EUROPEAN CHEMICALS AGENCY

Helsinki, 5 October 2017

Addressee: [redacted]

Decision number: CCH-D-2114373437-42-01/F
Substance name: PIN-2(3)-ENE
EC number: 201-291-9
CAS number: 80-56-8
Registration number: [redacted]
Submission number: [redacted]
Submission date: 17.02.2017
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. **Completed robust study summaries of the sub-chronic toxicity studies (90-day), inhalation route, in rats and mice with the registered substance, NTP 2006 (Annex IX, Section 8.6.2.; test method: OECD TG 413, in conjunction with Annex I, Section 3.1.5), as specified in Appendix I, section 4;**

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

3. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2., column 2; test method: EU B.31./OECD TG 414) in a second species that is appropriate, oral route with the registered substance;**

4. **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) with extension to mate the Cohort 1B animals to produce the F2 generation;**

5. **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;**

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 **12 April 2021**. 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.

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Appendix 1: Reasons

1. **Completed robust study summaries of the sub-chronic toxicity studies (90-day), inhalation route, NTP 2006 (Annex IX, Section 8.6.2. in conjunction with Annex I, Section 3.1.5)**

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

Pursuant to Article 10(a)(vii) of the REACH Regulation, the information set out in Annex VII to XI must be provided in the form of a robust study summary. Article 3(28) defines a robust study summary as a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report. Guidance on the preparation of the robust study summaries is provided in the Practical Guide on "How to report robust study summaries".

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 provided study records for two sub-chronic toxicity study (90-day), inhalation route in rats and mice with the registered substance (NTP, 2006, similar to OECD TG 413) to meet the standard information requirement of Annex IX, Section 8.6.2.

ECHA notes that, contrary to Article 3(28) of the REACH Regulation, the documentation of these studies provided by you is insufficient and does not allow an independent assessment of the adequacy of these studies, their results and their use for hazard assessment. In particular, in the IUCLID dossier submitted study summaries do not provide an adequate coverage of some key parameters. The following deficiencies have been observed:

- no information on the GLP status of the studies have been provided;
- food consumption, hematology, ophthalmological examination and some organ weights were not recorded;
- neurobehavioral examination was not performed;
- type of the inhalation administration (i.e. nasal only or full body) and physical form of the test material (i.e. vapour or aerosol) is not reported;
- cage-side observations included only moribundity and death but did not include changes in the skin and fur, eyes, and mucous membranes, changes in the respiratory and circulatory systems, changes in the nervous system, and changes in the somatomotor activity and behaviour pattern;
- the body weight measurement time schedule deviated from the guideline recommendation: examination were performed on Day 1 of the test, after 7 days and at weekly intervals thereafter instead of on Day 1 of the test and twice weekly intervals thereafter.

In addition, ECHA notes that a technical report on the alpha-pinene studies, including a detailed characterisation of the substance identity, the methods applied and the above mentioned sub-chronic inhalation studies (NTP Toxicity Report Series Number 81, May 2016) became publically available. In this report several findings are described, which are not contained in the current study summaries in section 7.5.2 of the IUCLID dossier nor reflected in the CSR but relevant for hazard identification and risk management purposes, e.g. on page 33, the NTP report summarizing the findings in the sub-chronic inhalation studies:

"There were significantly decreased numbers of cauda sperm in 200 and 400 ppm males with 19% lower sperm per cauda in the 200 and 400 ppm groups compared to the chamber controls (Tables 5 and E1). Females in the 400 ppm group displayed an apparent increase in cycle length and a slight increase in the percentage of the cycle spent in metestrus, relative to the chamber control group (Table E2). However, the apparent increase in cycle length may be secondary to stress, as evidenced by lower body weight and mortality in the 400 ppm group. Alternatively, the apparent changes in the 400 ppm females may have been an artifact of having too few animals available to allow for meaningful interpretation. In addition, consideration of the complete cycle using a Markov analysis indicated that these exposed females did not spend significantly more time in any of the estrous stages than did the chamber control group (Table E3). The minor changes in cycle length observed only in the exposure concentration group exhibiting overt toxicity, combined with a lack of ovarian histopathology findings, did not provide sufficient evidence for female reproductive toxicity potential under the conditions of the study. Based on these results, α-pinene exposure by inhalation exhibits the potential to be a reproductive toxicant in male rats, but not in female rats."

"There were significantly decreased numbers of sperm per mg cauda in 200 and 400 ppm males (24% and 37%, respectively) and cauda sperm in 100, 200, and 400 ppm males (25%, 33%, and 40%, respectively; Tables 10 and E4). There were no changes in the proportion of regularly cycling females, estrous cycle length, or percentage of time spent in the individual stages of the estrous cycle of female mice at any exposure concentration (Table E5) and there were no ovarian histopathologic findings. Therefore, α-pinene exposure via inhalation exhibits the potential to be a reproductive toxicant in male mice, but not in female mice."

ECHA notes that, contrary to Article 3(28) of the REACH Regulation the documentation of these studies is insufficient and does not allow an independent assessment of the adequacy of these studies, its results and its use for hazard assessment. In particular, the above mentioned elements regarding the quality criteria are missing.

In your comments to the draft decision you did not disagree to submit the requested information. In the updated IUCLID dossier, ECHA noted there was no change in adaptation in this endpoint from the original registration dossier used for the draft decision submitted to you.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information: Completed robust study summaries for the sub-chronic toxicity studies (90-day), inhalation route in rats and mice with the registered substance (NTP, 2006), as specified above in this section.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to 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 pre-natal developmental toxicity studies results with proposed analogue substances:

1) Developmental toxicity/teratogenicity, [redacted], 1992, key study, according to OECD 414, GLP, reliability 2, 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.
2) Developmental toxicity/teratogenicity, [redacted] 1978, supporting study, no guideline followed, no GLP, reliability 4, conducted with rowachol (CAS 65546-74-9). The reporting is not sufficient to draw conclusions.

The studies under 1) and 2) have the described shortcomings and 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. ECHA considers that the test should be performed with rats as a first species as the rabbit may not be an appropriate species to investigate prenatal developmental toxicity of the substance (for explanation please see request 3).

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) 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. In the updated IUCLID dossier, ECHA noted there was no change in adaptation in this endpoint from the original registration dossier used for the draft decision submitted to you. 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) by the oral route.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to 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 and ECHA *Guidance on information requirements and chemical safety assessment* (version 4.1, October 2015), Chapter R.7a, section R.7.6.2.3.2, Stage 4.5 the rat and the rabbit are the preferred species. The species selection is discussed further below.

In your comments to the draft decision you agreed to perform the requested study.

In addition, in your updated IUCLID dossier you indicate that: "*... Then, an OECD 414 study in rats is proposed in this dossier. Moreover, alpha-pinene is not expected to be well tolerated by rabbits, whose digestive tract is particularly sensitive to such molecules as excessive toxicity may occur and prevent from accurately discriminate between potential developmental toxicity and species-specific toxicity.*
Therefore, no OECD 414 study in rabbits should be conducted before the data of the OECD 414 study in rats become available."
ECHA notes that the timeline to submit the requested information in an updated registration dossier has been set to allow for sequential testing. Regarding the registered substance, "*not expected to be well tolerated by rabbits ...*", you have not provided any substance specific justification why you deem that the rabbit will be "*particularly sensitive to such molecules as excessive toxicity may occur and prevent from accurately discriminate between potential developmental toxicity and species-specific toxicity*".
However, ECHA Guidance further indicates that "*The selection of the species for the prenatal developmental toxicity study should be made taking into account substance-specific aspects. If a species other than the rat and the rabbit is selected as the first or second species, the selection should be justified.*"

ECHA considered based on the above presented that if there is evidence that the rabbits is not a suitable species (e.g. based on a range-finding study), the information requirement for prenatal developmental toxicity on a second species should be covered using information from another suitable species to address the species differences.

During the decision making procedure ECHA received proposals for amendments discussing whether testing the registered substance for pre-natal developmental toxicity in rabbit is appropriate. ECHA notes that there are indications ([redacted], 2006) that the registered substance exhibits antimicrobial activity and therefore the rabbit might indeed not be an appropriate species as already brought forward by you.

The supposition that rabbit is an inappropriate species for testing antimicrobials has been addressed by [redacted] 2002 as well: *"the rabbit is regarded as inappropriate for the testing of antibiotics (e.g. those occurring as veterinary residues) and for poorly absorbed materials because they often cause diarrhoea and reduced food consumption, which can, in themselves, result in abortion, foetal resorption and occasionally maternal death"*

ECHA considers that in light of the results of the above mentioned publications, it can be assumed that the rabbit is indeed not an appropriate species to investigate prenatal developmental toxicity of the substance.

In your comments to the proposal for amendments you provide further information ([redacted], 2007; [redacted] 2014) indicating that alpha-pinene has lower antimicrobial activity than alpha-terpineol and hence would be better tolerated by rabbits. You also emphasise the difficulties (in terms of statistical power, laboratory experience and availability of historical control data) to find a suitable non-rodent species other than the rabbit for testing developmental toxicity. You conclude that in view of the additionally presented information *"it is worth performing a preliminary study in order to assess alpha-pinene tolerance by female rabbits"*.

ECHA reminds you that the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017, R.7a, chapter R.7 6.4.2.2) indicates that *"if both or one of the default species (the rat or the rabbit) are not suitable species for prenatal developmental toxicity testing, a more suitable species considering the human relevance should be selected for testing. An adequate justification must be provided for other species other than the rat or the rabbit"*.

Therefore, if based on the preliminary studies you consider that the rabbit may not be a suitable species for testing the registered substance, you should select an appropriate species. ECHA further considers that for fulfilling the standard information requirement of a prenatal developmental toxicity, there is no explicit requirement for a non-rodent species in REACH Regulation.

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) 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.

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 that is appropriate, by the oral route.

Notes for your consideration

ECHA considers that for fulfilling the standard information requirement of a prenatal developmental toxicity, species 1-7 listed in Annex 1 of Directive 2010/63/EU on the protection of animals used for scientific purposes would be the starting point for species selection (mouse, rat, Guinea pig, Syrian hamster, Chinese hamster, Mongolian gerbil and the rabbit). In all cases, the species selection should be duly justified.

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.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to 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 6.0, 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], 1992) with the proposed analogue substance camphene (CAS 79-92-5), with the proposed analogue substance rowachol (CAS 5546-74-9, RL4, [redacted] 1974) and study summaries of two sub-chronic 90 days inhalation studies (OECD 413, RL1, NTP, 2006) in rats and mice with the registered 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 provided prenatal developmental studies have been performed with proposed analogue substances (see Appendix 1, section 0 above). However, since the read-across approach for those studies is rejected (see Appendix 1, section 0 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, alpha-pinene.

ECHA notes that the prenatal developmental studies (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 are 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.

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

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 6.0, July 2017). In this specific case ten weeks exposure duration is supported by the lipophilicity (logPow ~ 4.5 at 25 °C) of the substance and by substance specific information in the dossier indicating accumulation potential of the substance in lipid rich tissues (please also refer to section 0 *"Grouping of substances and read-across approach"*), 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, 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.

Extension of Cohort 1B

If the column 2 conditions of 8.7.3., Annex X are met, Cohort 1B must be extended, which means that the F2 generation is produced by mating the Cohort 1B animals. This extension provides information also on the sexual function and fertility of the F1 animals. The extension is inter alia required, if the use of the registered substance is leading to significant exposure of consumers and professionals (column 2, first paragraph, lit. (a) of section 8.7.3., Annex X) and if there are indications that the internal dose for the registered substance will reach a steady state in the test animals only after an extended exposure (column 2, first paragraph, lit. (b), second indent of section 8.7.3., Annex X).

The use of the registered substance in the joint submission is leading to significant exposure of consumers and professionals because the registered substance is used by professionals as e.g. formulation of fragranced products and uses of fragranced products itself (PROCs 1, 2, 3, 4, 5, 8a, 8b, 9, 11, 13, 14) and consumers as e.g. washing and cleaning products, air care products, biocides, polishes and wax blend, as well as textile dyes.

In addition, there are indications which point towards endocrine-disrupting modes of action of alpha-pinene because in the sub-chronic inhalation studies (NTP, 2006) with the registered substance, both in rats and mice significant decreased number of cauda sperm were observed. The above described effects were observed from mid dose for rats *"There were significantly decreased numbers of cauda sperm in 200 and 400 ppm males with 19% lower sperm per cauda in the 200 and 400 ppm groups compared to the chamber controls";* whereas in mice similar effects were described from the low dose *"There were significantly decreased numbers of sperm per mg cauda in 200 and 400 ppm males (24% and 37%, respectively) and cauda sperm in 100, 200, and 400 ppm males (25%, 33%, and 40%, respectively".*

Therefore, ECHA concludes that Cohort 1B must be extended to include mating of the animals and production of the F2 generation because the uses of the registered substance is leading to significant exposure of professionals and consumers and there are indications that the internal dose for the registered substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure, and there are indications of modes of action related to endocrine disruption from available studies for the registered substance.

The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/triggers must be documented. *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 6.0, 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 perform the requested study, 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.
You explain that *"a) According to the current Chemical Safety Report of the registered substance, the safe use for professional and consumer applications was shown."* However, as described above, the substance is used both by professionals and by consumers which is in line with ECHA's Guidance R.7a, chapter R.7.6 (version 6.0, July 2017). Hence ECHA concludes that the criteria of the legal text "uses leading to significant exposure of consumers or professionals, taking into account, inter alia, consumer exposure from articles..." is met. Furthermore, you comment 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"* suggesting that the criteria as listed in the guidance are not fulfilled. In addition, you comment on ECHA's argument related to *"indications of one or more relevant modes of action related to endocrine disruption from available in vivo studies or non-animal approaches".*

You suggest that due to the technical limitation of the sub-chronic inhalation studies (NTP, 2006, see above) - in which the indications pointing towards endocrine disrupting mode of actions were observed - the relevance of observed effects can be questioned.
ECHA notes that you have classified these studies as key studies in your registration dossier. Furthermore, ECHA considers that the applied method to prepare the cauda samples for sperm counts is acceptable i.e. the applied heat fixation might affect the integrity of the cells nevertheless does not prevent the determination of the exact sperm count. Moreover, the observed effects as detailed above are consistence in both studies and in both species, pointing toward endocrine disrupting mode of action, hence adequate to trigger the extension of cohort 1B.

Based on your comments, ECHA amended the justification of the extension of cohort 1B to mate the Cohort 1B animals to produce the F2 generation.

Furthermore, ECHA observes that in your updated IUCLID dossier you indicate that a screening for reproductive / developmental toxicity study according to OECD TG 421 is currently ongoing and that *"further testing will be discussed depending on the OECD 421 study results."*

ECHA notes that the conduction of a screening for reproductive / developmental toxicity study is at your discretion. In addition, ECHA notes that a reproduction/developmental toxicity screening test" (test method: OECD TG 421) does not provide the information required by Annex X, Section 8.7.3. because it does not cover key elements, such as exposure duration, life stages and statistical power of an extended one-generation reproductive toxicity study. More specifically, the main missing key elements are: 10 weeks pre-mating exposure duration, at least 20 pregnant females per group, and an extensive postnatal evaluation of the F1 generation. Hence this study cannot be used to adapt this information requirement.

Finally, ECHA notes that the current decision requests you to submit an extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443) with the registered substance, as specified above. There is no requirement to submit a testing proposal for the same endpoint.

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) with extension to mate the Cohort 1B animals to produce the F2 generation;

No triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including Cohorts 2A and 2B and/or Cohort 3 if new information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the new information shows triggers which are described in column 2 of Section 8.7.3., Annex X and further elaborated in ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 6.0, July 2017). You may also expand the study to address a concern identified during the conduct of the extended one-generation reproduction toxicity study and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the expansion 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.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to 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.
ECHA notes that during the Registrant's 30-day commenting period you indicated that you had updated the dossier and submitted new information for this endpoint. This new information includes an update of the adaptation submitted according to Annex XI, section 1.5 of the REACH regulation. In this specific case, as the updated dossier contains new and substantial information for a number of endpoints, ECHA has exceptionally accepted and evaluated the update. ECHA notes the following concerning the information submitted in the updated dossier for the present endpoint.

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 record for an Alga, Growth Inhibition Test (OECD TG 201) with the proposed analogue substance (-)-alpha-pinene (IUPAC name (1R,5R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, CAS No 7785-26-4, EC No 232-077-3). .

ECHA-S notes that the updated dossier mentioned above contains a new read-across justification document "[redacted]", as a separate attachment in IUCLID section 13. ECHA has assessed the information provided and considers that, although the new read-across approach is plausible, the source study submitted in the updated dossier for the present endpoint is not valid, as explained below.

You have used the following arguments to support the prediction of properties of the registered substance from data for the source substance:

- The target is a multi-constituent substance composed of (-) and (+) enantiomers of alpha-pinene, present in typical concentrations of 22%w/w and 74%w/w, respectively. The source (-)-alpha-pinene is a mono-constituent substance (typical concentration 88.6%w/w) where (+)-alpha-pinene is present as an impurity (typical concentration 9.9%w/w). In addition, other common impurities are not expected to influence the prediction (section 3 of the read-across justification document).

- On the basis of structural similarity and similarity in physico-chemical, ecotoxicological and environmental fate properties, it is possible to predict the ecotoxicological properties of the registered substance (section 4 of the read-across justification document).

ECHA considers that this information is the read-across hypothesis, which provides the basis whereby you predict the properties of the registered substance from the source substance.

ECHA agrees that the the two substances are structurally similar, since they are composed mainly by (-) and (+) enantiomers of alpha-pinene. ECHA further agrees that the other common impurities are not expected to influence the prediction. You indicates that "*All other impurities are the same and are in the same concentration range except (-)-beta-pinene*", which can be present at higher concentrations in the source (up to 10%w/w in the source and up to 3%w/w in the target). You have further provided short-term aquatic toxicity data for (-)-beta-pinene showing that it is less toxic than source and target substances (at least twice less toxic). ECHA notes that this impurity is present in test material of the new source study on algae growth inhibition only at about 0.2%w/w, thus it is not expected to underestimate the prediction for the target substance.

Furthermore, ECHA acknowledges that you have provided a data matrix in Tables 2 and 3 of the read-across document to allow comparison of physicochemical and and ecotoxicity properties, respectively, between the target and the source substances.

ECHA agrees that the target and source substances have similar physico-chemical properties (e.g. low water solubility, high vapour pressure, high Log Kow of about 4.5). ECHA-S further agrees that the target and source substances have similar effect values (96h EC50 about 0.3 mg/L, measured) in short-term toxicity tests on fish. In addition, you indicate that the expected mode of toxic action of (-)-alpha-pinene and (+)-alpha-pinene is "non-specific, for which acute toxicity is driven by their hydrophobicity". Since no reactive groups are present in the alpha-pinene entiomers, ECHA considers it plausible that acute toxicity is driven by LogKow. Hence, a similar acute aquatic toxicity profile can be expected for source and target substances, as shown by the similar effect values in short-term toxicity tests on fish.

Based on the above, ECHA considers that you have provided adequate information to support the read-across hypothesis. Hence the read-across approach is considered plausible.

However, concerning the "OECD / Toxicity to aquatic algae and cyanobacteria / Data source RA 7785-26-4, Toxicity to aquatic algae and cyanobacteria, [redacted] 2013, RS, K", test method: OECD Guideline 201 (Alga, Growth Inhibition Test) study submitted, ECHA notes the following. In the endpoint study record (ESR) in section 6.1.5. Toxicity to aquatic algae and cyanobacteria in IUCLID technical dossier, you have indicated that as a deficiency that "*chemical analyses revealed severe test item losses in the presence and absence of algae*" and that "*This study is considered reliable with restrictions*".

In the read-across justification document you give the water solubility of the source substance as 2.75 mg/L. While you indicate that the definitive study was a limit study at the solubility limit of the source substance at t=0h the measured concentration in the "*biotic system*" (with test organisms) was 0.905 mg/L and in the "*abiotic system*" (without tests organisms) 2.259 mg/L.

You have furthermore indicated that the measured values at 24 h were "b*elow the LOQ (0.1 mg/L) but above the LOD (0.03 mg/L)*" in both biotic and abiotic systems and the same at 72 and 96 h at the biotic system. In the abiotic, control, systems concentrations of 0.108 and 0.104 mg/L were measured at 48 and 72 h, respectively. You consider that the losses are likely due to "*a photodegradation phenomenon or another abiotic degradation characteristic of the test item*". Under method preparation you also indicate that "*Given the volatility of the test item (vapour pressure: 530 Pa at 25 °C), the stock and test solutions were prepared under closed conditions*".

As the results of the study you indicate that "*A slight inhibition (38.5 %) was recorded at 24 h at the limit test concentration in comparison to control cultures, but no significant inhibition of the growth rate was recorded during the remainder of the test (8 % at t = 48 h and 0 % at t = 72 h)".* You also indicate that "*After 72 h, no effect of the test item on Pseudokirchneriella subcapitata was observed at the solubility/saturation limit of the test item in the test water*".

Based on the above, ECHA notes that at 48 and 72 h it was not possible to detect the test substance in the test system. It is therefore not possible to know the level of substance the test organisms were exposed to.
You indicate that the concentrations measured were in between the limit of quantification of 0.1 mg/L and the limit of detection of 0.03 mg/L. Therefore, based on the information provided, it is unclear to ECHA whether any of the test substance remained in the test solutions and whether the test organisms were exposed to the test item. ECHA hence considers the study submitted as not valid.

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) 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.

The OECD 201 guideline defines that "*For testing of substances that are volatile, strongly adsorbing, coloured, having a low solubility in water or substances that may affect the availability of nutrients or minerals in the test medium, certain modifications of the described procedure may be required (e.g., closed system, conditioning of the test vessels)".* The guideline provides reference for further guidance on how studies for substances difficult to test may be designed. ECHA notes that based on substance properties the substance has high potential to be lost from solution due to volatilisation" making the substance concentration unstable in test solutions.

ECHA notes further that in the 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 further advice on testing difficult substances, including those susceptible to photodegradation, in algae studies is provided. Therefore, you should consult these sources for choosing the design of the requested ecotoxicity test and for calculation and expression of the result of the test(s).

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).

Notes for your consideration

Due to the low solubility of the substance in water and high volatility 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).

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 draft decision was notified to the Member State competent authorities pursuant to Article 51(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).

In your comments, you indicated that you would update the registration dossier with new studies. You updated your registration with the submission number Submission number: [redacted] (Submission date: 17.02.2017). Given the exceptional circumstances, where you have updated your dossier with new and substantial information, ECHA took into account your update of 17 February 2017 and your comments on the draft decision. This has resulted in the removal of the following decision requests: In vitro gene mutation study in bacteria; In vitro cytogenicity study in mammalian cells; In vitro gene mutation study in mammalian cells; Ready biodegradability; Long-term toxicity testing on aquatic invertebrates; Long-term toxicity testing on fish; Bioaccumulation in aquatic species, with the registered substance; and amendment to the following decision request: Pre-natal developmental toxicity study in a second species (preferred rabbit); and modified the following decision requests in Appendix 1: Completed robust study summaries of the sub-chronic toxicity studies (90-day), inhalation route, in rats and mice with the registered substance, NTP 2006; Pre-natal developmental toxicity study in a first species (rat); Pre-natal developmental toxicity study in a second species that is appropriate; Extended one-generation reproductive toxicity study in rats; Growth inhibition study aquatic plants with the registered substance;

ECHA notified the draft decision to the competent authorities of the Member States for proposal(s) for amendment.

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s).

ECHA referred the draft decision to the Member State Committee.

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

In addition, you provided comments on the draft decision. These comments were not taken into account by the Member State Committee as they were considered to be outside of the scope of Article 51(5).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-55 meeting and ECHA took the decision according to Article 51(6) 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.