EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114300159-59-01/F Helsinki, 30 July 2015

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

For bisisobutyryl peroxide, CAS No 3437-84-1 (EC No 222-340-0), 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 jointly submitted registration dossier in accordance with Articles 10(a)(ix) and 12(1)(d) thereof for bisisobutyryl peroxide, CAS No 3437-84-1 (EC No 222-340-0, submitted by [redacted] (Registrant).

- Viscosity;
- 90-day oral toxicity study (OECD 408);
- *In vivo* micronucleus study (Incorporated into OECD 408, equivalent to OECD 474);
- Developmental toxicity / teratogenicity study (OECD 414).

This decision is based on the registration dossier 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 15 January 2015, the date upon which ECHA notified its draft decision to the Competent Authorities of the Member States pursuant to Article 51(1) of the REACH Regulation.

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 27 March 2013.

ECHA held a third party consultation for the testing proposals from 4 April 2014 until 19 May 2014. ECHA received information from third parties (see section III below).

On 12 September 2014 ECHA sent the draft decision to the Registrant and invited him to provide comments within 30 days of the receipt of the draft decision.

On 15 October 2014 ECHA received comments from the Registrant agreeing to ECHA's draft decision.

On 15 January 2015 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, proposals for amendment to the draft decision were submitted.

On 20 February 2015 ECHA notified the Registrant of the proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposals for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposals for amendment received and did amend the draft decision.

On 2 March 2015 ECHA referred the draft decision to the Member State Committee.

By 23 March 2015, in accordance to Article 51(5), the Registrant provided comments on the proposals for amendment. The Member State Committee took the comments of the Registrant on the proposals for amendment into account.

A unanimous agreement of the Member State Committee on the draft decision was reached on 7 April 2015 in a written procedure launched on 26 March 2015.

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

II. Testing required

A. Tests required pursuant to Article 40(3)

The Registrant shall carry out the following proposed tests pursuant to Article 40(3)(a) 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 via the oral route, with examination of liver and either glandular stomach or duodenum/jejunum;
3. Sub-chronic toxicity study (90-day) in rats, oral route (Annex IX, Section 8.6.2.; 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-2-microglobulin nephropathy;
4. Pre-natal developmental toxicity study in rats or rabbits, oral route (Annex IX, Section 8.7.2.; test method: EU B.31/OECD 414).

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 **6 August 2017** an update of the registration dossier containing the information required by this decision. The timeline has been set to allow for sequential testing as appropriate.

III. 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.

1. Viscosity (Annex IX, Section 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 Viscosity study (Annex IX, Section 7.17.; test method OECD 114, Viscosity of Liquids).

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

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 bisisobutyryl peroxide: Viscosity of liquids (test method: OECD 114).

2. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 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.

"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."

Hence, the Registrant has submitted a testing proposal for an in-vivo micronucleus test with the following justification:

"Two in vitro mutation studies were conducted with the registered substance in phlegmatizer. In a bacterial reverse mutation assay, there was an increase in the number of revertants, in the absence of metabolic activation, in one tester strain. In an in vitro mouse lymphoma assay, the registered substance in phlegmatizer, did not induce an increase in mutation frequency with or without metabolic activation.

In accordance with Endpoint Specific Guidance Chapter R.7A, Figure R.7.7-1 "Flow chart of the mutagenicity testing strategy", no further testing (i.e. no in vivo testing) need be proposed in the event of a negative mouse lymphoma assay or hprt assay, regardless to whether or not the gene mutation test in bacteria is positive or negative. This therefore implies that when considering whether an in vivo gene mutation request is required for substances requiring Annex IX test proposals due to their volume bands, a negative mouse lymphoma assay or hprt assay is sufficient evidence to waive the need for an in vivo gene mutation test. In an in vitro micronucleus assay, the registered substance in phlegmatizer induced micronuclei frequency in binucleated cells only at a prolonged exposure time without metabolic activation.
The registered substance is thermally and hydrolytically unstable. The peroxide will completely decompose within half an hour at ambient temperature with a significant amount decomposing within 10 minutes primarily isobutyric acid which is not considered mutagenic or genotoxic. Propene may also be a major breakdown. However, due to its volatility, this could not be verified. Other decomposition products may include, to a lesser degree, isopropanol and acetone which are not considered mutagenic or genotoxic. Under the conditions of this assay, the parent compound would quickly decompose. The positive results are likely due a thermal decomposition product.

An in vivo micronucleus study, Annex IX, will be proposed as part of an OECD 408, 90 -day oral gavage study.

The following information is taken into account for any hazard / risk assessment: Bisisobutyryl peroxide, 40% in phlegmatizer, induced the formation of micronuclei in human lymphocytes in the absence of metabolic activation at a prolonged exposure period in binucleated cells only and is considered aneugenic or clastogenic under the conditions of the assay."

ECHA Secretariat notes the possible clastogenicity and aneugenicity of bisisobutyryl peroxide solution was tested in two independent experiments. The study procedures described in the report were based on the most recent OECD guideline 487. The results indicate that bisisobutyryl peroxide solution is positive in the in vitro micronucleus study and can be considered an aneugenic or clastogenic compound under the conditions of this assay.

In his comments of 20 March 2015 to the proposal for amendment received from a Member State Competent Authority, the Registrant stated "We are revising our test proposal from an *in vivo* MN assay to the Comet assay." He thereby proposed to carry out an *in vivo* mammalian alkaline comet assay (OECD 489) instead of the previously proposed *in vivo* micronucleus test (OECD 474). Moreover, the Registrant has proposed to combine this genotoxicity study with the 90-day oral gavage study (OECD 408).

ECHA Secretariat considers the comet assay test guideline (TG) OECD 489 gives the possibility to be integrated in a repeated dose study, e.g., 90-d (OECD 408) subject to the following considerations to be taken into account by the Registrant:

- the maximum tolerated dose (MTD) in the 90-day subchronic toxicity study may be lower than the MTD in a standard comet assay. For instance if the chemical does not induce toxicity, the top dose allowed by the TG is 1000 mg/kg for the 90 day sub-chronic toxicity study and 2000 mg/kg for the standard comet assay.

- The age of the animals and the corresponding historical controls: the laboratory performing the study should have historical control data for animals at the end of the 90-day chronic toxicity study (i.e. 13 weeks older than in the comet assay).
- An additional group of animals, i.e. positive control group, should be added to the 90-day sub chronic toxicity study protocol to demonstrate that the induced response are compatible with those generated in the historical positive control database.
- Careful consideration should be given to the logistics involved in tissue sampling for comet analysis alongside the requirements of tissue sampling for other types of toxicological assessments. Harvest 24 hours after the last dose, which is typical of a general toxicity study, is not appropriate for the comet assay where samples are usually collected 2-6 h after the last treatment (see OECD 489, paragraph 33).

As a general principle ECHA Secretariat notes there are no grounds to reject the proposal by the Registrant because this combination possibility is foreseen in the OECD 489 TG and in ECHA guidance R7a section R.7.7.6.3. However, the Registrant will need to take into account the issues referred to above to ensure that the generated data will be acceptable to cover the data requirement for the *in vivo* mammalian alkaline comet assay.

As regards the route of administration, paragraph 39 of the OECD test guideline 489 states that "*the 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)*". In light of the information provided in the dossier on the nature of the substance (low vapour pressure liquid), the uses and the human exposure, 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 "*the 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 an OECD 407 study in rat has been performed using the registered substance. ECHA considers that testing in the rat is appropriate.

As regards the tissues to be studied, paragraph 42 of the draft OECD test guideline 489 states that *the liver has been the tissue most frequently studied and for which there are the most data. Therefore, in the absence of any background information, and if no specific tissues of interest are identified, sampling the liver would be justified as this is a primary site of xenobiotic metabolism and is often highly exposed to both parent substance(s) and metabolite(s). In some cases examination of a site of direct contact (for example, for orally-administered substances the glandular stomach or duodenum/jejunum, or for inhaled substances the lungs) may be most relevant.*" ECHA considers that the comet assay should be performed in liver and either glandular stomach or duodenum/jejunum. It was noted that the conducted OECD 407 subacute oral toxicity study demonstrated toxic effects in the stomach and therefore duodenum/jejunum may be a preferable target tissue to the glandular stomach in order to avoid possible false positive effects due to the cytotoxic effects.

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: *In vivo* mammalian alkaline comet assay in rat via the oral route (test method: OECD 489), with examination of liver and either glandular stomach or duodenum/jejunum. The Registrant can incorporate this study into the proposed OECD 408 (90 day) oral gavage study if the bulleted considerations above are taken into account.

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, Section 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 rodents via the oral route (EU B.26/OECD 408) with the following justification:

"The study will include evaluation of extra reproductive parameters including sperm analysis, estrus cycle and sperm staging. In addition, a micronucleus test will be carried, in blood, as an in vitro micronucleus study was positive, as well as urinalysis and a full histopathological examination of the kidney which will include immunohistochemical investigation of renal pathology to determine if the pathology is mediated by alpha-2-microglobulin nephropathy."

The Registrant did not specify the species to be used for testing. 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. The Registrant proposed testing by the oral route. The testing is incorporating extra parameters that impact on the selection of route of administration. The physico-chemical properties of the substance show it to be a thermally unstable liquid (self-accelerating decomposition above 0°C) with low vapour pressure, classified as corrosive to the skin and damaging to the eyes. The information provided on the uses and human exposure indicate there is a spray use but it will be to a low concentration of the substance included in a polymerising matrix as a free radical initiator. On balance, ECHA considers that testing by the oral route is most appropriate.

In the short-term repeated dose toxicity 28 day study, increase in incidence and severity of cortical hyaline droplets in the kidneys of males at 300 and 1000 mg/kg were observed in male rats. The fact that these effects were only observed in male rats indicates that the registered substance may induce alpha-2-microglobulin-mediated nephropathy. Since humans do not excrete alpha-2-microglobulin, this mode of action is not relevant to humans. For this reason, and as proposed by the Registrant, ECHA requires inclusion of

urinalysis (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 kidney function, and a full histopathological examination (paragraph 36 of OECD 408, Section 1.5.2.4. of EU Method B.26), which is to include immunohistochemical investigation of renal pathology to determine if the pathology is indeed mediated by alpha-2-microglobulin.

Further the Registrant proposed to extend the sub-chronic toxicity study (90 day) by including additional examinations/parameters corresponding to the genotoxicity study. In his comments of 20 March 2015 to the proposal for amendment received from a Member State Competent Authority, the Registrant has changed the previously proposed genotoxicity test, i.e. *in vivo* micronucleus test (OECD 474), to an *in vivo* mammalian alkaline comet assay (OECD 489). ECHA notes, that it is at the Registrant's discretion to perform the intended additional examinations during the testing program, in line with the requirements of section III.2 of this decision, and to use the results to ensure the safe use of the substance.

The Registrant proposed to extend the sub-chronic toxicity study (90 day) by including additional examinations/parameters or reproductive parameters including sperm analysis, estrus cycle and sperm staging. ECHA notes, that it is at the Registrant's discretion to perform the intended additional 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 has received third party information concerning the testing proposal during the third party consultation.

A third party has commented: "Based on the registrant's assessment an oral 28-day repeated dose toxicity study with the diacyl peroxide did not show systemic toxicity effects which are relevant to humans (NOAEL 1000 mg/kg bw/d). Comparable adaptive liver effects and rat specific nephropathy were observed in an oral 90-day sub-chronic toxicity study with an isomer of the phlegmatizer additive. Furthermore, the substance is hydrolytically instable and expected to be rapidly decomposed to isobutyric acid which has been reported to be substantially eliminated as CO2. Therefore, and in view of the corrosive nature of the parent compound the proposed study may scientifically not be justified, and read-across to existing data for the phlegmatizer and the conversion product isobutyric acid (or the related isobutanol) is suggested."

ECHA acknowledges that the third party has proposed a testing strategy including a read across approach for the Registrant to consider.

ECHA notes that it is the Registrant's responsibility to consider and justify any adaptation of the information requirements in accordance with the relevant conditions as established in Annex XI, Section 1.5.. Therefore, the Registrant should assess whether he can justify a read-across as suggested by the third party. If the adaptation can be justified, he should include the adaptation argument with all necessary documentation in the registration dossier. Such update can only be taken into consideration in the decision-making if it is submitted before the draft decision is sent to the Member State Competent Authorities pursuant to Article 51(1) of the REACH Regulation.

ECHA notes that the information provided by the third party is currently insufficient for demonstrating that the conditions of Annex XI, Section 1.5. of the REACH Regulation are

met. For example, organic peroxides are free radical initiators and the toxicological significance of free radical generation could not be assessed through read across to the hydrolysis products and the phlegmatiser. The registered substance and the hydrolysis product may not have sufficiently similar toxicological properties and the criteria of similarity as specified in Annex XI, Section 1.5 is thus not met. The breakdown products may have some overall toxicological relevance but it is the mechanism by which they are produced that remains untested and the requirements of Annex XI 1.5.2 are also not met.

Therefore, the information provided by the third party in itself would not be sufficient to adapt the standard information requirement.

Further, the third party has referred to the corrosive property of the substance.

ECHA acknowledges that – as specified in the general part of Annexes VII-X – "*in vivo* testing with corrosive substances at concentration/dose levels causing corrosivity shall be avoided". The test methods for repeated dose toxicity and reproductive toxicity specify that the highest dose level should induce "toxicity but not death or severe suffering". Therefore, it is the Registrant's responsibility to ensure that appropriate dose/exposure levels are used in the requested studies.

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-2-microglobulin nephropathy.

4. Pre-natal developmental toxicity study (Annex IX, Section 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 rabbits 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 rabbits. He did not specify the route for testing. 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).

IV. Adequate identification of the composition of the tested material

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. If the registration of the substance covers different grades, the sample used for the new studies must be suitable to assess these.

Furthermore, 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.

V. General requirements for the generation of information and Good Laboratory Practice

ECHA reminds registrants of the requirements of Article 13(4) of the REACH Regulation that ecotoxicological and toxicological tests and analyses shall be carried out in compliance with the principles of good laboratory practice (GLP).

According to Article 13(3) of the REACH Regulation, tests that are required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the European Chemicals Agency as being appropriate. Thus, the Registrant shall refer to Commission Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 as adapted to technical progress or to other international test methods recognised as being appropriate and use the applicable test methods to generate the information on the endpoints indicated above.

VI. 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://echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[1] by Leena Ylä-Mononen, Director of Evaluation

[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.