EUROPEAN CHEMICALS AGENCY

Helsinki, 12 December 2019

Addressee: [redacted]

Decision number: CCH-D-2114493103-54-01/F
Substance name: tert-butyl 3,5,5-trimethylperoxyhexanoate
EC number: 236-050-7
CAS number: 13122-18-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 24 October 2017
Registered tonnage band: 100-1000

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. **In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, EU B.13/14. / OECD TG 471) with the registered substance;**

2. **Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation**

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 ***20 December 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.

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

[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. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes 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.

An "*In vitro* gene mutation study in bacteria" is a standard information requirement as laid down in Annex VII, Section 8.4.1. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided a study record for a "Mutagenic evaluation of Trigonox 42 S in Salmonella/microsome mutagenicity test" [redacted] 1977). However, this study does not provide the information required by Annex VIII, Section 8.4.1. for the reasons presented below.

According to the information reported in the robust study summary of that study included in your dossier, ECHA observes that no positive controls have been used in the study protocol. The OECD test guideline 471 indicates that "concurrent strain-specific positive and negative controls" should be included in each assay in order to demonstrate effective performance of these tests. ECHA considers that in the absence of positive controls confirming the performance of the test systems used in each assay, the results obtained from this study cannot be considered as reliable for hazard identification purposes.

Furthermore, according to paragraph 13 of the current OECD TG 471 test guideline (updated 1997) at least five strains of bacteria should be used: S. typhimurium TA1535; TA1537 or TA97a or TA97; TA98; TA100; S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). This includes four strains of S. typhimurium (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by *E.coli* WP2 strains or *S. typhimurium* TA102 which have an AT base pair at the primary reversion site.

The test reported in your technical dossier used five different strains of S. typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100 and it did not include tests with strains S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). However, since the test was conducted, significant changes have been made to OECD TG guideline 471 so that additionally testing with S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101) is now required. Therefore, the provided study does not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

ECHA concludes that a test using *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 has not been submitted and that the test using one of these is required to conclude on *in vitro* gene mutation in bacteria.

For the reasons presented above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA considers that the bacterial reverse mutation test (test method EU B.13/14. / OECD TG 471) is appropriate to address the standard information requirement of Annex VII, Section 8.4.1. of the REACH Regulation.

In your comments you agreed to perform the requested study.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471).

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes 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.

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 IX 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 concerns in relation with reproductive toxicity are observed. More specifically, reduced fetal body weight and increased post-natal death of pups were observed in the mid and high dose groups, 160 mg/kg/d and 500/400 mg/kg/d respectively, in the reproduction and developmental toxicity screening test conducted with the registered substance [redacted] 2010). You have considered that the mortality of pups is "*related to the maternal toxicity*" observed at these doses in this study. Based on the information provided, ECHA understands that you refer to the reduction in food consumption and in body weight gain of dams during the lactation period. ECHA stresses that these findings on maternal food consumption and body weight gain were detected during the lactation period only. No such effects were observed during the pre-mating, mating and gestation period. ECHA considers it unlikely that the reduction in food

consumption during the lataction period immediately causes offspring mortality. Furthermore, consistent with the findings from the screening study for reproductive and developmental toxicity, a statistically significant reduction in fetal body weight was detected in the mid and high dose groups, 50 mg/kg/d and 150 mg/kg/d respectively, in the absence of maternal toxicity in a pre-natal developmental toxicity study conducted with the registered substance in rats ([redacted] 2013). According to the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a/R.7.6: "Reduced body weight of offspring independent of litter size" and "Reduced survival of offspring" constitute triggers for an extended one-generation reproductive toxicity study at REACH Annex IX level. Therefore, 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.

You did not consider the information requirement for reproductive toxicity in Annex IX, Section 8.7.3., column 1, because no adverse effects on reproductive organs or tissues have been observed in the available repeated dose toxicity studies and these studies did not reveal other concerns in relation with reproductive toxicity. You indicated in the technical dossier and in your Chemical Safety Report that "*The study does not need to be conducted according to REACH Annex IX, 8.7.3 column 1, since available studies do not indicate any concerns in relation with reproductive toxicity*". You considered that "*there is no evidence of substance-related effects with regard to reproductive toxicity as demonstrated in the available Screening on Reproduction/Developmental Toxicity Study (OECD 421), the Prenatal Developmental Toxicity study (OECD 414), as well as the Repeated Dose 90-Day Toxicity study (OECD 408), and the Repeated Dose 28-Day Toxicity study (OECD 407) in rats*".

However, ECHA considers that such adverse effects on reproductive organs or tissues or other concerns in relation with reproductive toxicity are observed from these studies. The reduction in fetal body weight detected in the screening study for reproductive and developmental toxicity and in the pre-natal developmental toxicity study and the icreased offspring mortality in the screening study constitute a concern in relation with reproductive toxicity. Hence, an extended one-generation reproductive toxicity study is an information requirement.

In your comments to the draft decision you reiterated your views that the reduced fetal body weight and increased post-natal death of pups observed in the mid and high dose groups, in the reproduction and developmental toxicity screening test conducted with the registered substance are secondary to maternal toxicity. You consider that the dams were not in a condition to nurse and nourish their offspring, thereby causing the observed reduced fetal body weight and post-natal mortality. With regard to the effects observed in the OECD TG 414 study reported in the draft decision as supporting the findings from the OECD TG 421 study, you argue that the reduced fetal weights, although reaching statistical significance, were within the range of historical control data and considers these observations as being of no toxicological relevance.

Detailed information on the results obtained in the OECD 421 and OECD 414 studies were included in the comments. However, no new scientific arguments other than reference to historical control data were brought forward in your comments to dismiss the effects observed in these studies. Whilst historical control data provide valuable information on the incidence of findings in test animals used across multiple studies, this information does not overwrite the statistical significance of effects observed against concurrent controls in the conditions of a particular study.

As indicated in this decision, according to the ECHA Guidance on information requirements and chemical safety assessment Chapter R.7a/R.7.6: "*Reduced body weight of offspring independent of litter size*" and "*Reduced survival of offspring*" constitute triggers for an extended one-generation reproductive toxicity study at REACH Annex IX level.

You have also submitted a category justification document as part of your comments to this draft decision. The category includes four structurally similar peroxyesters for which EOGRT studies have all been requested.

According to you, "*the basic structures of all four substances are the same: a carboxylic acid moiety is linked to tert-butyl hydroperoxide forming a peroxyester. Adequate and reliable scientific information indicates that all four compounds have comparable toxicity profiles. Based on identical technical function (radical initiators of polymerisation processes), similar chemical structure, assumed similar metabolic pattern and a comparable toxicological profile the category approach is applicable. Results obtained from toxicity studies with one peroxyester may thus be applied to another peroxyester of the category*".

For the endpoint reproductive toxicity, you indicated in your category justification document that "*organs of the reproductive system were not affected by any of the compounds. The same applies for all parameters examined regarding fertility. Effects on offspring were only present at doses of pronounced maternal toxicity and as such are attributed to be of secondary nature. Maternal toxicity included reduced food consumption as well as depressed body weight and/or body weight gain, mostly severe at the end of gestation and during lactation period. This is a common finding among all four substances of the category.*"

You specify that an EOGRT study is ongoing on the category member TBPEH (EC 221-110-7; CAS 3006-82-4). ECHA understands from the information provided in the comments that you intend to use the information obtained from this study to predict the properties of the registered substance. You asked ECHA to postpone the decision on the need for further studies with the registered substance until results of the EOGRT study with TBPEH are available. The results from this study are due to be provided to ECHA by 09 September 2019.

ECHA has taken into account the information provided in the category justification document attached to the comments on the draft decision. The final results from the EOGRT study conducted with TBPEH have been submitted to ECHA in a dossier update on 09 September 2019. The observation of impairment of the female reproductive performance during the course of the study has led to the modification of the study design and extension of cohort 1B to produce a second generation. Specifically, a reduction in the number of developing follicles and an 1892increase in follicular atresia was noted in high-dose dams of the P generation. Reduced fertility index was noted in the P (high dose) and F1 (high dose and mid dose) generations. Increased incidence of post-natal mortality was detected in the high dose groups of the F1 and F2 generations suggesting inadequate nursing behaviour of dams. These findings have triggered a self-classification of TBPEH as Repr. 1B - H360F: May damage fertility.

In the category justification document provided alongside the comments to the draft decision, you considered that, on the basis of the data set available at that time, "*organs of the reproductive system were not affected by any of the compounds. The same applies for all parameters examined regarding fertility*". These conclusions are contradicted by the

findings of the EOGRT study conducted on TBPEH.

The findings from the EOGRT study on TBPEH and the results from the OECD TG 421 studies raise a concern on the reproductive toxicity of the other members of this category, including the registered substance. In the absence of self-classification of the registered substance as Repr. 1B on the basis of the data obtained on TBPEH, further information on the reproductive toxicity of the registered substance needs to be generated.

Hence, 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 to 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 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).

You have contested in your comments the need for a ten-week pre-mating exposure period. You indicate that "*according to the respective OECD guideline (the golden standard) 2 weeks are sufficient. A prolongation to 10 weeks based on the available data is not justified (e.g. testicular toxicity or effects on sperm integrity as mentioned in the respective OECD guideline) for the substance as this would unnecessary prolong the stress to the animals*".

The OECD TG 443 provides generic recommendations on the study protocol for an extended one-generation reproductive toxicity study. As indicated in the ECHA guidance, a two-week pre-mating exposure period is equivalent to the time required for epididymal transit of maturing spermatozoa and should allow the detection of post-testicular effects on sperm (during the final stages of spermiation and epididymal sperm maturation) at mating. According to the OECD TG 443, at the time of termination, when testicular and epididymal histopathology and analysis of sperm parameters are scheduled, the P and F1 males, will have been exposed for at least one entire spermatogenic process.

However, a two-week pre-mating exposure period in the context of an EOGRT study with a basic design does not provide information on the impact of exposure to the substance over the duration of the entire spermatogenic process on the reproductive function. According to the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a/R.7.6 "*Ten weeks cover the full spermatogenesis, sperm maturation and folliculogenesis before the mating allowing a meaningful assessment with the full spectrum*

of the effects after the same exposure history". Furthermore, the information generated under REACH need to be adequate for risk assessment and for classification and labelling purposes. According to the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a/R.7.6, in the asbence of substance specific justification, "a two-week premating period may be too short to produce results appropriate to conclude whether the substance meets the criteria for a category 1B reproductive toxicant, and thus may not be sufficient for classification and labelling purposes".

Therefore, a ten-week pre-mating exposure period is appropriate in this specific case. 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 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.

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) 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 EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

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

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 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

The timeline indicated in the draft decision to provide the information requested is 24 months from the date of adoption of the decision.

In your comments on the draft decision you consider that sequential testing is appropriate and that the genotoxic properties of the substance need to be clarified before deciding on the need to conduct the EOGRT study. The timeline set in the decision already allows for conducting the *in vitro* mutagenicity study before starting the EOGRT study. The outcome of the *in vitro* mutagenicity study can then be taken into account when reassessing the need for and the design of the requested EOGRT study.

In the comments on the draft decision, you requested an extension of the timeline to 44 months because 24 months would not allow to conduct the experimental phase of the EOGRT study and the subsequent revisions of the risk assessment and of the technical dossier. You also referred to an ongoing compliance check decision on a structurally similar substance (CAS 927-07-1) also requesting an EOGRT study, and request that the deadlines to provide both studies allows for conducting these studies in the same testing facilities for consistency reasons.

According to the statement from the testing laboratory attached to your comments, the performance of the preliminary work and of the experimental phase of an EOGRT study requires 14 to 18 months. The timeline of 24 months set in the decision exceeds the duration specified by the testing laboratory and accommodates time to update your risk assessment and technical dossier. It is the your responsibility to identify the appropriate test facilities to conduct the studies requested in regulatory decisions and to provide the results within the indicated timeline. In this case, it is the your decision to perform both EOGRT studies in a single test laboratory.

ECHA has not extended the timeline to provide the information.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 19 March 2018.

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 within 30 days of the notification.

ECHA took into account your comments and did not amend the requests or the deadline.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

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.