does not act as sedative but as teratogen. Without experimental evidence to the contrary, ECHA therefore assumes that the (-)- and the (+)-alpha pinenes may have different toxicological properties or may have different potencies to induce adverse effects.
3. In addition, ECHA understands that you claim that the target substance, the (-)-isomer, contains also the (+)-isomer and the source substance (i.e. the multi-constituent substance) contains also the (-)-isomer. Therefore, a test with the source substance would assess also the toxicological properties of the target substance.
   Composition of the target substance: ECHA points out that the (+)-isomer has a concentration range between [redacted] % in the target substance according to the legal entity composition. The boundary composition does not list the (+)-isomer at all. Instead, the boundary composition indicates that there may be [redacted] % of non-identified impurities present. So in the extremes, the composition of the target substance could almost only consist of the (-)-isomer, or have [redacted] % non-identified impurities.
   Composition of the source substance: ECHA points out that the (-)-isomer in the source substance has a concentration of 'ca. [redacted] %' as indicated in your testing proposal. Therefore, ECHA assumes that at least [redacted] % of the composition of the source substance is different from (-)-alpha pinene.
   Conclusion: Your claim that testing with the source substance assesses also the toxicity of the target substance is not supported, because the major portion of the source substance does not contain the enantiomer constituent of the target substance,

[2] Nguyen LA;He H and Pham-Huy C, 2006. Chiral drugs: an overview. Int J Biomed Sci 2006;2(2):85-100 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3614593/
[3] Smith SW, 2009. Chiral toxicology: it's the same thing...only different. Toxicol Sci 2009;110(1):4-30 https://doi.org/10.1093/toxsci/kfp097

as pointed out above. Assuming that the target substance is covered by the composition of the source substance will lead to an underestimation of the hazard because of the low concentrations at which the target substance constituents are present in the source substance. Without evidence to the contrary ECHA assumes that the enantiomers have different hazard properties.

ECHA concludes that you have not addressed the obvious structural differences between the source substance and the target substance. You did not explain why those differences would not lead to differences in the toxicity profile of target and source substances. ECHA does not consider the provided explanations valid to establish a scientific credible link between the structural similarity and the prediction.

b) Support of a similar or regular pattern as a result of structural similarity

Annex XI, Section 1.5. provides that "*substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances.* One prerequisite for a prediction based on read-across therefore is that the substances involved are structural similar and are likely to have similar properties. One important aspect in this regard is the analysis of the data matrix to compare the properties of source and target substances and to establish whether indeed they are similar or follow a regular pattern.

ECHA notes the following observations:

1. Currently, there are no data on any toxicological properties for the registered substance.
2. There are also no hazard data on any of the possible metabolites formed from the parent compounds (see section d.).

ECHA concludes that the presented evidence in the data matrix does not support a similar or regular pattern of toxicity because of structural similarity. Therefore, it cannot be verified that the proposed analogue substance can be used to predict properties of the registered substance.

c. Reliability and adequacy of the source studies

ECHA notes the following observations:

The proposed source study was requested under a compliance check (CCH-D-2114373437-42-01/F) for the source substance and ECHA assumes that this study is ongoing. Currently, results are not available.

ECHA concludes that the proposed source study currently cannot be assessed.

d. Toxicokinetics

One important aspect in establishing that substances have similar effects or follow a regular pattern is the comparison of absorption, distribution, metabolism and elimination of source and target substances. This allows assessing the qualitative and quantitative internal systemic exposure of the test organism when exposed to source and target substances.

You did indicate, but not specifically claim, that the two enantiomers are metabolised to the same products. ECHA has analysed the information on toxicokinetics with a view on this aspect and makes the following observations on the information submitted.

Information regarded as not adequate and/or not reliable by ECHA:

- A study with (+)-, (-)-, and (+/-)-alpha pinene studied the metabolism in the rabbit [redacted] 1981). You report that the main metabolites in urine were determined after orally administered doses of 400 – 700 mg/kg bw. The metabolite for all alpha-pinenes is reported to be (-)-transverbenol. No methods or other details are reported and therefore the results are difficult to interpret. However, ECHA notes that (-)-alpha-pinene has two chiral carbons. In contrast, the metabolite (-)-transverbenol, has three chiral carbons. Furthermore, ECHA notes that it appears that the metabolism that has occurred is a hydroxylation of (-)-alpha-pinene and that the two chiral carbons from (-)-alpha-pinene remain intact. ECHA concludes that this study has not measured metabolites stemming from (+)-alpha-pinene.
- A report of a patient attempting suicide ingested pine oil containing [redacted]% alpha-pinene (Koeppel, 1981). The identity of alpha-pinene in terms of isomer ratio was not reported. ECHA considers the results as not adequate or reliable due to missing details of methods and results.
- The distribution of turpentine containing [redacted] % alpha-pinene was investigated (Savolainen, 1978). Alpha-pinene was found in the perinephric fat and brain. The brain pinene content remained similar throughout the experiment and it was about 10% of that in fat. No methods nor the enantiomer ratio of the test material was reported. ECHA considers the results as not adequate or reliable due to lacking details of methods and results.
- In a human volunteer study ([redacted], 1996) the uptake and blood clearance of turpentine and several monoterpenes including alpha-pine was studied. The enantiomer ratio of the test material was not reported and conclusions cannot be drawn with regard to the results for the individual enantiomers.
- In humans, the renal elimination of verbenols after exposure to (+)-alpha-pinene and (-)-alpha-pinene was studied at 10, 225 and 450 mg/m3 (Levin, 1992; only reported in the justification document). You report that the pulmonary uptake was about 60 %. About 8 % was exhaled unchanged. The renal excretion of unchanged material was less than 0.001 %. Depending on the exposure level, about [redacted] % of the total uptake was eliminated as cis-and trans-verbenol. Most of the verbenols were eliminated within 20 hours and the differences between the measured toxicokinetic parameters were very close for both isomers studied. The methods used in the study were not reported in the justification document and a robust study summary is missing from the registration dossier. ECHA therefore cannot verify the reported results from the dossier.

Information regarded as adequate and reliable:

- In a human volunteer study ([redacted] 1990; only reported in the justification document) the toxikokinetics of (-)- and (+)-alpha pinene was studied. Two volunteers were exposed to 450 mg/m3 of each enantiomer for two hours. There was rapid uptake of 58 % of the total exposure dose for both isomers. The elimination kinetics had three phases with t1/2 of 4.8 and 5.6 min for the first phase, 38 and 40 min for the second phase, and 695 and 555 min for the third phase for (+)-alpha pinene and (-)-alpha-pinene, respectively. Less than 0.001 % of the dose was excreted unchanged in urine. The blood clearance values 1.09 and 1.16 L/h/kg for (+)-alpha-pinene and (-)-alpha-pinene, respectively, indicated that both substances are readily metabolised. Metabolites were not analysed in the study. The methods used in the study were not reported in the justification document and a robust study summary is missing from the registration dossier. ECHA therefore cannot verify the reported results from the dossier. However, ECHA has access to the publication and regards the results reported in the publication as adequate and reliable.

- In a recent publication identified by ECHA, but not reported in the dossier or justification document, the human in vivo metabolism and the elimination kinetics of alpha-pinene after oral administration was studied.[4] The authors did not report the ratio of enantiomers in the test material. The results indicate rapid uptake and rapid elimination with storage of the unchanged substance in adipose tissue. A large proportion of the dose appeared to be eliminated via exhalation. The renal elimination accounted to only 22 % of the dose, followed a biphasic kinetics, and the analytics applied resulted in the detection of carboxylic acid derivatives as metabolites. These metabolites must have been formed via various intermediates starting with hydroxylation of the parent substance at several positions of the ring system. ECHA regards the metabolism scheme of alpha-pinene as quite complex, as also reported by Vespermann et al.[5]

Based on the available information, ECHA concludes that it is likely that the enantiomers of alpha-pinene show similar kinetic behaviour with regard to uptake and elimination. However, the same uptake and elimination characteristics of enantiomers do not indicate that they have the same toxicological properties.

Both enantiomers are apparently taken up and are present in systemic circulation after uptake, as indicated by the storage in adipose tissue, the elimination kinetics, and the exhalation. However, many aspects of the metabolism are not clear for the individual enantiomers, such as which metabolites are formed after inhalation of one of the enantiomers alone. ECHA assumes that the metabolites also have chiral centres leading to distinct enantiomers depending on the stereochemistry of the parent compound. As consequence, the metabolic profile and the systemic exposure to the metabolites are assumed to be different after administration of the individual enantiomers. There is no information on toxicological properties of any metabolites in the registration dossier. As explained above under point a. different enantiomers of a substance may have different adverse effects or different potencies for the same effects. This is also valid for enantiomeric metabolites.

ECHA concludes that different toxicological profiles or different potencies for the same effect cannot be excluded for the individual enantiomers on the basis of the available toxicokinetic data. Therefore, it is not possible to identify the substances, which are likely to govern the toxicity profiles of source and target substances. In the absence of such information there is not an adequate basis for predicting the properties of the registered substance from the data obtained with the source substance.

iii. Conclusion on the read-across approach

The testing proposal for a pre-natal developmental toxicity study in the technical dossier is based on the proposed grouping and read-across approach examined above. ECHA does not consider that testing the proposed analogue substance will provide a reliable basis to predict the properties of the registered substance for the reasons set out above. ECHA therefore concludes that the criteria of Annex XI, 1.5. are not met, and the grouping and read-across approach is not considered plausible to meet the information requirements.

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

[4] Schmidt L and Goen T, 2017. Human metabolism of alpha-pinene and metabolite kinetics after oral administration. Arch Toxicol 2017;91(2):677-687 https://doi.org/10.1007/s00204-015-1656-9
[5] Vespermann KA;Paulino BN;Barcelos MC;Pessoa MG;Pastore GM and Molina G, 2017. Biotransformation of alpha- and beta-pinene into flavor compounds. Appl Microbiol Biotechnol 2017;101(5):1805-1817 https://doi.org/10.1007/s00253-016-8066-7

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.

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 OECD TG 414 with the analogue substance ALPHA-PINENE MULTICONSTITUENT (Reaction mass of (1R,5R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene and (1S,5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene) (EC No 201-291-9).

ECHA requested your considerations for alternative methods to fulfil the information requirement for reproductive toxicity (pre-natal developmental toxicity). ECHA notes that you provided your considerations and you applied read-across to fulfil the respective information requirement, and no other alternative methods were available. ECHA has taken these considerations into account.

ECHA has evaluated your proposal to perform the test with the proposed analogue substance and as explained above under 'Grouping of substances and read-across approach' ECHA does not consider that testing the proposed analogue substance will provide a reliable basis to predict the properties of the registered substance.

In addition, ECHA notes that the results of a sub-chronic inhalation study in rats and mice with the proposed source substance (NTP Toxicity Report Series Number 81, May 2016) is reported in the dossier on the registered substance. The test material was [redacted] % alpha pinene of which [redacted] % was (-) alpha pinene and [redacted] % was (+)-alpha pinene. Besides other effects, in both species significantly decreased numbers of cauda sperm were observed. Although you question the relevance of these findings in your robust study summary, ECHA considers that the applied method to prepare the cauda samples for sperm counts is acceptable and the observed effects are consistent in both studies and in both species, pointing towards an endocrine disrupting mode of action. It is however not known, whether only one enantiomer of alpha-pinene is causing such effects or whether one enantiomer is more potent than the other. Moreover, such endocrine disrupting mode of action is also relevant for pre-natal developmental toxicity. Therefore, the potency of the registered substance in a pre-natal developmental toxicity study needs to be investgated.

ECHA considers that a study performed with the registered substance is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

According to the test method 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 the rat or rabbit 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 6.0, July 2017) 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, you consider that the proposed read-across approach rejected in this decision still could be supported by results obtained by the studies requested in the parallel compliance check decision on the same dossier. You state, if the results of the requested OECD TG 421 study provide evidence for similar toxicity between target and source substances, you intend to conclude that the ongoing OECD 414 study with the source study could be used to cover this information requirement for the target substance according to Annex XI, Section 1.5. Furthemore, you consider that the future results of the OECD TG 471 study would strenghten the read-across approach. If different toxicity is observed, you consider the read-across approach is not validated and the OECD TG 414 study would be conducted with the registered substance.

There are several issues to be taken into account with regard to your comment:

(1) ECHA points out that also the comparison of the results obtained in the OECD TG 413 requested in the parallel compliance check decision with the registered substance with the existing OECD 413 study on the proposed source substance is mandatory, prior to conclude on similarity in toxicity. A distinct toxicity pattern was observed in the study with the source substance (significantly decreased numbers of cauda sperm, liver and kidney weight increases, alpha-2u-globulin induced nephropathy proposed as mechanism for the male kidney effects). Therefore, the results obtained in the OECD TG 413 study with the registered substance will in particular be suitable for a comparison of systemic toxicity profiles.

(2) Results obtained in the OECD TG 471 or in other genoxicity studies will be of limited value in assessing similarity for systemic or reproductive toxicity.

(3) Results from the OECD TG 421 and OECD TG 413 will allow the assessment of whether the toxicity profiles (including type and strength of effects) observed for the substances are indeed similar for systemic toxicity and, at a screening level, for reproductive toxicity. In that respect, ECHA considers that the following criteria are decisive for the actual determination of similarity in toxicity:

- No adverse effects are observed in any organ or tissue for the both source and target substances when tested up to the limit dose; or
- Comparable effects (i.e. in terms of type of effect, severity and incidence) are observed in the same organ(s) tissue(s) or parameters at similar dose level for both source and target substances.

Verifying that these criteria are met is an essential condition for the valid justification of the similarity of toxicity for the substances and, hence, for meeting the provisions in Annex XI, Section 1.5 to adapt the information requirement.

(4) A data matrix and the comparison of results in this matrix for source and target substance is one essential element for presenting an adaptation based on a grouping and reas-across approach. However, in addition it has to be explained why and how a prediction can be made. The RAAF explains the elements to be covered[6]. Therefore, an updated justification complying with the provisions in Annex XI, Section 1.5, and integrating all new information generated need to be developed by you, in case you consider that the information requirement on pre-natal developmental effects may be covered by the study results obtained with the proposed source substance.

[6] RAAF, https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

Since currently, neither the results of the studies with the proposed source substance nor the results of the studies requested in the parallel compliance check are known to ECHA, ECHA has not amended the request.

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, you are requested to carry out the 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: OECD TG 414).

Your originally proposed test for a Pre-natal developmental toxicity study (OECD TG 414) using the analogue substance reaction mass of (1R,5R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene and (1S,5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene is rejected, according to Article 40(3)(d) of the REACH Regulation.

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* (version 6.0, July 2017), Chapter R.7a, Section R.7.6.2.3.2.

Deadline

In order to follow the sequential testing approach proposed by you and described above, you requested to extend the deadline for submitting the information requested in this decision. In the draft decision communicated to you the time indicated to provide the requested information was 12 months from the date of adoption of the decision. In your comments on the draft decision, you requested a 24 months delay from the the date of the final decision before performing the OECD TG 414 study requested in this decision. You provide the reasoning that only with an extended deadline it is possible to consider the results of the studies requested in the parallel compliance check decision for sequential testing.

ECHA considers that your proposed approach will integrate the timelines for the parallel compliance check decision and this testing proposal decision in order to allow for sequential testing. The default deadline for submitting the results of an OECD TG 421, an OECD TG 413 study and an OECD TG 414 study is 30 months when requested in combination in one decision. ECHA considers this deadline also applicable in the current case, when the same studies are requested in two parallel decisions.

Therefore, ECHA has set the deadline to 30 months.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 19 September 2017.

ECHA held a third party consultation for the testing proposals from 25 October 2017 until 11 December 2017. ECHA did not receive information from third parties.

This decision does not take into account any updates after **8 August 2018**, 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 amended the deadline.

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 decision does not imply that the information provided in your 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 otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of the Member States.

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.