EUROPEAN CHEMICALS AGENCY

Helsinki, 21 November 2018

Addressee: [redacted]

Decision number: TPE-D-2114449853-39-01/F
Substance name: 4-tert-butylpyrocatechol
EC number: 202-653-9
CAS number: 98-29-3
Registration number: [redacted]
Submission number: [redacted]
Submission date: 15/12/2017
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 accepted and you are requested to carry out:

1. **In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum using the registered substance.**

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

2. **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:**
   - **Ten 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) without extension to mate the Cohort 1B animals to produce the F2 generation.**

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 an adequate and reliable documentation.

You have to submit the requested information in an updated registration dossier by ***29 November 2021***. You also have to update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

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, Evaluation E3

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

Appendix 1: Reasons

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

1. In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2)

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

"Mutagenicity" is an information requirement as laid down in Section 8.4. of Annexes VII to X of the REACH Regulation. Column 2 of Annex X, Section 8.4. provides that "If there is a positive result in any of the in vitro genotoxicity studies in Annexes VII or VIII, a second in vivo somatic cell test may be necessary, depending on the quality and relevance of all the available data."

The technical dossier contains several endpoint study records for *in vitro* genetic toxicity:

- four negative *in vitro* bacterial (Ames) tests (OECD TG 471) of which one is compliant (2002);
- two negative *in vitro* chromosomal aberration studies: one according to OECD TG 473 (1992) and one disregarded non-guideline study (1985);
- two *in vitro* gene mutation assays in mammalian cells: one *in vitro* study mouse lymphoma assay (OECD TG 490, 2017, reliability 1, according to and GLP) with the registered substance which show positive results with metabolic activation. The positive results are an indication that the substance is inducing gene mutations under the conditions of the tests; one supporting publication (similar to OECD TG 476, 1988, reliability 2, GLP not specified) publication performed similar to OECD TG 476 (GLP not specified) which shows ambiguous results without metabolic activation. Therefore the test item is considered to be mutagenic with metabolic activation in the first study and in the second study an ambiguous result without metabolic activation is obtained;

The IUCLID dossier also contains two *in vivo* micronucleus assays (similar to OECD TG 474): one in rat (intraperitoneal route, 2002) and one in mice (oral route, feed, 2002). However, ECHA considers that the studies are inconclusive since, in the first study,no information is reported on the ratio of PCE/NCE, there is a lack of reproducibility of the results and no historical control data are provided; and in the second study, there is inconsistency the in the findings reported (i.e. no increase in micronucleus in NCE (= mature erythrocytes) when the frequency in immature PCEs should be analysed) indicating a lack of bone marrow toxicity.

An appropriate second *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance but may be necessary to meet the information requirements. Consequently there is an information gap and you proposed to generate information for this endpoint.

Hence, you have submitted a testing proposal for a comet (single cell gel electrophoresis) assay (OECD TG 489) in rats by gavage with the registered substance 4-tert-butylpyrocatechol (EC number 202-653-9; CAS RN 98-29-3) and provided the following justification: *"GLP* in vitro *and* in vivo *studies are available. However, the recent MLA study performed in 2017 showed positive effects with metabolic activation at toxic doses. Therefore, in order to clarify this result, an additional* in vivo *test is needed. Furthermore, an* in vivo *micronucleus test on rats is unclear. Therefore, in order to clarify these two*

points an additional in vivo test is suggested. The Comet assay appears to be the more appropriate test".

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. 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.

ECHA notes that, according to ECHA *Guidance document on information requirements and chemical safety assessment* (version 6.0, July 2017), Chapter R.7a, Section R.7.7.6.3, the proposed test is an appropriate test to follow up positive *in vitro* result for gene mutation. Furthermore, this test is suitable to detect genotoxic effects at the site of contact.

You proposed testing in rats and by the oral route. According to the test method OECD TG 489, the test shall be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s), performance of the test by the oral route is appropriate.

In line with the test method OECD TG 489, the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, you are requested to carry out the proposed study with the registered substance subject to the present decision:
In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

Notes for your consideration

You are reminded that according to Annex X, Section 8.4., column 2 of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, *"the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered".*

You may consider examining gonadal cells, as it would optimise the use of animals. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

2. 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 8.7.3., Annex X of the REACH Regulation. 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 in ECHA *Guidance on information requirements and chemical safety assessment,* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

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 (gavage) route to be performed with the registered substance and to extend of Cohort 1B to produce a F2 generation. You have submitted the following justification and specification of the study design:
"The testing proposal is an extended one-generation reproductive toxicity study with cohort 1B to mate the F1 animals to produce the F2 generation.

The substance does not meet the criteria for specific adaptation according to column 1 and 2 of Annex VIII and IX. The registered substance is not known to be a genotoxic carcinogen, to be a germ cell mutagen and no extended one generation or a two-generation study is available. Furthermore, the substance is not of low activity and the data required for classification as toxic for reproduction 1A or 1B are not available.""

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.

ECHA considers that, based on the currently available information, the proposed study designs requires modification to fulfil the information requirement of Annex X, Section 8.7.3. of the REACH Regulation, as outlined below.

Premating exposure duration and dose-level setting

You did not specify a 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, 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 because 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). The registered substance has a measured partition coefficient of 1.98 and is considered as inherent ultimately biodegradable.

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 results from a 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.

You proposed to include an extension of Cohort 1B and did not provide any justification following the criteria described in column 2 of Section 8.7.3 of Annex X and detailed in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

The first condition of the column 2, Annex X, Section 8.7.3 outlines that "*the substance has uses leading to significant exposure of consumers and professionals, taking into account, inter alia, consumer exposure from articles*". ECHA notes that you have only reported industrial uses of the registered substance in your registration dossier. No significant professional or consumers uses are reported in the dossiers of the member registrants and you did not provide any information on the exposure of consumers via articles. Hence, ECHA notes that the first condition of the column 2, Annex X, Section 8.7.3 is not met.

Therefore, ECHA does not agree that the criteria to extend Cohort 1 B to produce a F2 generation are met and concludes that the extension of Cohort 1B does not need to be conducted.

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 X. When there are triggers for developmental neurotoxicity, both the Cohorts 2A and 2B are to be conducted as they provide complementary information.

You proposed not to include Cohorts 2A and 2B and provided justifications following the criteria described in column 2 of Section 8.7.3 of Annex X and detailed in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Therefore, ECHA agrees that the criteria to include Cohorts 2A and 2B are not met and concludes that the developmental neurotoxicity Cohorts 2A and 2B need not to be conducted.

Cohort 3

The developmental immunotoxicity Cohort 3 needs to be conducted in case of a particular concern on (developmental) immunotoxicity as described in column 2 of 8.7.3., Annex X.

You proposed not to include Cohort 3 and provided justifications following the criteria described in column 2 of Section 8.7.3 of Annex X and detailed in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Therefore, ECHA agrees that the criteria to include Cohort 3 are not met and concludes that the developmental immunotoxicity Cohort 3 needs not to be conducted.

Species and route selection

You proposed testing in rats and by the oral route. According to the test method OECD TG 443, the rat is the preferred species. On the basis of this default consideration, ECHA considers that testing should be performed in rats.

Also ECHA agrees 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 solid, ECHA concludes that testing should be performed by the oral route.

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 has indicated that, in addition to the extended one-generation reproductive toxicity study (EOGRTS), you have also proposed a study of genetic toxicity in vivo. The third party noted that a step-wise approach to testing should therefore be followed; the EOGRTS should not be initiated until a conclusion on the genotoxicity potential of the substance has been reached.

ECHA notes - that such adaptation may be considered in case the substance is known to be a germ cell mutagen (which corresponds to a classification as germ cell mutagen category 1A or 1B) and appropriate risk management measures are implemented

However, ECHA considers that results of a positive *in vivo* comet assay may contribute to a classification as germ cell mutagen, but this test is usually not sufficient on its own for classification as germ cell mutagen category 1B.

In any case, the timeline in this decision has been set to allow for sequential testing. It is your responsibility to consider and justify any adaptation that may become available in accordance with Annex X, Section 8.7., column 2, second indent.

Therefore, the information provided by third parties is not sufficient to adapt 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 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:
- Ten 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) without extension to mate the Cohort 1B animals to produce the F2 generation.

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

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, 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 the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if 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 on information requirements and chemical safety assessment*, Chapter R.7a, Section 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.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 14 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 **4 July 2018**, 30 calendar days after the end of the commenting period.

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

ECHA did not receive any comments by the end of the commenting period.

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

As no amendments were proposed, ECHA took the decision according to Article 51(3) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. The substance subject to the present decision is provisionally listed in the Community rolling action plan (CoRAP) for the start of substance evaluation in 2018.

2. 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.

3. 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.

4. 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.