EUROPEAN CHEMICALS AGENCY

Helsinki, 23 November 2018

Addressee [redacted]

Decision number: CCH-D-2114449797-29-01/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 COMPLIANCE CHECK

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

1. **In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, EU B.13/14. / OECD TG 471) with the registered substance;**

2. **In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;**

3. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance provided that both studies requested under 1. and 2. have negative results;**

4. **Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: OECD TG 421 or 422 in rats, oral route with the registered substance;**

5. **Sub-chronic toxicity study (90-day), inhalation route, whole body exposure (Annex IX, Section 8.6.2.; test method: OECD TG 413) in rats with the registered substance modified to include urinalysis and a full histopathological examination which is to include immunohistochemical investigation of renal pathology to determine if the pathology is mediated by alpha-2u globulin nephropathy.**

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 **30 November 2020**. 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 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
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.

Your registration dossier contains for endpoints

- In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1);
- In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.);
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.);
- Screening for reproductive/developmental toxicity; and
- Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.)

adaptation arguments in form of a grouping and read-across approach under Annex XI, Section 1.5. of the REACH Regulation. ECHA has considered first the scientific and regulatory validity of your read-across approach in general before assessing the individual properties in sections 1 – 5.

For the property pre-natal developmental toxicity (Annex IX, Section 8.7.2) you provided a testing proposal. This testing proposal is evaluated in a separate decision (communication number TPE-D-2114423420-66-01/D).

Grouping of substances and read-across approach

You have sought to adapt the information requirements 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 consider to achieve compliance with the REACH information requirements 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' using data of a structurally similar 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 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 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. According to the information provided in your registration dossier the composition 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 at [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 your registration dossier 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 requirements. 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:

- 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.
- 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.
- 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-

[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

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 registration dossier. 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, 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:

- Currently, there are no data on any toxicological properties for the registered substance.
- Thera are also no hazard data on any of the possible metabolites formed from the parent compounds (see point 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

Annex XI, Section 1.5 provides with regard to the reliability and adequacy of the source studies that in all cases the results of the read-across should:

- *be adequate for the purpose of classification and labelling and/or risk assessment;*
- *have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3);*
- *cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3) if exposure duration is a relevant parameter, and*

- *adequate and reliable documentation of the applied method shall be provided.*

ECHA notes the following observations:

- For genetic toxicity you provided studies on gene mutation in bacteria (OECD TG 471), on chromosomal aberration in mammalian cells (OECD TG 487) and gene mutation in mammalian cells (OECD TG 476) according to GLP (2016). All studies were conducted with the proposed source substance at a purity of 96.2 % of which [redacted] % was (-)-alpha pinene and [redacted] % (+)-alpha-pinene. ECHA regards these studies as adequate and reliable to investigate genetic toxicity for the source substance.
- For toxicity to reproduction you state that a reproduction toxicity study in rats is currently on-going according to guideline OECD TG 421. No results are reported and the study cannot be assessed.
- For sub-chronic toxicity, you provided study records for two weeks and 90-day inhalation studies in rats and mice with the proposed source substance at a purity of 96 % of which [redacted] % was (-)-alpha pinene and [redacted] % (+)-alpha pinene. ECHA regards these studies as adequate and reliable to investigate sub-chronic toxicity for the source substance.

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 notes 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 lacking 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 missing 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

[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

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.

In your comments to the draft decision, you state that toxicokinetic studies are not required by the REACH Regulation, only available data can be considered, and it is not possible to gather more data with new experimental studies. In particular for read-across approaches ECHA points out, although not required as standard information, toxicokinetic data are valuable and often urgently needed for supporting predictions and the REACH Regulation does not impose restrictions on the generation of new data in this regard.

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 adaptation of the standard information requirements in the technical dossier is based on the proposed read-across approach examined above. ECHA does not consider the read-across justification to be a reliable basis to predict the properties of the registered substance for the reasons set out above. Thus, the adaptation does not comply with the general rules of adaptation as set out in Annex XI, 1.5. Therefore, ECHA rejects the adaptation in the technical dossier that are based on Annex XI, 1.5.

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. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

An "*In vitro* gene mutation study in bacteria" is a standard information requirement as laid down in Annex VII, Section 8.4.1. 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 record for a Bacterial Reverse Mutation Assay (OECD TG 471) with the proposed source substance. However, as explained above in the 'Grouping of Substances and Read-Across Approach', your adaptation of the information requirement is rejected.

Therefore, 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.

ECHA considers that the bacterial reverse mutation test (test method EU B.13/14. / OECD TG 471) is appropriate to address the standard information requirement of Annex VII, Section 8.4.1. of the REACH Regulation.

In your comments to the draft decision, you agree to conduct the study in order to collect more toxicological data on the target substance.

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: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471).

2. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)

An "*In vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study" is a standard information requirement as laid down in Annex VIII, Section 8.4.2. 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 record for an in vitro mammalian cell micronucleus tes (OECD TG 487) with the proposed source substance. However, as explained above in the 'Grouping of Substances and Read-Across Approach', your adaptation of the information requirement is rejected.

Therefore, 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.

ECHA considers that the *in vitro* mammalian chromosome aberration test (test method OECD TG 473) and the *in vitro* mammalian cell micronucleus test (OECD TG 487) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.2. of the REACH Regulation.

In your comments to the draft decision, you consider it possible to justify a read-across approach based on the hypothesis "different compounds have the same type of effects" by completing the data matrix. ECHA observes that currently there is not a single data point for any human health hazard for the target substance.

You propose to use the results obtained in the future study according to OECD TG 471 to predict the outcome of the *in vitro* cytogenicity study in mammalian cells. Although not so clearly stated in your comments, ECHA understands that you propose to use the future OECD TG 471 study with the target substance to compare results obtained with the target and proposed source substance. This comparison is supposed to verify that the results in bacteria are similar between the source and target substance and therefore you expect that also the results in other genotoxicity tests conducted with source and target substances would be similar.

ECHA emphasises that the information requirements to assess genotoxicity are based on separate mechanisms/test organisms, which are used to detect genotoxic damage. The in vitro gene mutation study in bacteria dectects point mutations in several strains of bacteria, whereas the *in vitro* cytogenicity study detects chromosomal aberrations in mammalian cells. It is therefore concluded that:
(1) a result obtained for point mutations in bacteria is in principle not predictive for the outcome of the study on chromosomal aberrations in mammalian cells, and
(2) in view of the lacking information on the possible differences in the interaction of (-)- and (+)-alpha-pinene with the biologically occuring optical isomers (see II.a) and the lack of any mechanistic information which could support a prediction, the result in the OECD TG 471 obtained with the target substance cannot be confidently used as bridging result and a test with the target substance is needed to exclude genotoxicity based on chromosomal aberrations.

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 vitro* mammalian chromosome aberration test (test method: OECD TG 473) or *in vitro* mammalian cell micronucleus study (test method: OECD TG 487).

3. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

An "*In vitro* gene mutation study in mammalian cells" is an information requirement as laid down in Annex VIII, Section 8.4.3. of the REACH Regulation, "if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2." is obtained.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for an in vitro mammalian gene mtation test (OECD TG 476) with the proposed source substance. However, as explained above in the 'Grouping of Substances and Read-Across Approach', your adaptation of the information requirement is rejected.

Therefore, 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.

ECHA considers that the *in vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes (OECD TG 476) and the *in vitro* mammalian cell gene mutation tests using the thymidine kinase gene (OECD TG 490) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.3.

In your comments to the draft decision, you propose to use the results obtained in the future study according to OECD TG 471 to predict the outcome of the *in vitro* gene mutation study in mammalian cells. ECHA understands your approach is in analogy the one described under 2.

The *in vitro* gene mutation study in bacteria dectects point mutations in several strains of bacteria, whereas the *in vitro* gene mutation study in mammalian cells detects point mutation in mammalian cells, i.e. in a different test system. It is therefore concluded that:
(1) a result obtained for point mutations in bacteria is in principle not predictive for the outcome of the study on gene mutations in mammalian cells, and

(2) in view of the lacking information on the possible differences in the interaction of (-)- and (+)-alpha-pinene with the biologically occuring optical isomers (see II.a) and the lack of any mechanistic information which could support a prediction, the result in the OECD TG 471 obtained with the target substance cannot be confidently used as bridging result and a test with the target substance is needed to exclude genotoxicity based on gene mutation in mammalian cells.

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 vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490) provided that both studies requested under 1. and 2. have negative results.

4. 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.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. by a groupig and read-across approach based on results from the proposed source substance. However the proposed source study is indicated in the registration dossier as ongoing and no results are reported. In any case, as explained above in the 'Grouping of Substances and Read-Across Approach', your adaptation of the information requirement is rejected.

Therefore, 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.

ECHA considers that the Reproduction/Developmental Toxicity Screening Test according to OECD TG 421 and the Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test according to OECG TG 422 are appropriate to address the standard information requirement of Annex VIII, Section 8.7.1.

According to the test methods OECD TG 421 and 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 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.

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 2017).

In view of the parallel decision for the registered substance on the testing proposal for a pre-natal developmental toxicity you should also carefully consider the order of testing of the requested screening (OECD TG 421/422) and the developmental toxicity studies (OECD TG 414) to ensure that unnecessary animal testing is avoided, paying particular attention to the endpoint specific guidance.
(https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf) Section R.7.6.2.3.2., pages 484 to 485 of version 6.0 – July 2017."

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

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. 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 study records for a two weeks and 90-day inhalation studies in rats and mice conducted by NTP (Toxicity Report Series Number 81, May 2016) with the proposed source substance. However, as explained above in the 'Grouping of Substances and Read-Across Approach', your adaptation of the information requirement is rejected.

Therefore, 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.

In the NTP studies on the multi-constutuent alpha-pinene, the test material was [redacted] % alpha pinene of which [redacted] % was (-) alpha pinene and [redacted] % was (+)-alpha pinene. The test concentrations were 0, 25, 50, 100, 200, and 400 ppm. Liver weight increases were observed in rats and mice of both sexes, which were not associated with histopathological findings and were attributed to the induction of liver metabolising enzymes. Male and female relative kidney weight increases were observed in a dose dependent manner for rats starting at 50 ppm. In male rats, the kidney effects were accompanied by histopathological findings and are supposedly related to alpha-2u-globulin induced nephropathy. However, the kidney weight changes also in females could indicate also other mechanims of toxicity.

In mice, the primary effects was an increased incidence of transitional eithelium hyperplasia of the urinary bladder in both sexes.

In both species significantly decreased numbers of cauda sperm were observed. In rats the absolute sperm per cauda deacrease by about 20 % at the two highest concentrations compared to the control animals. 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 toward an endocrine disrupting mode of action.

It is however not known, whether one enantiomer of alpha-pinene is more potent than the other to cause such effects or whether there may be masking of adverse effects by the presence of both isomers simultaneously, or whether a different toxicity profile will be observed for the registered substance, containing the (-)-isomer as main constituent. A 90-day study in rats conducted with the registered substance will provide the information for this endpoint, will clarify the potency of the registered substance to cause more severe or other toxicity in this type of study.

ECHA has evaluated the most appropriate route of administration for the study. The registered substance is a liquid with a vapour pressure of 652 Pa at 25°C. The substance is used as a fragrance. There are spray applications for professionals and consumer uses in washing and cleaning products, air care products, biocides, polishes and waxes, and cosmetics leading to inhalative exposure. Furthermore, the information on the proposed analogue substance indicates that inhalation is a relevant route to investigate the subchronic toxicity. Finally, the toxicity of the registered substance and the source substance should be investigated using the same administration route to conclude on possible differences. Hence, the test shall be performed by the inhalation route using the test method OECD TG 413. Since the study on the multiconstituent alpha-pinene was performed as whole body exposure study, this exposure condition should also be used in the study with the registered substance.

According to the test method OECD TG 413 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

As explained above in the description of the NTP study results adverse effects were observed in the kidneys of male rats and the findings were consistent with alpha-2u-globulin-mediated nephropathy. It is not known, which enantiomer is causative for this effects. ECHA accordingly considers that the kidney may also be a target organ of the registered substance. Since humans do not excrete alpha-2u-globulin and this mode of action is considered not relevant to humans, the involvement of alpha-2u-globulin in the kidney effects is a key parameter for establishing the relevance of the kidney effects for risk assessment. It is further relevant to clarify the mechanism of kidney toxicity, since in the NTP study in rats increased kidney weight were also observed in females, which do not develop alpha-2u-globulin-mediated nephropathy. For these reasons, ECHA considers that urinalysis is required to investigate kidney function (which is optional in paragraphs 49 of OECD TG 413). Additionally, a full histopathological examination (paragraph 57 of OECD TG 413), which is to include immunohistochemical investigation of renal pathology to determine, if the pathology is indeed mediated by alpha-2u globulin.
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. Sub-chronic inhalation toxicity: 90-day study (test method: OECD TG 413, whole body exposure) in rats, modified to include urinalysis and a full histopathological

examination which is to include immunohistochemical investigation of renal pathology to determine if the pathology is mediated by alpha-2u globulin nephropathy.

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 30 May 2018.

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 request(s).

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.