EUROPEAN CHEMICALS AGENCY

Helsinki, 27 July 2017

Addressee: [redacted]

Decision number: CCH-D-2114366659-31-01/F
Substance name: 3,7,7-TRIMETHYLBICYCLO[4.1.0]HEPT-3-ENE
EC number: 236-719-3
CAS number: 498-15-7
Registration number: [redacted]
Submission number: [redacted]
Submission date: 01.03.2016
Registered tonnage band: 1000+T

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. **Name or other identifier of the substance (Annex VI, Section 2.1.) of the registered substance; as specified in Appendix 1, section 1;**

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 the study requested under point 2 has negative results;**

4. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats with the registered substance;**

5. **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;**

6. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species (rat or rabbit), oral route with the registered substance;**

7. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance ; specified as follows:**
    - **Ten weeks premating exposure duration for the parental (P0) generation;**
    - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
    - **Cohort 1A (Reproductive toxicity);**
    - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;**
8. **Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: Alga, growth inhibition test, EU C.3./OECD TG 201) with the registered substance;**
9. **Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: Fish, acute toxicity test, OECD TG 203) with the registered substance;**
10. **Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: Daphnia magna reproduction test, EU C.20./OECD TG 211) with the registered substance;**
11. **Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) with the registered substance;**
12. **Bioaccumulation in aquatic species (Annex IX, Section 9.3.2.; test method: Bioaccumulation in fish: aqueous and dietary exposure, OECD TG 305, aqueous exposure or dietary exposure, as specified under Appendix 1, section 13, 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 are required to submit the information requested under point 1 in an updated registration dossier by **3 November 2017**.

You are required to submit the requested information on human health and environment in an updated registration dossier by **4 May 2021** except for the information requested under point 4 for a sub-chronic toxicity study (90-day) which shall be submitted in an updated registration dossier by **5 November 2018**. You may only commence the extended one-generation reproductive toxicity study as requested under point 7 after **4 February 2019**, unless an indication to the contrary is communicated to you by ECHA before that date. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Authorised[1] by 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

IDENTIFICATION OF THE SUBSTANCE

1. Name or other identifier of the substance (Annex VI, Section 2.1.)

Pursuant to Article 10(a)(ii) of the REACH Regulation, the technical dossier shall contain information on the identity of the substance as specified in Annex VI, Section 2 of the REACH Regulation. In accordance with Annex VI, Section 2 the information provided shall be sufficient to enable the identification of the registered substance.

"Name or other identifier of the substance" is an information requirement as laid down in Annex VI, Section 2.1 of the REACH Regulation. Adequate information needs to be present in the technical dossier for the registered substance to meet this information requirement.

The registration is currently linked to EC number (i.e. 236-719-3) which refers to the generic chemical name "3,7,7-trimethylbicyclo[4.1.0]hept-3-ene".

However, the other information provided in Section 1.1 of the IUCLID dossier corresponds to a mono constituent substance with the chemical name [redacted] and the CAS number "[redacted]". These identifiers correspond to the information in section 1.2 and 1.4 of your IUCLID dossier respectively on the reported composition and the analytical data. These identifiers in the EC inventory are linked to the EC number [redacted].

The EC identifier 236-719-3 does not correspond with any of the other information provided in Sections 1.1, 1.2 and 1.4 of the registration dossier.

You are accordingly requested to clarify the identity of the registered substance. In that respect, ECHA foresees two possibilities:

If, based on the present decision, you still consider that the substance subject to this registration is the well-defined substance corresponding to EC number 236-719-3, you are required to adapt accordingly all the name and all the identifiers of Section 1.1 according to that specific substance. This includes the revision of the chemical name assigned to the registered substance. You shall ensure that the chemical name is representative of this substance. You are furthermore requested to replace the CAS information and structural information currently assigned to the substance and provide instead any available CAS information and structural information specifically corresponding to the substance. The information provided shall be sufficient to enable the identification of the registered substance. You shall ensure that the information is consistent throughout the dossier.

If, based on the present decision, you otherwise consider that the substance subject to this registration is the substance with the chemical name [redacted] and the CAS number "[redacted]", you are required to adapt the EC identifier of Section 1.1 according to that specific substance.

However, for technical reasons, at this stage you cannot remove or modify the EC number, because the registration is linked to that number in REACH-IT. To ensure unambiguous identification of the registered substance, you shall however indicate, in the "Remarks" field of the reference substance in IUCLID section 1.1, the following: "The EC number 236-719-3 currently assigned does not specifically correspond to the registered substance. This identifier cannot be modified or deleted at this stage in the present registration update for technical reasons". You shall also specify, in the same "Remarks" field, the appropriate EC number for the substance.

You should note that ECHA has established a process, subject to certain conditions, enabling registrants to adapt the EC identifier of an existing registration, while maintaining the regulatory rights already conferred to the substance concerned.

However, pending the resolution of all the incompliances highlighted in the present decision, the adaptation of the identifier can only be effective once ECHA is at least in a position to establish unambiguously the identity of the substance intended to be covered by you with this registration. Should the information submitted by you as a result of the present decision enable ECHA to identify the substance unambiguously, the process of adapting the identifier will be considered relevant. In that case, ECHA will inform you in due time as to when the identifier adaptation process shall be initiated.

In any case, you should note that the application of the process of adapting the identifier does not affect your obligation to fulfil the requirements specified in this decision.

Further technical details on how to report the identity substances in IUCLID are available in the Manual "How to prepare registration and PPORD dossiers" on the ECHA website.

In your comments to the draft decision according to Article 50(1) of the REACH Regulation you have agreed with the information requirements in the draft decision. In addition, you have indicated your intention to revise Section 1.1 of your IUCLID dossier addressing the information requirement in an update of the registration. ECHA will examine such information only after the deadline set in the adopted decision has passed and all the substance information requested in this decision has been submitted.

PROPERTIES OF THE SUBSTANCE

Grouping of substances and read-across approach

ECHA based its decision on the evaluation of your registration dossier that contains adaptation arguments in form of a grouping and read-across approach under Annex XI, 1.5. of the REACH Regulation, for certain toxicological endpoints which are addressed in the current decision. ECHA has assessed first the scientific and regulatory validity of your read-across approach in general before the individual endpoints (sections 2-7).
The proposed read-across is discussed in the following section of this decision. The corresponding sections 2 –7 refer back to this section.

Article 13(1) of the REACH Regulation provides that information on intrinsic properties of substances may be generated by means other than tests. Such other means include the use of information from structurally similar substances (grouping of substances and read-across), "provided that the conditions set out in Annex XI are met".

According to Annex XI, section 1.5. there needs to be structural similarity among the substances within a group or category and furthermore, it is required that the relevant properties of a substance within the group can be predicted from the data for the reference substance(s), and the data should be adequate for the purpose of classification and labelling and/or risk assessment. The REACH Regulation aims at promoting wherever possible the use of alternative means, where equivalent results to the prescribed test are provided on health and environmental hazards. In accordance with these objectives and the objectives of the Compliance Check process, ECHA shall assess whether a prediction of the relevant properties of the substance subject to this decision by using the results of the proposed read-across is acceptable based on the information currently available.

Description of the grouping and read-across approach proposed by the Registrant

In your registration dossier you intend to adapt the following standard human health information requirements subject to the current decision:

- *In vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2) or in vitro micronucleus study (Annex VIII, Section 8.4.2);
- *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3);
- Sub-chronic toxicity (90-day) study (Annex IX, Section 8.6.2.);
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2) in a first species ;
- Extended one-generation reproductive toxicity study (Annex IX/X, Section 8.7.3);

by applying a read-across adaptation.

You have proposed read-across between the substance subject to this decision, DELTA-3-CARENE (EC No 236-719-3, CAS No 498-15-7) as target substance and

- the structurally similar substance, PIN-2(3)-ENE (EC 201-291-9, CAS 80-56-8, hereafter referred to as alpha-pinene) as source substance for the sub-chronic toxicity (90-day) study (Annex IX, 8.6.2.);
- the structurally similar substance, camphene (EC 201-234-8, CAS 79-92-5) as source substance for the pre-natal developmental toxicity study (Annex IX, Section 8.7.2.); and
- both alpha-pinene and camphene for the *In vitro* cytogenicity study in mammalian cells or *in vitro* micronucleus study (Annex VIII, Section 8.4.2.) and for the *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.) studies.

Your dossier contains read-across documentation as a separate attachment in the updated registration, in IUCLID, Section 13, which is according to you relevant for both source substances.

You use the following arguments to support the prediction of properties of the registered (target) substance from data for source substances: *"The review of physico-chemical, toxicological and toxicokinetic data as well as Toolbox profiling showed that all three substances have physico-chemical, toxicological and toxicokinetic properties similar enough to consider that alpha-pinene and camphene would have the same toxicological properties as delta-3-carene, especially for repeated dose toxicity and reproductive toxicity*

endpoints.". ECHA understands that according to you the sources and target substances have similar properties for the above mentioned information requirements.

ECHA notes that you have grouped some *"bicyclic terpenes",* in ECHA's understanding including the substance subject to the current decision, in an analogue group. Based on the information provided by you, ECHA understands that no category hypothesis and justification has been included in your registration dossier for the registered substance, as ECHA understands it. ECHA rather considers that the proposed predictions are based on the analogue approach and the above presented information is your read-across hypothesis, which provides the basis whereby you predict the properties of the target substance from the source substances.

Information submitted to support the grouping and read-across approach

Your dossier contains read-across documentation as a separate attachment in the updated registration, in IUCLID Section 13. The documentation contains the identification of the source and target substances; comparison of the structural features, physico-chemical properties, toxicokinetic properties, toxicological properties of the target and source substances and conclusion on your read-across approach. In addition your technical dossier contains toxicological studies on the target and source substances, which are further discussed under the endpoints specific sections. The following analysis presents your read-across hypothesis and justification together with ECHA's analysis concerning the above listed elements of your hypothesis and justification.

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

According to ECHA's understanding you suggest that based on their structural similarities target and source substances have *"similar enough"* physico-chemical, toxicological and toxicokinetic properties and hence the toxicological properties of the substances would be similar.

(i) Substance characterisation of source and target substances

The substance characterisation of the source substance(s) needs to be sufficiently detailed in order to assess whether the attempted prediction is not compromised by the composition and/or impurities. In the ECHA practical guide 6 "How to report on Read-Across" it is recommended to follow the ECHA Guidance for identification and naming of substances under REACH and CLP (version 1.3, February 2014) also for the source substances. This ensures that the identity of the source substance and its impurity profile allows an assessment of the suitability of the substances for read-across purposes.

ECHA notes that your read across justification document identifies the target substance delta-3-carene by a generic CAS number 13466-78-9, which refers to a racemate mixture of delta-carene. In contrast, your updated dossier of the target substance identifies the substance (and the main constituent) with the enantiomer specific CAS 498-15-7 number. Further details on the relevance of the above described aspect are explained in the section *"Name or other identifier of the substance"*.
In view of the issue outlined above, ECHA is not in the position to verify which substance is intended to be used as a target substance. Additionally, ECHA cannot be certain that your read-across justification is intended to justify read-across to the registered substance (i.e.

the enantiomer, and not the racemate). Consequently, ECHA is unable to verify that there is an adequate basis for predicting the properties of the registered substance.

(ii) **Structural** (dis)similarities and their impact on prediction

Structural similarity is a prerequisite for applying the grouping and read-across approach, but ECHA does not accept in general or this specific case that structural similarity *per se* is sufficient to enable the prediction of human health properties of a substance, since structural similarity does not always lead to predictable or similar human health properties. 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.

You describe the structural similarities between target and source substances as *"all of them belong to the same structurally-related bicyclic terpenes"*. ECHA notes that in addition to the structural similarities, you describe structural differences observed in the saturation of the substances and position of the double bound, carbon number and reactivity of the rings.

ECHA notes that you acknowledge the structural differences between the target and source substances however, you do not provide any information on how the structural differences may impact the toxicity of the substances and thus affect the possibility to predict properties of the target substance from the data obtained with the source substances. The provided explanation is therefore not sufficient to establish a scientifically credible link between the structural similarity and the prediction.

(iii)Similar **properties** 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 structurally 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.

In the following, ECHA examines to which extent similar patterns are indeed demonstrated for physico-chemical properties, toxicokinetics and toxicological properties.

In your read-across justification you state that target and source substances *"share very similar **physico-chemical** properties"*. You propose that the *similar pattern in the physico-chemcal properties strengthen the reliability and robustness of the read-across with these two molecules"* [i.e. the source substances].

ECHA observes that the presented physico-chemical properties of target and source substances are in the same range.
ECHA considers that the fact that physico-chemical and other parameters are similar may support the structural similarity, but cannot be used alone to justify a prediction on properties related to human health.

You claim that **toxicokinetic** properties of target and source substances are "*similar enough to consider that alpha-pinene and camphene would have the same toxicological properties as delta-3-carene.*"

In chapter "5. Similarity of toxicokinetics" of your read across justification document you conclude that "*... these bicyclic terpenes are rapidly absorbed, efficiently metabolised through hepatic first-pass effect to polar oxygenated metabolites that are subsequently conjugated with glucuronic acid and excreted mainly in the urine.*" You provide, *inter alia,* toxicokinetic information **after inhalation** exposure in human for turpentine oil, alpha-pinene and delta-carene separately in a comparative study ([redacted], 1996, supporting study) and for delta-carene only ([redacted], 1996, supporting study). After inhalation exposure the pulmonary adsorption of target and source substances represents 50 -70% of the inhaled dose; it appears that a relative low percentage of the total uptake (i.e. parent substance) is excreted unchanged (2-8%) via the lung and the renal excretion of the parent substances is lower (e.g. less than 0.001% of the uptake is reported in one study). The mean half-lives (t1/2) of the last phase in blood appears to be long and averaged 32, 25, and 42 h for alpha-pinene, beta-pinene, and 3-carene, respectively.

ECHA notes that you have not provided comprehensive information about the metabolites formed in vivo from the individual source and target substances, and you do not compare the broad range of metabolites formed from the target and source substances and to which the organisms will be exposed, in addition to the parent substances. ECHA further notes that your hypothesis of rapid absorption and efficient metabolism of terpenoids is not substantiated by the provided information. Therefore, it is not possible to verify the similarity in the toxicokinetic profile of the substances in view of the absence of sufficient data on metabolism, and ECHA considers that on this basis, the argument of similar toxicokinetics does not provide a basis for predicting the toxicological properties of the substance.

ECHA further notes that toxicokinetic information does not by itself provide information about the toxicodynamic effects of systemically available chemicals. The source substances and the registered substance (and their metabolites) are systemically available, and irrespective of (proposed) common toxicokinetic pathways/ similarity, there is no provided basis which would explain why these structurally different substances would cause similar toxicological effects. This is an additional basis for rejecting the argument of "Similarity of toxicokinetics" as a reliable basis for predicting the toxicological properties of the substance.

You further propose that "*alpha-pinene and camphene would have the* ***same toxicological properties*** *as delta-3-carene, especially for repeated dose toxicity and reproductive toxicity endpoints.*"

ECHA notes that the dossier contains for the **target** substance results of an *in vitro* Ames test (OECD 471, RL1, GLP, [redacted], 2010) and several non-GLP acute dose toxicity studies (via oral and dermal route) are provided in the technical dossier. You determined the reliability of the acute dose toxicity studies as "*non assignable, (RL4)*". ECHA observes that for the target substance in animals *in vivo,* only an unreliable acute dose toxicity study is available. ECHA notes that acute toxicity data alone is not sufficient to establish the toxicological profile of a substance with regard to repeated dose and/or developmental toxicity reproductive toxicity. On this basis, ECHA does not accept that you have shown toxicological similarity, and your basis for predicting toxicological properties fails.

Further, your proposed adaptation argument is that the toxicological similarity between the source and target substance is a sufficient basis for predicting the properties of the substance. This argument is limited and is in principle not capable of being sufficient. Toxicological similarity is a prerequisite for applying the grouping and read-across approach, but ECHA does not accept in general or this specific case that toxicological similarity per se is sufficient to enable the prediction of human health properties of a substance. This is because toxicological similarity does not always lead to predictable or similar human health properties. Further elements are needed[2], such as a well-founded hypothesis of (bio)transformation to a common compound(s), or that different compounds have the same type of effect(s), to allow a prediction of human health properties that does not underestimate risks. You have not provided such elements in your dossier.

Therefore, ECHA concludes that based on the presented information it is not possible to confirm that the substances would have similar properties or they would follow a regular pattern in their properties. In the absence of such information there is not an adequate basis for predicting the properties of the target substance from the data obtained with the source substances.

Conclusion on the grouping and read-across approach

For the reasons as set out above, and taking into account data available in your registration dossier, ECHA considers that this grouping and read-across approach does not comply with the general rules of adaptation as set out in Annex XI, 1.5. of the REACH Regulation. Therefore, this adaptation cannot be accepted and there is a data gap for the endpoints covered by this read-across approach.

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

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.
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 governing grouping of substances and read-across by providing in results from in vivo studies conducted with proposed analogue substances:

1) mammalian erythrocyte micronucleus test in mice, conducted with alpha-pinene (CAS 80-56-8), similar to OECD 474, study report, NTP 2005, reliability 3, supporting study no data on GLP, negative results; you report the following deviations: "no data on test material purity; age, body weight, housing and exposure conditions of animals; duration of exposure/day; positive/negative controls; polychromatic

[2] Please see for further information ECHA *Guidance on information requirements and chemical safety assessment* (version 1, May 2008), Chapter R.6: QSARs and grouping of chemicals and ECHA's Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across).

erythrocytes not scored." ECHA notes that contrary to the guideline which defines that at least 2000 immature erythrocytes per animal should be scored for the incidence of micronucleated immature erythrocytes, the percentage of normochromatic erythrocytes (%NCEs) and micronucleus cells in a population of 1000 erythrocytes was reported.

2) mammalian erythrocyte micronucleus test in mice, conducted with camphene (CAS 79-92-5) according to OECD 474, study report, [redacted], 1991, reliability 2, key study, GLP, negative results; you report the following deviations: "no data on evaluation criteria". In addition, ECHA notes that evidence that the test material reached the bone marrow is not present; moreover the analytical purity of the applied test material was 78 % and there is no further information on the composition of the test material.

Due to the shortcomings described under 1) and 2) the results of these studies are not adequate for use as source studies. In any case, as explained in the section '*Grouping of substances and read-across approach*' of this decision, your adaptation of the information requirement is not accepted also on due to other reasons. .

For these reasons 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 agree to conduct an *in vitro* mammalian cell micronucleus study (test method: OECD TG 487).

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* cytogenicity study in mammalian cells (test method: EU B.10./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.)

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

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 not provided any study record of an *in vitro* gene mutation study in mammalian cells in the dossier that would meet the information requirement of Annex VIII, Section 8.4.3.

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.

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 agree to conduct an i*n vitro* mammalian cell gene mutation test (test method: OECD TG 476).

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 study requested under point 2. has negative results.

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

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.
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 governing grouping of substances and read-across by providing sub-chronic inhalation toxicity study results with the proposed analogue substance alpha-pinene (CAS 80-56-8) in mice and rats. ECHA notes that the provided study summaries of the source studies on alpha-pinene you have used in your read-across approach (OECD 413 in rat and mouse (NTP 2006) do not provide an adequate coverage of some key parameters for the 90-day sub-chronic toxicity study. Furthermore it does not reflect the results as reported by NTP recently in 2016 (NTP Toxicity Report Series Number 81, May 2016), in particular that alpha-pinene was found to be a reproductive toxicant in male mice and rats.

In any case, as explained in the section *'Grouping of substances and read-across approach'* of this decision, your adaptation of the information requirement cannot be accepted.

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 has evaluated the most appropriate route of administration for the study. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers that the oral route - which is the preferred one as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 6, July 2017) Chapter R.7a, section R.7.5.4.3 - is the most appropriate route of administration. More specifically, even though the information indicates that human exposure to the registered substance by the inhalation route is likely, the exposure concentrations reported in the chemical safety report for the inhalation route is low (maximum 0.8 mg/m3). Hence, the test shall be performed by the oral route using the test method EU B.26./OECD TG 408.

According to the test method EU B.26./OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

In your comments to the draft decision you do not disagree to conduct the requested study.

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: Repeated dose 90-day oral toxicity study (test method: EU B.26./OECD TG 408) in rats.

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

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

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 governing grouping of substances and read-across by providing study results from a proposed analogue substance: developmental toxicity/teratogenicity, [redacted] 1992, key study, reliability 2, according to GLP and OECD 414, conducted with camphene (CAS 79-92-5), ECHA notes that in contrast to the guideline requirements only 2 doses were tested and the exposure was from gestation day 6 to 15 only.

In any case, as explained in the section *'Grouping of substances and read-across approach'* of this decision, your adaptation of the information requirement cannot be accepted. 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, July 2017) R.7a, chapter 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 agree to conduct the requested study.

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.

6. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.) in a second species

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

Pre-natal developmental toxicity studies (test method EU B.31./OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

As explained above (section 5) the technical dossier does not contain information on a pre-natal developmental toxicity study on the first species with the registered substance and the adaptation provided is rejected. In addition there is no information provided for a pre-natal developmental toxicity study in a second species.

The technical dossier does not contain an adaptation in accordance with column 2 of Annex X, Section 8.7.2. or with the general rules of Annex XI for this standard information requirement.
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 consideration, ECHA considers testing should be performed with rabbits or rats as a second species, depending on the species tested in the first pre-natal developmental toxicity study.

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, July 2017) R.7a, chapter 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 agree to conduct the requested study.

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 second species (rabbit or rat) by the oral route.

Notes for your consideration

You are reminded that before performing a pre-natal developmental toxicity study in a second species you must consider the specific adaptation possibilities of Annex X, Section 8.7., column 2 and general adaptation possibilities of Annex XI. If the results of the test in the first species with other available information enable such adaptation, testing in the second species should be omitted and the registration dossier should be updated containing the corresponding adaptation statement.

7. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

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

The basic test design of an extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex X.

If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in the ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 6, July 2017

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information provided

ECHA observes that you have not provided any study record of an extended one-generation reproductive toxicity study in the dossier that would meet the information requirement of Annex X, Section 8.7.3.
You have sought to adapt the information requirements by providing the following justification:

"In a GLP teratogenicity study conducted according to OECD guideline 414 with camphene and in a teratogenicity/postnatal development study using rowachol (terpene mixture of alpha/beta-pinene (■%)), no teratogenic/postnatal development effects were identified. Moreover, in a 90-day repeated toxicity study conducted with alpha-pinene, no effects were

observed on reproductive organs (tissues examined microscopically: epididymidis, preputial gland, prostate, seminal vesicle and testes for males, clitoral gland, ovary and uterus for females). Thus, a reproductive toxicity study is not deemed necessary based on the results of these studies."

While you have not explicitly claimed an adaptation, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this provision.

In order to support your adaptation you provided in the technical dossier study summaries of a prenatal developmental study (OECD TG 414, RL2, [redacted] KE, 1992, see section 5.) with the proposed analogue substance camphene (CAS 79-92-5) and study summaries of two sub-chronic 90 days inhalation studies (OECD 413, RL1, NTP, 2006, see section 4.) in rat and mice with the proposed analogue substance alpha-pinene (CAS 80-56-8).

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property with respect to the information requirement in question including an adequate and reliable documentation.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance at equivalent level as investigated in an extended one-generation reproductive toxicity study (EU B.56./OECD TG 443) as requested in this decision. ECHA considers that this study provides relevant information on two aspects, namely on sexual function and fertility in P1 and F1 generations (further referred to as 'sexual function and fertility') and on developmental toxicity observable peri- and postnatally in the F1 and F2 generation. Relevant elements for 'sexual function and fertility' are in particular functional fertility (mating behaviour, conception, pregnancy, parturition, and lactation) in the P0 and F1 parental generations after sufficient pre-mating exposure and histopathological examinations of reproductive organs in both P and F1 generations. Relevant elements for 'post-natal developmental toxicity' are in particular peri- and post-natal investigations of the F1 generation up to adulthood, postnatal development of F2 generation.

Furthermore, the relative values/weights of different pieces of the provided information needs to be assessed as indicated in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.4.4. In particular relevance, reliability and adequacy for the purpose as well as consistency of results/data need to be considered.

ECHA notes that the above mentioned studies were conducted on the proposed analogue substances (see Appendix 1, section *"Grouping of substances and read-acorss approach"* above). However, since the read-across approach for those studies is rejected (see Appendix 1, section *"Grouping of substances and read-acorss approach"* above) this information can not be used as reliable source of information within a weight of evidence adaptation.

In your statement you propose that sufficient evidence is available to conclude on the reproductive toxicity potential of the registered substance, delta-carene (CAS 498-15-7). In addition to the rejection of the proposed source studies due to the rejected read-across ECHA notes that the prenatal developmental study (OECD TG 414) and the two sub-chronic 90 days inhalation studies do not address key information required by Annex X, Section 8.7.3., as information on relevant aspects is missing, such as: information on hazardous properties to the postnatal development including sexual maturation, histopathological integrity of the reproductive organs at adulthood or changes in sperm parameters and investigations of the F2 generation. Hence based on the information it is not possible to conclude if the registered substance has or has not a hazardous property on sexual function and fertility and developmental toxicity.

Therefore, the general rules for adaptation laid down in Annex XI, Section 1.2. of the REACH Regulation are not met and 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. Thus, an extended one-generation reproductive toxicity study according Annex X, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) The specifications for the study design

Information from studies to be conducted before the extended one-generation reproductive toxicity study

The sub-chronic toxicity study shall be conducted before the extended one-generation reproductive toxicity study and the results from that study shall be used, among other relevant information, to decide on the study design of the extended one-generation reproductive toxicity study following ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 6, July 2017).
The sub-chronic toxicity study may provide information on effects that is relevant for triggers (e.g. weight changes and histopathological observations of organs as indication(s) of one or more modes of action related to endocrine disruption which may meet the toxicity-trigger for extension of Cohort 1B or as evidence of specific mechanism/modes of action and/or neurotoxicity and/or immunotoxicity which may meet the particular concern criteria for developmental neurotoxicity and/or developmental immunotoxicity cohorts).

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA Guidance, the starting point for deciding on the length of premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required if there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter

R.7.6 (version 6, July 2017). In this specific case ten weeks exposure duration is supported by the lipophilicity of the substance (and by substance specific information in the dossier indicating accumulation potential of the substance in lipid rich tissues), to ensure that the steady state in parental animals has been reached before mating.

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a conducted range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Species and route selection

According to the test method EU B.56./ OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in 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, July 2017) R.7a, chapter 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 agreed to perform the requested study with 10 weeks premating exposure, however you propose to omit the extension of Cohort 1B to mate the Cohort 1B animals to produce the F2 generation. In your comments you provide details to support your proposal. ECHA examined your line of arguments.
Your comments on ECHA's argument related to the *"indications that the internal dose for the substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure"* indicates that the criteria listed in the guidance are not fulfilled.

Based on your comments, ECHA amended the study design by removing the request to extend cohort 1B to mate the Cohort 1B animals to produce the F2 generation.

c) Outcome

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.

Currently, the extension of Cohort 1B to produce the F2 generation, and the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) are not requested. However, the sub-chronic toxicity study (90-day) requested in this decision (request 4) and/or any other relevant information may trigger changes in the study design. Therefore, the sub-chronic toxicity study (90-day) is to be conducted first and the study results submitted to ECHA in a dossier update by **5 November 2018**. If, on the basis of this update and/or other relevant information, a need for changes to the study design is identified, ECHA will inform you by **4 February 2019** (i.e. within three months after expiry of the 15-month deadline to provide the sub-chronic toxicity study (90-day)) of its intention to initiate a new decision making procedure under Articles 41, 50 and 51 of the REACH Regulation to address the design of the extended one-generation reproductive toxicity study. If you do not receive a communication from ECHA by **4 February 2019**, the request of the present decision for the extended one-generation reproductive toxicity study remains effective and you may commence the conduct of the study and the results will need to be submitted by the deadline given in this decision **4 May 2021**.

Notes for your consideration

When submitting the study results of the sub-chronic toxicity study (90-day) you are invited to also include in the registration update your considerations whether changes in the study design are needed (see also *ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter R.7.6 (*version 6, July 2017*)*).

Furthermore, after having commenced the extended one-generation reproduction toxicity study in accordance with the ECHA decision, you may also expand this study to address a concern identified during the conduct of it and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the changes in the study design must be documented. The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/ triggers must be documented.

8. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)

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

"Growth inhibition study aquatic plants" is a standard information requirement as laid down in Annex VII, Section 9.1.2. 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.

Column 2 of Annex VII, Section 9.1.2 specifies that the study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur for instance if the substance is highly insoluble in water or the substance is unlikely to cross biological membranes.

In the technical dossier you have provided the following study record to fulfil the standard information requirement of Annex VII, section 9.1.3.:

- Key study, a QSAR prediction, "13466-78-9, Toxicity to aquatic algae and cyanobacteria, [redacted] 2014, RS, K", *"Prediction of Toxicity of Δ-3-carene in an Algal Growth Inhibition Test (72-hour ErC50)"*, reliability 1, Kreatis/iSafeRat QSAR prediction

Concerning the QSAR prediction, ECHA has compared the QSAR information provided for delta-3-carene with the requirements set for acceptance of QSAR models in Annex XI section 1.3 as follows:

- Adequate and reliable documentation of the applied method is provided: You have submitted a QPRF.
- Results are derived from a (Q)SAR model whose scientific validity has been established: You have submitted a detailed and clear QMRF. You consider that the model is based on regression, however, the precise equation has not been provided. For ECHA to decide on the scientific validity of the model also the equation would need to be submitted and assessed. It is therefore not possible to conclude on scientific validity of the model submitted.
- The substance falls within the applicability domain of the (Q)SAR model: You have explained in the QMRF the stepwise approach used to check whether a chemical falls into the applicability domain of the model and concluded in the QPRF that the registered substance is within the domain. However, as no training set has been submitted it is not possible for ECHA to conclude whether this is true.

Due to the above short-comings it is not possible to evaluate whether the results are reliable and adequate for the purpose of classification and labelling and risk assessment. In conclusion, the QSAR information submitted is currently not sufficient to fulfil the requirements of Annex XI section 1.3. ECHA advises you to consider the above were you to justify that this study fulfills the standard information requirement of Growth inhibition study aquatic plants for the registered substance, the enantiomer.

In your comments on the draft decision you indicate that the above model can be used to fulfill the present information requirement. However, in your comments you provide no further information on the model. ECHA notes that due to missing information on the model used, it is not possible for ECHA to assess the validity of the prediction. Nevertheless, ECHA notes that if you decided to continue using this adaptation for the present endpoint, ECHA will assess any information submitted to us at the follow up stage.

While in the initial draft decision ECHA had concerns over how the QSAR prediction relates to the registered substance, a specific enantiomer, in your comments you have indicated that the model was used to calculate the inhibition of growth to the registered substance. Furthermore, ECHA acknowledges that normally 2D QSAR models do not distinguish different steric configurations and therefore give the same prediction for the racemate and the two single enantiomers.
Under section 6.1.5. Toxicity to aquatic algae and cyanobacteria you have also included the following disregarded study "13466-78-9, Toxicity to aquatic algae and cyanobacteria, [redacted] 2010, RS, D". reliability 3, GLP, test method: OECD Guideline 201 (Alga, Growth Inhibition Test). You have indicated that the study is "*disregarded due to major*

methodological deficiencies". You have defined further that "*Unsuitable methods used for the preparation of test solutions in case of difficult substance to test (volatile, poorly soluble), concentration not maintained. No concentration / response. Results were expressed based on the solubility limit of the test substance.*" You have stated that the effects observed in this study were above the water solubility limit and are hence not relevant. ECHA agrees that the study submitted is not valid.

In conclusion and 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 ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) Algae growth inhibition test (test method EU C.3. / OECD TG 201) is the preferred test to cover the standard information requirement of Annex VII, Section 9.1.2.

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: Algae growth inhibition test, EU C.3./OECD TG 201).

9. Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.)

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

"Short-term toxicity testing on fish" is a standard information requirement as laid down in Annex VIII, Section 9.1.3. 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.

In the technical dossier you have provided the following study record to fulfil this information requirement:

- Key study, a QSAR prediction, "13466-78-9, Short-term toxicity to fish, [redacted], 2014, RS, K", "*Prediction of Toxicity of Δ-3-carene in an Algal Growth Inhibition Test (72-hour ErC50)*", Kreatis QSAR prediction

Concerning the QSAR prediction, ECHA has compared the QSAR information provided for delta-3-carene with the requirements set for acceptance of QSAR models in Annex XI, section 1.3 as follows:

- Adequate and reliable documentation of the applied method is provided: You have submitted a QPRF.
- Results are derived from a (Q)SAR model whose scientific validity has been established: You have submitted a detailed and clear QMRF. You consider that the model is based on regression, however, the precise equation has not been provided. For ECHA to decide on the scientific validity of the model also the equation would

need to be submitted and assessed. It is therefore not possible to conclude on scientific validity of the model submitted

- The substance falls within the applicability domain of the (Q)SAR model: You have explained in the QMRF the stepwise approach used to check whether a chemical falls into the applicability domain of the model and concluded in the QPRF that the registered substance is within the domain. However, as no training set has been submitted it is not possible for ECHA to conclude whether this is true.

Due to the above short-comings it is not possible to evaluate whether the results are reliable and adequate for the purpose of classification and labelling and risk assessment. In conclusion, the QSAR information submitted is currently not sufficient to fulfil the requirements of Annex XI, section 1.3.

In your comments on the draft decision you indicate that the above model can be used to fulfill the present information requirement. However, in your comments you provide no further information on the model. ECHA notes that due to missing information on the model used, it is not possible for ECHA to assess the validity of the prediction. Nevertheless, ECHA notes that if you decided to continue using this adaptation for the present endpoint, ECHA will assess any information submitted to us at the follow up stage.

While in the initial draft decision ECHA had concerns over how the QSAR prediction relates to the registered substance, a specific enantiomer, in your comments you have indicated that the model was used to assess the acute toxicity of the registered substance to fish. Furthermore, ECHA acknowledges that normally 2D QSAR models do not distinguish different steric configurations and therefore give the same prediction for the racemate and the two single enantiomers.

Under section 6.1.1. Short-term toxicity to fish you have also included the following disregarded study "13466-78-9, Short-term toxicity to fish, [redacted], 2010, SS, D", (reliability 3, GLP, [redacted] (2010), test method: OECD Guideline 203 (Fish, Acute Toxicity Test)You have indicated that this study is "*disregarded due to major methodological deficiencies*". You have defined further that "*Measured concentrations didn't stay in the 80-100% limit. Results were expressed based on the solubility limit of the test substance*". You have stated that the effects observed in this study were above the water solubility limit and are hence not relevant.

In the initial draft decision ECHA asked you to further justify as to why the study was disregarded. In your comments on the draft decision you have discussed further that it is not possible to asses to which concentrations of test substance the organisms were exposed and what cause the effects observed at the highest test suspension prepared from 100 mg/L water accommodated fraction. ECHA agrees with you that the study is not valid.

In conclusion and as explained above, the QSAR 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 ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) fish acute toxicity test (test method EU C.1. / OECD

TG 203) is the preferred test to cover the standard information requirement of Annex VIII, Section 9.1.3.

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: Fish, acute toxicity test (test method: EU C.1./OECD TG 203).

10. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)

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

"Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex IX, Section 9.1.5. 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 not provided any study record of a long-term toxicity on aquatic invertebrates in the dossier that would meet the information requirement of Annex IX, Section 9.1.5.
You have sought to adapt this information requirement according to Annex IX, Section 9.1.5., column 2. You provided the following justification for the adaptation: "*the quantitative risk assessment indicated that the risks are controlled for the aquatic compartment (PEC/PNEC < 1). In addition, the substance is not PBT/vPvB. Therefore, a long-term toxicity to invertebrates does not need to be proposed.*"

However, ECHA points out that your claim that the quantitative risk assessment shows that all risks are controlled for the aquatic compartment does not meet the specific rules for adaptation of Annex IX, Section 9.1.5., column 2 or general adaptation set out in Annex XI.

In addition, as discussed in sections 8 and 9 above, there is no valid acute data on aquatic algae and fish. Consequently the risk assessment is not valid and cannot be used to adapt the standard information requirement for Annex IX, Section 9.1.5.

Therefore, your adaptation of the information requirement cannot be accepted.

In your comments on the draft decision you consider that long-term testing is not needed due to the registered substance not being a poorly water soluble substance. ECHA notes that the latest water solubility value of 3.7 mg/L has been considered in the decision. However, as explained above, in this case the request for chronic testing is based on the fact that in absence of valid acute data on three trophic levels, it is not possible to use the CSA to adapt long-term testing. ECHA notes further that in the "*Notes for aquatic toxicity testing, requests 8-11"* section at the end of this decision, further advice on your possibilities on how to fulfil the current standard information requirement is provided.

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 ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) *Daphnia magna* reproduction test (test method EU C.20. / OECD TG 211) is the preferred test to cover the standard information requirement of Annex IX, Section 9.1.5.

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: Daphnia magna reproduction test (test method: EU C.20./OECD TG 211).

11. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

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

"Long-term toxicity testing on fish" is a standard information requirement as laid down in Annex IX, Section 9.1.6. of the REACH Regulation. Adequate information on Fish, early-life stage (FELS) toxicity test (Annex IX, 9.1.6.1.), or Fish, short-term toxicity test on embryo and sac-fry stages (Annex IX, 9.1.6.2.), or Fish, juvenile growth test (Annex IX, 9.1.6.3.) 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 long-term toxicity on fish in the dossier that would meet the information requirement of Annex IX, Section 9.1.6.1 / 9.1.6.2 / 9.1.6.3. You have sought to adapt this information requirement according to Annex IX, Section 9.1.6., column 2. You provided the following justification for the adaptation: "*the quantitative risk assessment indicated that the risks are controlled for the aquatic compartment (PEC/PNEC < 1). In addition, the substance is not PBT/vPvB. Therefore, a long-term toxicity to fish does not need to be proposed*".

However, ECHA points out that your claim that the quantitative risk assessment shows that all risks are controlled for the aquatic compartment does not meet the specific rules for adaptation of Annex IX, Section 9.1.6., column 2 or general adaptation set out in Annex XI.

In addition, as discussed in sections 8 and 9 above, there is no valid acute data on aquatic algae and fish Consequently the risk assessment is not valid and cannot be used to adapt the standard information requirement for Annex IX, Section 9.1.5.

In your comments on the draft decision you consider that long-term testing is not needed due to the registered substance not being a poorly water soluble substance. ECHA notes that the latest water solubility value of 3.7 mg/L has been considered in the decision. However, as explained above, in this case the request for chronic testing is based on the fact that in absence of valid acute data on three trophic levels, it is not possible to use the CSA to adapt long-term testing. ECHA notes further that in the "*Notes for aquatic toxicity testing, requests 8-11*" section at the end of this decision, further advice on your possibilities on how to fulfil the current standard information requirement is provided.

Therefore, your adaptation of the information requirement cannot be accepted.

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 ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) fish early-life stage (FELS) toxicity test (test method OECD TG 210), fish short-term toxicity test on embryo and sac-fry stages (test method EU C.15. / OECD TG 212) and fish juvenile growth test (test method EU C.14. / OECD TG 215) are the preferred tests to cover the standard information requirement of Annex IX, Section 9.1.6.

However, the FELS toxicity test according to OECD TG 210 is more sensitive than the fish, short-term toxicity test on embryo and sac-fry stages (test method EU C.15 / OECD TG 212), or the fish, juvenile growth test (test method EU C.14. / OECD TG 215), as it covers several life stages of the fish from the newly fertilized egg, through hatch to early stages of growth (see ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), *Chapter R7b, Figure R.7.8-4).*

Moreover, the FELS toxicity test is preferable for examining the potential toxic effects of substances which are expected to cause effects over a longer exposure period, or which require a longer exposure period of time to reach steady state (ECHA *Guidance Chapter R7b,* version 4.0, June 2017).

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: Fish, early-life stage (FELS) toxicity test (test method: OECD TG 210).

Notes for aquatic toxicity testing, requests 8-11

Pursuant to column 2 of Annex VII, Section 9.1.1. and Annex VIII, Section 9.1.3. the short-term toxicity testing on invertebrates and fish need not be conducted if a long-term study on invertebrates and fish, respectively, is available. Furthermore, Column 2 of Annex VII, Section 9.1.1 specifies that long-term aquatic toxicity study on Daphnia (Annex IX, section 9.1.5) shall be considered if the substance is poorly water soluble. Due to the relatively low water solubility of the substance, you may thus choose to continue directly with long-term testing and waive the short-term toxicity on fish.

However, if you decide to carry out the short-term study requested under section 9 before the long-term studies, before initiating the test mentioned above in points 10 and 11 ayou should consult the ECHA *Guidance on information requirements and chemical safety assessment (version 4.0, June 2017)*, Chapter R7b, Section R.7.8.5 to determine the sequence in which the aquatic long-term toxicity tests are to be conducted and the necessity to conduct long-term toxicity testing on fish.

Once results of the tests on short-term/long-term toxicity to aquatic organisms are available, you shall revise the chemical safety assessment, as necessary according to Annex I of the REACH Regulation.

Due to the relatively high volatility of the substance you should consult OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO

(2000)6 and ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), Chapter R7b, Table R.7.8-3 summarising aquatic toxicity testing of difficult substances for choosing the design of the requested ecotoxicity test(s) and for calculation and expression of the result of the test(s).

14. Bioaccumulation in aquatic species (Annex IX, Section 9.3.2.)

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

"Bioaccumulation in aquatic species, preferably fish" is a standard information requirement as laid down in Annex IX, Section 9.3.2.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 1.3 by providing results obtained from the application of three quantitative structure activity relationship models ((Q)SARs):

1. "*BCF Prediction according to TGD part. II eq. (74) DELTA 3 CARENE, Cas# 13466-78-9*", reliability 4, result BCF 1054.4 L/kg wet-wt,
2. "*QSAR Prediction Reporting Format, BCFBAF v.3.00, DELTA 3 CARENE, Cas# 13466-78-9*", Episuite, regression based estimate, reliability 2, result log BCF = 2.557, BCF (L/kg) = 360.5, and
3. "QSAR Prediction Reporting Format, BCFBAF v.3.00, SANDEROL, Cas# 13466-78-9", Episuite, Arnot-Gobas lower trophic level, reliability 2, result log BCF = 3.031, BCF (L/kg) = 1075.

ECHA has compared the QSAR information provided for delta-3-carene with the requirements set for acceptance of QSAR models in Annex XI, section 1.3 as follows:

- Adequate and reliable documentation of the applied method is provided: Whilst you have submitted QPRFs for the two Episuite predictions (2. and 3. as listed above) you have not submitted any documentation for the first prediction. You have indicated that the first prediction is reliability 4 due to the missing documentation.
- Results are derived from a (Q)SAR model whose scientific validity has been established: The models used in the EPIsuite predictions are scientifically valid, however, the validity of the prediction used in study No. 1 cannot be assessed due to lack of documentation.
- The substance falls within the applicability domain of the (Q)SAR model: the predictions for the delta-3-carene (CAS No 13466-78-9, EC No 248-908-8) fall within the applicability domain of the EPISuite (BCFBAF) calculations (studies No 2 and 3 listed above). In absence of documentation it is not possible to assess whether delta-3-carene falls in the applicability domain of the model used in the first prediction.
- Results are adequate for the purpose of classification and labelling and/or risk assessment: ECHA notes that in the results provided you have only considered the predictions for BCF (lower trophic) obtained assuming a biotransformation rate. However, using a biotransformation rate of zero, as is recommended for a

conservative approach, the BCF estimate would be Log BCF 3.374, BCF 9604. This value is above both the threshold values of 2000 for B and 5000 for vB.
- ECHA hence concludes that taking into account the uncertainty of the predictions and the B threshold for PBT/vPvB assessment, as well a conservative approach, we consider the predictions not adequate for the purpose of PBT assessment.

In your comments on the draft decision you have provided further information on the models used. Concerning study 1. listed above you have provided information on the regression used but have not provided any additional documentation. While you have provided some information on the training set used you have still not addressed whether the model is applicable for the registered substance and whether there are any structurally similar analogues in the training set. Hence ECHA considers that this prediction is still not sufficiently reliable on its own to conclude on the present endpoint.

Concerning studies 2. and 3. ECHA notes that as indicated above ECHA initially considered these predictions not acceptable since you had used a biotransformation rate in the calculations which lead to a non-conservative approach. In your comments you have provided further information regarding the applicability of the models to the registered substance and further explained why a biotransformation rate can be used in the model. You have compared the fragments of the substance with the training set and refer to two monoterpenes in the validation set data on which show that the biotransformation correction is in line with experimental study results for similar chemicals. You also refer to publications for further support.

ECHA agrees that metabolism is crucial in the assessment of the bioaccumulation for this substance. If the substance is not metabolised, there is a bioaccumulation potential above the B-criterion threshold to be expected, as also indicated by ECHA in the initial draft decision. The referenced literature points towards general metabolisation potential, though it still does not address specifically metabolisms in fish, or the rate of metabolism. Still, the fragments of the substance are covered by the models used in studies 2. and 3., and experimental data of two analogue substances in the validation set are in line with predictions which take into account biotransformation. These aspects support using the Arnot-Gobas model (Study 3) with the biotransformation rate.

ECHA considers that based on the information provided in your comments, studies 2. and 3. Could be used to estimate the bioaccumulation potential of the registered substance. However, ECHA notes that the justification for using the biotransformation rate is not included in the technical dossier and ECHA does not accept any dossier updates at this stage of the decision making process. ECHA suggests you include this information in the technical dossier. Any dossier updates including improved adaptations will be examined by ECHA after the deadline set in the adopted decision has passed, as also indicated at the *Notes for your consideration* Section at the end of this request.

Furthermore, in your comments you also indicate that achieving and maintaining a test substance concentration of [redacted] % of acute fish LC50, requested by the guideline, would be technically not possible since such sensitive analytical method is not available. ECHA notes that you have not provided any further information on this claim, and as such it is not possible for ECHA to verify whether it would be technically not possible to carry out the study requested. However, ECHA notes that if you wish to pursue such adaptation it needs to fulfil the requirements of Annex XI section 2. Testing is technically not possible.

ECHA notes further that as the substance is readily biodegradable, there is no need to assess its PBT/vPvB properties further.

While in the initial draft decision ECHA had also concerns over how the QSAR prediction relates to the registered substance, a specific enantiomer, in your comments you have indicated that the models were used to assess the bioaccumulation potential of the registered substance. Furthermore, ECHA acknowledges that normally 2D QSAR models do not distinguish different steric configurations and therefore give the same prediction for the racemate and the two single enantiomers.

In conclusion, the QSAR information submitted in the technical dossier is currently not sufficient to fulfil the requirements of Annex XI, section 1.3.

Therefore, your adaptation of the information requirement cannot be accepted.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement since it is unclear how the data submitted relates to the substance as registered and as the data itself cannot be used to fulfil this standard information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7c* (version 3.0, June 2017) bioaccumulation in fish: aqueous and dietary exposure (test method EU C.13. / OECD TG 305) is the preferred test to cover the standard information requirement of Annex IX, Section 9.3.2. ECHA Guidance defines further that results obtained from a test with aqueous exposure can be used directly for comparison with the B and vB criteria of Annex XIII of REACH Regulation and can be used for hazard classification and risk assessment. Comparing the results of a dietary study with the REACH Annex XIII B and vB criteria is more complex and has higher uncertainty. Therefore, the aqueous route of exposure is the preferred route and shall be used whenever technically feasible. If you decided to conduct the study using the dietary exposure route, you shall provide scientifically valid justification for your decision. Data obtained from a dietary study will also need to be used to estimate BCF values.

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

Bioaccumulation in fish: aqueous or dietary bioaccumulation fish test (test method: OECD TG 305), aqueous exposure or dietary exposure, as specified above.

Notes for your consideration

Before conducting the above test you are advised to consult the ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, June 2017), Chapter R.11.4. and Figure R.11-4 on the PBT assessment for further information on the integrated testing strategy for the bioaccumulation assessment of the registered substance. You should revise the PBT assessment when information on bioaccumulation is available. If you consider that the study requested above is not needed for the PBT/vPvB assessment and any other relevant purpose you may provide a scientifically valid adaptation to the

information requirement. Any such adaptation will be assessed by ECHA at the follow up stage.

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 17 November 2016.

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 request(s).

ECHA notified the draft decision to the competent authorities of the Member States for proposal(s) 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 request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new test(s) 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 test(s) 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 test(s) must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the test(s) to be assessed.