Helsinki, 21 February 2019

Addressee: [redacted]

Decision number: TPE-D-2114460918-36-01/F
Substance name: Citral
EC number: 226-394-6
CAS number: 5392-40-5
Registration number: [redacted]
Submission number: [redacted]
Submission date: 09/11/2017
Registered tonnage band: Over 1000

DECISION ON A TESTING PROPOSAL

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

Your testing proposal is modified and you are requested to carry out:

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 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); and**
- **Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation.**

You have to submit the requested information in an updated registration dossier by **1 March 2021**. You also have to update the chemical safety report, where relevant.

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

Appeal

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

Authorised[1] by Ofelia Bercaru, Head of Unit, Hazard Assessment C4

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

Appendix 1: Reasons

The decision of ECHA is based on the examination of the testing proposals you submitted and information submitted by third parties.

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

Pursuant to Article 40(3)(b) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test under modified conditions.

a) Examination of the testing proposal

The basic test design of an extended one-generation reproductive toxicity study (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 Section 8.7.3., Annex X of the REACH Regulation, whereas column 2 defines when the study design needs to be expanded.

The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

You have submitted a testing proposal for an extended one-generation reproductive toxicity study according to OECD TG 443 by the oral route, in rats to be performed with the registered substance according to the basic study design and by applying ten weeks premating exposure duration for the parental (P0) generation. You provided justifications by summarising the existing *in vivo* and *in vitro* information on the registered substance and providing literature references.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Reproductive toxicity (extended one-generation reproductive toxicity study). ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

Thus, an extended one-generation reproductive toxicity study according to columns 1 and 2 of Section 8.7.3., Annex X is required. The following refers to the specifications of this required study.

Premating exposure duration and dose-level setting

You proposed ten weeks premating exposure duration.

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[2], 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.

[2] ECHA Guidance *on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017)

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[2]. In this specific case, 2-week premating exposure duration for P0 animals is sufficient, because the F1 animals of Cohort 1B are mated to produce the F2 generation and, thus, the premating exposure duration will be ten weeks for these animals. Consequently 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 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.

If there is no relevant data to be used for dose-level setting, it is 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.

Species and route selection

You proposed testing by oral route in rats. ECHA agrees with your proposal, since the substance to be tested is a liquid, and the rat is the preferred species according to the test method OECD TG 443.

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.

You proposed that *"the conditions to include the extension of Cohort 1B to produce the F2 generation are currently not met."* because none of the column 2, first paragraph, section b) criteria of 8.7.3., Annex X are fulfiled. In addition you claimed that *"A second generation does not provide information of relevance for classification/labeling decisions and risk assessment (Piersma 2011, Rorije 2011) and should be omitted due to animal welfare reasons."*

ECHA observes that 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 cleaning agents (PROC 1, 2, 8a, 8b, 10, 11, 13) and consumers as cleaning agents, in air care, in cosmetics and in other not further specified consumer uses.

Furthermore, there are several indications for endocrine-disrupting modes of action of the substance. In particular, effects on reproductive organs and reproductive parameters were observed:

- In the OECD TG 414 via oral gavage (Nogueria, 1995): dose-dependent decrease in gestation ratio (pregnant rats/ sperm-positive rat) supported by reduced number of *corpora lutea*, reduced implantation sites and increased resorptions. The increased resorptions is considered to be treatment-related, as the dosing was reported to be started on gestation day 6, after implantation. The number of resorptions was increased statistically significantly at 60 and 125 mg/kg bw/day. Clear statistically

significant reduction in corrected maternal body weight gain was observed only at and above 500 mg/kg bw/day. The corrected lower body weight gain in the early gestational period (day 6-11) was not dose–dependent. Hence ECHA does not agree with your conclusion that the reproductive toxicity observed can be attributed to maternal toxicity over the whole tested dose range.

- In a series of dermal repeated dose toxicity studies (Servadio 1986; Abramovici 1987; Geldof 1992; Scolnik, 1994), citral was consistently shown to induce different types of prostatic hyperplasia (atypic prostate hyperplasia and benign prostate hyperplasia).
- In a dietary carcinogenicity study in rats (OECD 453, [redacted] 2003c), negative trends in incidences of neoplasms in hormonally-sensitive organs (mammary gland fibroadenoma and clitoral adenoma or carcinoma) were found. (Significantly decreased incidence of the mammary gland, fibroadenoma at 210 mg/kg bw/day; and clitoral hyperplasia at 50 and 210 mg/kg bw/day.) In the study summary you concluded that the biological significance of these phenomena is unknown but "relationship to a possible antioestrogenic effect of citral was discussed".

Based on all of the above-mentioned findings, 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 information from *in vivo* studies with the registered substance indicates one or more modes of action related to endocrine-disruption.

In your comments to the draft decision you disagree with the extension of Cohort 1B to produce the F2 generation by mating the Cohort 1B animals, and argue that:
(i) the treatment-related increase in the resorption rates observed during the OECD TG 414 study via oral gavage in rats (Nogueria, 1995) is to be attributed to the maternal body weight parameters and that the study has limitation in term of validity;
(ii) the induction of prostatic hyperplasia in the different dermal repeated dose toxicity studies contradict the results from other studies;
(iii) the substance-related changes in hormonally-sensitive organs were not confirmed in the corresponding carcinogenicity study in mice; and
(iv) an EOGRTS without F2 is sufficient for risk assessment and classification purposes, since the F2 generation has limited added scientific and regulatory value (and you provided references to support this hypothesis).

Nonetheless, (i) the changes in the resorption rates do not correspond to the changes in maternal body weight parameters and the changes in resorption cannot be attributed exclusively to the maternal body weight parameters;
(ii) the consistent findings in the *in vivo* studies outweigh the results of *in vitro* studies;
(iii) the negative trends in incidence in hormonally sensitive organs was decreased in all three dose groups when compared to untreated and vehicle control groups combined. Furthermore, ECHA considers that this in conjunction with the other *in vivo* findings as explained above is sufficient to raise concern that the registered substance indicates one or more modes of action related to endocrine description; and
(iv) finally the provided publications on the need on F2 do not inform on the properties of the registered substance, nor do they inform why and how the information from these publications should be used to address the information requirement for production of the second filial generation (extension of Cohort 1B) which is triggered because the regulatory scientific criteria are met for the registered substance.

ECHA considers that taking together the different pieces of evidence, the weight of evidence sufficiently supports the concerns for reproductive toxicity. Therefore, ECHA maintains that

the Cohort 1B must be extended to include mating of the animals and production of the F2 generation.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation.

The third party considers the basic study design as proposed by the registrant (Cohorts 1A, and 1B without extension) *"to be appropriate in the absence of any triggers or conditions necessitating the inclusion of additional cohorts or a further generation"* and did not provide further scientific information.

ECHA considers that the comment acknowledges the need to conduct the study. Nonetheless the information provided by third parties is not sufficient to fulfil this information requirement.

c) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the modified study with the registered substance subject, as specificed above.

Notes for your consideration

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, Cohort 3 if new 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 X and further elaborated in ECHA Guidance[2]. 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.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 9 November 2017.

ECHA held a third party consultation for the testing proposals from 28 February 2018 until 16 April 2018. ECHA received information from third parties (see Appendix 1).

This decision does not take into account any updates after 12 September 2018, 30 calendar days after the end of the commenting period.

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

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

ECHA took into account your comments and amended the request.

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.

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). As stated above, the comments you submitted on the draft decision at a previous step in the decision making process (according to Article 50(1)) were all considered by ECHA at that process step.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-63 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 decision does not imply that the information provided in your registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of the Member States.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.