EUROPEAN CHEMICALS AGENCY

Helsinki, 21 December 2018

Addressee: [redacted]

Decision number: CCH-D-2114453561-52-01/F
Substance name: 3,5,5-trimethylcyclohex-2-enone
EC number: 201-126-0
CAS number: 78-59-1
Registration number: [redacted]
Submission number: [redacted]
Submission date: 13/06/2016
Registered tonnage band: [redacted] (submission number [redacted] with latest tonnage band)

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. **Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **At least two weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce systemic 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; and**
   - **Cohorts 2A and 2B (Developmental neurotoxicity).**

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 **4 *January 2021***. You also have to update the chemical safety report, where relevant.

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.

The scope of this compliance check decision is limited to the toxicological standard information requirements of Annex IX to the REACH Regulation.

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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 Claudio Carlon, Head of Unit, Evaluation E2

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

TOXICOLOGICAL INFORMATION

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

1. Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.)

The basic test design of an extended one-generation reproductive toxicity study (test method 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 Annex IX, Section 8.7.3. of the REACH Regulation, if the available repeated dose toxicity studies (e.g. 28-day or 90-day studies, OECD TGs 421 or 422 screening studies) indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. If the conditions described in column 2 of Annex IX 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 in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section 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 requirement

ECHA considers that there are 'other concerns in relation with reproductive toxicity' and in addition 'adverse effects on reproductive organs or tissues'. In respect of adverse effects, the significantly lower reproductive organ weight for seminal vesicles is adverse. In respect of other concerns, a dose-related decrease in serum testosterone levels (statistically significant at the highest dose of 800 mg/kg bw/day; -58%) was seen in juvenile/peripubertal rats in the Pubertal Development and Thyroid function assay ([redacted], 2011). Furthermore, the study shows a statistically significant increase in the adjusted age at preputial separation (+1.8 days) and significantly decreased seminal vesicle weights (~24%) at the highest dose. In view of all the aforementioned effects, there are indications of other concerns in relation with reproductive toxicity.

In view of the above, pursuant to Annex IX, Section 8.7.3. an extended one-generation reproductive toxicity study is thus an information requirement for registrations of the registered substance.

In the technical dossier you have provided a study record (publication) for a one-generation reproductive toxicity study with the registered substance (Duterte-Catella, 1976). ECHA believes you sought to adapt the information requirement according to Annex XI, Section 1.1.2. based on exsiting data derived from a study not carried out according to GLP or the test methods referred to Article 13(3) REACH. However, ECHA notes that this study does not provide the information required by Annex IX, Section 8.7.3. because it does not cover

key parameters that are foreseen to be investigated in the corresponding test method (Annex XI, Section 1.1.2. (2)). For the P0 the organ weights and body weight ratios were not measured and no information was provided on the reproductive success and maternal survival. For the F1 generation the body weight and weight changes were not measured. Furthermore, the study was performed with only one dose. Therefore, your adaptation of the information requirement is rejected.

You have also sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this provision.

You have provided the following justification for the weight of evidence adaptation:

"According to section 1.2 of Annex XI, the study need not be done if there is a weight of evidence to conclude the substance does not have a particular property, and further testing on vertebrate animals may be omitted. [...] For isophorone a one-generation study was conducted. Treatment with isophorone did not influence pregnancy rates and litter sizes nor were there any abnormalities in the pups (Dutertre-Catella, 1976, chapter 7.8.1 of IUCLID 5). In an inhalation teratogenicity study with rats and mice, isophorone was neither embryotoxic nor teratogenic up to the highest concentration tested in the study ([redacted] 1984, see chapter 7.8.2 of IUCLID 5). Furthermore there are no indications of adverse effects on the examined reproductive organs of rats, mice and dogs in several 90-day studies (NTP, 1986 and [redacted], 1972, see chapter 7.8.3 of IUCLID 5). All this findings lead to the conclusion that effects on fertility of the substance isophorone at doses, which do not cause parental toxicity, are unlikely. Therefore further studies regarding effects on fertility (e.g. 2-generation study) are not necessary for isophorone."

To support your weight of evidence adaptation you refer to the following sources of information provided in the technical dossier with the registered substance:

i. Key study (Toxicity to reproduction endpoint): One-generation reproductive toxicity study (No test guideline; not GLP compliant) in rats, via the inhalation route, Duterte-Catella (1976), publication, reliability 2.
ii. Key study (Developmental toxicity endpoint): Inhalation teratology study in rats and mice (No test guideline; not GLP compliant), [redacted] (1984), study report, reliability 1.
iii. Key study (Repeated dose toxicity endpoint): 90-day subchronic toxicity of isophorone in the rat, via the oral route (No test guideline; not GLP compliant), [redacted] (1972), study report, reliability 1.

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 (hazardous) property with respect to the information requirement in question including an adequate and reliable documentation while the information from each single source alone is regarded insufficient to support this notion.

Your weight of evidence adaptation needs to address the specific intrinsic (hazardous) properties of the registered substance with respect to an extended one-generation reproductive toxicity study (OECD TG 443) as requested in this decision. ECHA considers that this study provides, in addition to information to general toxicity, information in particular on two aspects, namely on sexual function and fertility in P0 and F1 generations (further referred to as 'sexual function and fertility') and on development and toxicity of the offspring from birth until adulthood due to pre- and postnatal and adult exposure in the F1

generation (further referred to as 'effects on offspring').

Relevant elements for 'sexual function and fertility' are in particular functional fertility (oestrous cycle, sperm parameters, mating behaviour, conception, pregnancy, parturition, and lactation) in the parental generation after sufficient pre-mating exposure duration and histopathological examinations of reproductive organs in both P and F1 generations. Relevant elements for 'effects on offspring' are in particular peri- and post-natal investigations of the F1 generation up to adulthood including investigations to detect certain endocrine modes of action, and sexual development, and investigations on developmental neurotoxicity and postnatal development of F2 generation. Also the sensitivity and depth of investigations to detect effects on 'sexual function and fertility' and 'effects on offspring' needs to be considered.

As already mentioned above, the one-generation reproductive toxicity study (i.) (key study provided for this endpoint) does not provide the information required by Annex IX, Section 8.7.3. because it does not cover key elements of an extended one-generation reproductive toxicity study. One of the missing key elements is an extensive postnatal evaluation of the F1 generation.

You also refer to the developmental toxicity study (ii.) and the sub-chronic toxicity (90-day) study (iii.).

ECHA notes that the pre-natal developmental toxicity study (study ii.) provides only a focused evaluation of the potential effects on developing organism investigated at the end of pregnancy following prenatal exposure while the sub-chronic repeated dose toxicity study provides only very limited information on reproductive toxicity (only toxicity on gonads) and lacks information on functional fertility (for example mating, pregnancy, delivery, litter size and survival of offspring, lactation and nursing of pups).

Hence these studies do not adequately address the reproductive toxicity to the extent required at this tonnage level in order to conclude on the intrinsic properties of the registered substance. More specifically, these three studies do not provide the information for instance on extensive postnatal evaluation, developmental neurotoxicity or F2 generation which are critical elements required by Annex IX, Section 8.7.3. for your substance.

Thus, the sources of information you provided, together with your justification for the adaptation, do not allow to assume/conclude on the dangerous (hazardous) property of the registered substance with respect to the information requirement for Annex IX, Section 8.7.3.

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.

In your comments on the draft decision you indicated a tonnage band downgrade from Annex X to Annex IX. Moreover you stated that "*as the Extended one-generation reproductive toxicity study (acc. OECD 443) is a standard information requirement of Annex X, there is no information gap in the technical dossier of Annex IX and the current request is not relevant*". Hence, you did not consider the information requirement for reproductive toxicity in Annex IX, Section 8.7.3., column 1. However, as explained above, ECHA considers that 'other concerns in relation with reproductive toxicity' and 'adverse effects on

reproductive organs or tissues' are observed in the study by [redacted] (2011). Hence, an extended one-generation reproductive toxicity study is an information requirement already at Annex IX level.

One of the Member States Competent Authorities made a Proposal for Amendment (PfA) to include the request for the Extended One-Generation Reproductive Toxicity study. In your response to the PfA, you have argued that there is no trigger for conducting an Extended one-generation reproductive toxicity study or any concern in relation with reproductive toxicity. You have argued (a) that the existing one-generation study shows no effects on pregnancy rate, litter sizes nor abnormalities in the offspring (b) there are no indications of adverse effects in examined reproductive organs in several 90-day studies (c) an inhalation dose-range finder study for a PNDT was not associated with effects on foetal weights or abnormalities/variants (d) you conclude that the adverse effects in the male pubertal assay do not follow the profile of an anti-androgen (e) You quote from the US EPA Weight of Evidence assessment of the Endocrine disrupting screening programme, specifically "*Based on weight of evidence considerations, mammalian and wildlife EDSP Tier 2 testing are not recommended for isophorone since there was no convincing evidence of potential interaction with E, A or T pathways.*"

ECHA considers that the studies referenced in points (a), (b) and (c) do not address the key concerns that have been identified in the Pubertal Development and Thyroid function assay, specifically including treatment of rats between PostNatal Days (PND) 23-53, and measurement of the relevant parameters. Consequently, points (a)-(c) cannot remove the concern from the results of the Pubertal Development and Thyroid function assay. In respect of (d), ECHA notes that the mode of action analysis is not required to trigger extended one-generation reproductive toxicity study. The triggers are 'adverse effects on reproductive organs or tissues' or 'other concerns in relation with reproductive toxicity'. Moreover, ECHA does not assert that the adverse effects in the male pubertal assay follow the profile of an anti-androgen. In respect of (e), the US EPA has reached a decision as to whether to proceed with Tier 2 testing. However, this is a different legal framework from the REACH regulation, and does not inform on whether there are other concerns in relation with reproductive toxicity. In the context of the regulatory review, the US EPA considered particularly that the dose level (800 mg/kg/day) was close to 1000 mg/kg/day, which had unacceptable effects, that there was systemic toxicity (significantly increased liver weight), and whether or not endocrine-related responses occurred at dose(s) that also resulted in general systemic toxicity or overt toxicity. ECHA considers that a) there is no evidence that the maximum tolerated dose was exceeded in this study; b) the increase in liver weight does not reflect excessive toxicity; and c) there is no reason to discount the results seen on the basis of increased liver weight. ECHA does not assert that the effects seen (delay in preputial separation, reduced testosterone levels, reduced seminal vesicle weight) are a result of the observed increase in liver weight. Consequently, ECHA considers that the triggering remains for the Extended one-generation reproductive toxicity study at Annex IX.

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 IX, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) The specifications for the required study

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 the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017), the starting point for deciding on the length of the 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* Chapter R.7a, Section R.7.6 (version 6.0, July 2017). In this specific case, animals of Cohort 1B are mated to produce the F2 generation and, thus, the premating exposure duration will be 10 weeks for these Cohort 1B animals and the fertility parameters will be covered allowing an evaluation of the full spectrum of effects on fertility in these animals. Thus, shorter premating exposure duration for parental (P) animals may be considered. However, the premating period shall not be shorter than two weeks and must be sufficiently long to reach a steady-state in reproductive organs as advised in the ECHA Guidance. The consideration should take into account whether the findings from P animals after a longer premating exposure duration would provide important information for interpretation of the findings in F1 animals, e.g. when considering the potential developmental origin of such findings as explained in ECHA guidance.

The highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

The selected dose levels should aim to address the findings of the Pubertal Development assay ([redacted], 2011). It is also recommended that a range-finding study (or range finding studies) is performed and that its results 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 IX 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 IX) and there are indications of one or more relevant modes of action related to endocrine disruption from available *in vivo* studies or non-animal approaches (column 2, first paragraph, lit. (b), third indent of Section 8.7.3., Annex IX).

The use of the registered substance in the joint submission is leading to significant exposure of professionals because the registered substance is used by professionals for transfer and spraying as a co-formulant in Plant Protection Products (PROCs 8a, 8b, 11).

Furthermore, there are indications of modes of action related to endocrine disruption based on the effects observed in the Pubertal Development and Thyroid function assay ([redacted] 2011). The findings of this study (reduced seminal vesicle weights; reduced serum testosterone; increased age at preputial separation) indicate one or more relevant modes of action related to endocrine-disruption.

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 there are indications of relevant modes of action related to endocrine disruption from the available study ([redacted] 2011).

Cohorts 2A and 2B

The developmental neurotoxicity Cohorts 2A and 2B need to be conducted in case of a particular concern on (developmental) neurotoxicity as described in column 2 of 8.7.3., Annex IX. When there are triggers for developmental neurotoxicity, both the Cohorts 2A and 2B are to be conducted as they provide complementary information.

ECHA notes that existing information on the registered substance itself derived from an available *in vivo* study ([redacted] 2011) indicates a potential specific mode of action with an association to developmental neurotoxicity due to dose-dependent, markedly reduced testosterone levels in juvenile/pubertal animals.

Moreover, exencephalies were reported in a preliminary inhalation teratology studies in rats and mice ([redacted], 1984).

ECHA concludes that the developmental neurotoxicity cohorts 2A and 2B need to be conducted because there is a particular concern on (developmental) neurotoxicity based on the results from the above-identified *in vivo* studies ([redacted] 2011; [redacted], 1984).

Species and route selection

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

c) Outcome

Based on the available information, 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 OECD TG 443), in rats, oral route, according to the following study-design specifications:

- At least two weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce systemic 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; and
- Cohorts 2A and 2B (Developmental neurotoxicity).

While the specifications for the study design are given above, you shall also submit with the new endpoint study record a scientific justification on each of the following aspects: 1) length of the premating exposure duration and dose level selection, 2) reasons for why or why not Cohort 1B was extended, 3) termination time for F2 generation, and 4) reasons for why or why not Cohorts 2A/2B and/or Cohort 3 were included.

Notes for your consideration

No triggers for the inclusion of Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including Cohort 3 if relevant information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the available information, together with the new information, shows triggers which are described in column 2 of Section 8.7.3., Annex IX 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.

Deadline to submit the requested information in this decision

In the draft decision communicated to you the time indicated to provide the requested information was 30 months from the date of adoption of the decision. This period of time took into account the fact that the draft decision requested an *in vivo* mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method OECD TG 489) in rats, oral route and an Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: /OECD TG 443) in rats, oral route with the registered substance. Considering that the request for *in vivo* comet assay has been removed from the present decision, the deadline for providing the information to meet the request remaining in the draft decision has been set to 24 months from the date of the adoption of the decision. The decision was therefore modified accordingly.

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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. However, following your comments on the draft decision indicating a tonnage band downgrade, ECHA has taken into account the updated tonnage band (submission number [redacted] and date 20 August 2018) only. No assessment of the updated registration has occurred. Based on the average production and/or import volumes for the three preceding calendar years, ECHA has changed the tonnage band as basis for the draft decision from [redacted] per year (submission number: [redacted] from 13 June 2016) to [redacted] tonnes per year (submission number: [redacted]).

The compliance check was initiated on 05 October 2017.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and your information about tonnage band downgrade.

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

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-62 written procedure 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.