Helsinki, 14 December 2016

Decision number: TPE-D-2114348987-28-01/F

DECISION ON TESTING PROPOSAL(S) SET OUT IN A REGISTRATION PURSUANT TO ARTICLE 40(3) OF REGULATION (EC) NO 1907/2006

For OO-tert-butyl O-(2-ethylhexyl) peroxycarbonate, EC No 252-029-5 (CAS No 34443-12-4), registration number: [redacted]

Addressee: [redacted]

The European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 51 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

I. Procedure

Pursuant to Article 40(1) of the REACH Regulation, ECHA has examined the following testing proposals submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for OO-tert-butyl O-(2-ethylhexyl) peroxycarbonate, EC No 252-029-5 (CAS No 34443-12-4), submitted by [redacted] (Registrant):

- Viscosity of Liquids (OECD 114);
- Comet assay *in vivo* in male rats in liver, kidneys and forestomach;
- Repeated Dose 90-Day Oral Toxicity (OECD 408) in rats, including additional analyses of reproductive organs, accessory glands and sperm parameters as well as investigation of kidney toxicity with immunohistochemical determination of alpha-2-microglobulin;
- Prenatal developmental toxicity study (OECD 414) in rats;
- Daphnia magna Reproduction Test (OECD 211).

This decision is based on the registration as submitted with submission number [redacted], for the tonnage band of 100 to 1000 tonnes per year. This decision does not take into account any updates after **17 February 2016,** i.e. 30 calendar days after the end of the commenting period.

This decision does not imply that the information provided by the Registrant in his 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.

ECHA received the registration dossier containing the above-mentioned testing proposals for further examination pursuant to Article 40(1) on 28 March 2013.

ECHA held a third party consultation for the testing proposals from 21 March 2014 until 5 May 2014. ECHA received information from third parties (see section III below).

ECHA notified you of the draft decision on 27 November 2015 and invited you to provide comments. That draft decision was based on the registration dossier with submission number [redacted].

ECHA received your comments on the draft decision on 15 January 2016.

You updated your registration with submission number [redacted] on 13 February 2016.

The ECHA Secretariat considered your comments on removing the *Daphnia magna* Reproduction test (EU C.20/OECD 211) from your testing proposal in the updated REACH dossier, and considered your update where the removal became effective.

As a result, ECHA amended the information required in the draft decision. Annex 1 (Reasons) was changed accordingly.

On 21 July 2016 ECHA notified the Competent Authorities of the Member States of its draft decision and invited them pursuant to Article 51(1) of the REACH Regulation to submit proposals for amendment of the draft decision within 30 days of the receipt of the notification.

Subsequently, proposal for amendment to the draft decision was submitted.

On 26 August 2016 ECHA notified the Registrant of the proposal for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposal for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposal for amendment received and amended the draft decision.

On 5 September 2016 ECHA referred the draft decision to the Member State Committee.

By 26 September 2016, the Registrant did not provide any comments on the proposal for amendment.

A unanimous agreement of the Member State Committee on the draft decision was reached on 10 October 2016 in a written procedure launched on 29 September 2016.

ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

Testing required

A. Tests required pursuant to Article 40(3)

The Registrant shall carry out the following tests as proposed or modified pursuant to Article 40(3)(a) and (b) and 13(4) of the REACH Regulation using the indicated test methods and the registered substance subject to the present decision:

1. Viscosity (Annex IX, Section 7.17.; test method OECD 114);
2. *In Vivo* Mammalian Alkaline Comet Assay (Annex IX, Section 8.4., column 2; test method: OECD 489) in rat (male and female), oral route, on the following tissues: liver, glandular stomach, duodenum[1] and kidney;
3. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26/OECD 408) in rats; including urinalysis and a full histopathological examination which is to include immunohistochemical investigation of renal pathology to determine if the pathology is mediated by alpha-2u globulin nephropathy. It is at the Registrant's discretion to perform the intended additional reproductive toxicity examinations during the testing program;

[1] ECHA considers that the duodenum is the most appropriate part of the intestine to be tested, as it is the first part of the intestine and directly connected to the stomach. The duodenum tissue sampled may contain a small part of the jejunum.

4. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31/OECD 414) in rats or rabbits, oral route.

Note for consideration by the Registrant:

The Registrant 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 of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring to and conforming with the appropriate rules in the respective Annex, and an adequate and reliable documentation.

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

B. Deadline for submitting the required information

Pursuant to Articles 40(4) and 22(2) of the REACH Regulation, the Registrant shall submit to ECHA by **23 December 2019** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report. The timeline has been set to allow for sequential testing as appropriate.

Statement of reasons

The decision of ECHA is based on the examination of the testing proposals submitted by the Registrant for the registered substance and scientific information submitted by third parties.

A. Tests required pursuant to Article 40(3)

1. Viscosity of Liquids (Annex IX, 7.17)

a) Examination of the testing proposal

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

"Viscosity" is a standard information requirement as laid down in Annex IX, Section 7.17. of the REACH Regulation. The information on this endpoint is not available for the registered substance subject to the present decision 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.

The Registrant has submitted a testing proposal for a OECD Test Guideline 114 (Viscosity of Liquids).

ECHA considers the proposed test appropriate and testing should be performed with the registered substance.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed test using the registered substance subject to the present decision: Viscosity of liquids (test method: OECD 114).

2. In Vivo Mammalian Alkaline Comet Assay (Annex IX, 8.4., column 2, OECD 489)

a) Examination of the testing proposal

Pursuant to Article 40(3)(b) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test under modified conditions.
"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "If there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the registrant."

The technical dossier contains three Ames tests performed according to OECD 471 with the registered substance. The Registrant considers all three studies as key studies and reports weakly positive or positive results for them, respectively. In addition, the dossier contains an *in vitro* Mouse Lymphoma Assay (MLA) performed according to OECD 476 with the registered substance that also is reported with positive results. The *in vitro* positive results indicate that the substance is inducing gene mutations under the conditions of the tests.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance but shall be proposed by the Registrant. Consequently, there is an information gap and the Registrant proposed to generate information for this endpoint.

The Registrant has submitted a testing proposal for an *in vivo* Comet assay with the following justification: "*A comet assay is proposed to clarify the* in vitro *positive effects observed in the Ames test and the MLA. It is proposed to analyse effects on forestomach (first site of contact), on kidney (since toxicity was observed even if only in male rats). In addition, it is proposed to analyse the liver as it is a possible site of metabolism. It is proposed to administrate twice TBEC by oral route and to sample once, in male rats.* "

According to the ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.7.6.3 (July 2015), the Comet assay can be used to follow up *in vitro* positive results in mammalian cells. Therefore, ECHA considers a study according OECD TG 489 (Comet assay) as appropriate to fulfil the current information requirement.

As regards the route of administration, paragraph 39 of the OECD test guideline 489 states that "*[t]he anticipated route of human exposure should be considered when designing an assay*" and "*[i]n any case the route should be chosen to ensure adequate exposure of the target tissue(s)*". ECHA notes that there is no spray application indicating high inhalation exposure and therefore ECHA considers that testing by the oral route is appropriate.

As regards the species to be used, paragraph 23 of the OECD test guideline 489 states that "*[t]he choice of rodent species should be based on (i) species used in other toxicity studies (to be able to correlate data and to allow integrated studies), (ii) species that developed tumours in a carcinogenicity study (when investigating the mechanism of carcinogenesis), or (iii) species with the most relevant metabolism for humans, if known. Rats are routinely used in this test.*" ECHA notes that a 28-day repeated dose toxicity study in rat has been performed using the registered substance. Moreover, the comet assay has been most extensively validated in rats. Therefore ECHA considers that testing in the rat is appropriate.

Furthermore, the Registrant proposed male rats to be used for testing. ECHA notes that the OECD test guideline 489 indicates that "*Data demonstrating relevant differences between males and females (e.g. differences in systemic toxicity [...]) encourage the use of both sexes*". The Registrant did not further clarify the reason(s) for its intention to use male rats for testing. ECHA notes that 28-day repeated dose toxicity study kidney toxicity was only observed in male rats demonstrating relevant differences in systemic toxicity between males and females. Therefore, ECHA considers it is appropriate to perform the Comet assay using both male and female rats.

As regards the tissues to be studied, according to the test method (OECD 489), the comet assay can be performed by analysing tissues from liver, glandular stomach and duodenum. As set out in the OECD TG 489, the liver is recommended as the primary site of xenobiotic metabolism, and an often highly exposed tissue. There are several expected or possible variables between the glandular stomach and the duodenum/jejunum (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 sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract. Moreover, ECHA notes that the Registrant proposed also analysing kidney tissue due to kidney toxicity observed in male rats in the 28-day study. ECHA agrees with the Registrant on the relevance of examination of kidney tissue.

b) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: *In Vivo* Mammalian Alkaline Comet Assay (Annex IX, Section 8.4., column 2; test method: OECD 489) in rat (male and female), oral route, on the following tissues: liver, glandular stomach, duodenum and kidney.

Note for consideration by the Registrant

The Registrant is reminded that according to the column 2 of section 8.4 of Annex IX 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". ECHA notes that the examination of gonadal cells would optimize the use of animals. Positive results in whole gonad that contains a mixture of somatic and germ cells are not necessarily reflective of germ cell damage, but they indicate that tested substance(s) and/or its metabolites have reached the gonad. This type of evidence may still be relevant for the overall assessment of possible germ cell mutagenicity including classfication and labelling according to the CLP Regulation.

3. Sub-chronic toxicity study, 90-day (Annex IX, 8.6.2)

a) Examination of the testing proposal

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

A sub-chronic toxicity study (90 day) is a standard information requirement as laid down in Annex IX, Section 8.6.2. of the REACH Regulation. 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.

The Registrant has submitted a testing proposal for a sub-chronic toxicity study (90 day) in rats via the oral route (EU B.26/OECD 408) with the following justification: *"A 90-day oral toxicity study in rats is proposed. In order to better evaluate reproductive effects of repeated dose exposure, histopathology of the testes, as well as weights of reproductive organs and accessory glands will be taken (i.e. testis, epididymis, prostate, seminal vesicle). In addition sperm parameters such including sperm count, sperm morphology and sperm motility will be evaluated. In addition, it is proposed to investigate kidney toxicity with immunohistochemical determination of alpha-2-microglobulin, since male kindey toxicity was observed during the 28-day study by oral route."*

The Registrant proposed testing by the oral route. ECHA notes that the substance is a liquid with low vapour pressure, not classified as corrosive/irritating to the skin or damaging/irritating to the eyes, and that the information provided on the uses and human exposure does not include professional uses with spray application. In light of the physic-chemical properties of the substance, uses and human exposure, ECHA agrees that testing by the oral route is most appropriate. Hence, the test shall be performed by the oral route using the test method EU B.26./OECD 408.

The Registrant proposed testing in rats. According to the test method EU B.26/OECD 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

As explained above in the Registrant's justification, in the 28-day repeated dose toxicity study kidney toxicity was observed in male rats and the Registrant consequently proposed to investigate kidney toxicity in the proposed 90-day study. The fact that these effects were only observed in male rats indicates that the registered substance may induce alpha-2u-globulin-mediated nephropathy. Since humans do not excrete alpha-2u-globulin, this mode of action is not relevant to humans. In order to verify whether the observed effects on kidney are indeed alpha-2u-globulin-mediated or whether there the substance acts via another mode of action, ECHA decided to include in the request for a sub-chronic toxicity study the analysis of urin (which is optional in paragraph 30 of OECD 408, and the relevant part of Section 1.5.2.2. of EU Method B.26) to investigate the kidney function, as well as a full histopathological examination of the kidney (paragraph 36 of OECD 408, Section 1.5.2.4. of EU Method B.26), which is to include immunohistochemical investigation of renal pathology.

In addition, the Registrant proposed to extend the sub-chronic toxicity study (90 day) by including additional reproductive examinations/parameters (i.e. histopathology of testes, weights of reproductive organs and accessoryx glands and sperm parameters). ECHA notes that it is at the Registrant's discretion to perform these intended additional reproductive toxicity examinations during the testing program and use the results to ensure the safe use of the substance. However, the Registrant is reminded that, if the condition of Annex IX, Section 8.7.3., Column 1 is fulfilled, the proposed extension of the study presently requested does not fulfil the standard information requirement in the registration dossier for reproductive toxicity set out in Annex IX, Section 8.7.3.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

A third party has indicated that *"Based on registration data the substance displays a 'low toxicity profile' if the additionally proposed Comet assay will produce evidence of the absence of genotoxic effects in vivo. A review on more than 40 low toxicity chemicals has shown that the results of the 28-day study are predictive of low toxicity in the 90-day repeated dose toxicity study. Accordingly, the proposed 90-day sub-chronic toxicity study is not expected to add toxicologically meaningful information if the result of the Comet assay will be negative, suggesting that it may be waived in a weight-of-evidence approach. Therefore a sequential testing process is recommended which gives priority to the Comet assay."*

ECHA acknowledges that, in addition to sequential testing strategy, the third party has also proposed a weight of evidence approach based on a database search. The third party claims that this general weight of evidence approach can be used to predict the sub-chronic toxic properties of a substance based on observed "low toxicity" in a sub-acute (short-term repeated dose) toxicity study if the substance fulfils certain other criteria described as a "low toxicity profile".

However, ECHA notes that this predictive weight of evidence approach has shortcomings that prevent its application. First of all, ECHA notes that a weight of evidence approach requires substance-specific justification and cannot be addressed with a generic weight of evidence approach which e.g. does not explain whether it is applicable to the registered substance. Secondly, the proposed approach has a limited predictive power. Taking into account only the substances that fulfilled all the "low toxicity" criteria listed in the review, it is based on eighteen substances with a "low toxicity profile". Out of these eighteen substances, the prediction was incorrect for two substances. Thirdly, ECHA notes that the proposed general weight of evidence approach that a substance will not have an effect in a sub-chronic toxicity study based on results of a sub-acute toxicity study is not appropriate for the following reasons. The study design of sub-acute toxicity studies and sub-chronic toxicity studies differ in relevant key parameters, which affect the uncertainty and relevance of the information obtained from these studies. For example, the reduced number of animals used in a sub-acute toxicity study (5 animals per sex and dose) compared to the sub-chronic toxicity study (10 animals per sex and dose) results in a lower statistical power of the sub-acute toxicity study to detect effects. Similarly, the duration of exposure in a sub-chronic toxicity study (90 days) covers a prolonged period of the animals' lifespan as compared to the sub-acute toxicity study (28 days).

As a consequence of these differences in the study protocols, a sub-chronic toxicity study (90-day) may detect effects which were not observed in a sub-acute toxicity study (28 days) or . Therefore, the information provided by the third party is not sufficient to adapt the standard information requirement. Furthermore the test guideline of Comet assay was endorsed only in 2014, consequently no studies with this test guideline were included in the review mentioned by the third party and the value of the parameters of that study in the proposed weight of evidence cannot be asessed. ECHA further notes that as explained under section III.A.3.a above, there was kidney toxicity observed in the 28-day study and it is necessary to follow up that toxicity in the proposed 90-day study.

ECHA notes that the deadline to provide the requested information allows for sequential testing and that it is the Registrant's responsibility to consider the order of the tests to be performed. ECHA further notes that it is the Registrant's responsibility to justify any adaptation of the information requirements in accordance with the relevant conditions as established in Annex XI, Section 1.2. Therefore, the Registrant should assess whether he can justify weight of evidence as suggested by the third party. If the information requirement can be met by way of adaptation, he should include the adaptation argument with all necessary documentation according to Annex XI, Section 1.2. in the registration dossier.

c) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: Sub-chronic toxicity study (90-day) in rats, oral route (test method: EU B.26/OECD 408) modified to include urinalysis and a full histopathological examination, which is to include immunohistochemical investigation of renal pathology to determine if the pathology is mediated by alpha-2u globulin nephropathy.

4. Pre-natal developmental toxicity study (Annex IX, 8.7.2)

a) Examination of the testing proposal

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

A pre-natal developmental toxicity study for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. 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.

The Registrant has submitted a testing proposal for a pre-natal developmental toxicity study in rats according to EU B.31/OECD 414.

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

The Registrant proposed testing in rats. He proposed testing by the oral route. According to the test method EU B.31/OECD 414, the rat is the preferred rodent species, the rabbit the preferred non-rodent species and the test substance is usually administered orally. ECHA considers these default parameters appropriate and testing should be performed by the oral route with the rat or the rabbit as a first species to be used.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: Pre-natal developmental toxicity study in rats or rabbits, oral route (test method: EU B.31/OECD 414).

II. Adequate identification of the composition of the tested material

The process of examination of testing proposals set out in Article 40 of the REACH Regulation aims at ensuring that the new studies meet real information needs. Within this context, the Registrant's dossier was sufficient to confirm the identity of the substance to the extent necessary for examination of the testing proposal. The Registrant must note, however, that this information, or the information submitted by other registrants of the same substance, has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In relation to the proposed tests, the sample of substance used for the new studies must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is within the specifications of the substance composition that are given by the joint registrants. It is the responsibility of all joint registrants of the same substance to agree to the tests proposed (as applicable to their tonnage level) and to document the necessary information on their substance composition.

In addition, it is important to ensure that the particular sample of substance tested in the new studies 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 by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new studies must be suitable to assess these grades.

Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the studies to be assessed.

III. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Article 51(8) of the REACH Regulation. Such appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[2] by Claudio Carlon, Head of Unit, Evaluation E2

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