ECHA
EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114311133-70-01/F Helsinki, 09 December 2015

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

For p-tert-butylphenyl 1-(2,3-epoxy)propyl ether, CAS No 3101-60-8 (EC No 221-453-2), 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 p-tert-butylphenyl 1-(2,3-epoxy)propyl ether, CAS No 3101-60-8 (EC No 221-453-2), submitted by [redacted] (Registrant).

- 90-day oral toxicity study (OECD 408) in rat.
- In vivo alkaline single-cell gel electrophoresis assay for DNA strand breaks (Comet assay) in rat by oral route. In addition, the frequency of micronuclei in the rat bone marrow will be assessed.
- Developmental toxicity / teratogenicity study (OECD 414).
- Two-generation reproduction toxicity study (OECD 416).

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 6 June 2015, 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 15 March 2013.

ECHA held a third party consultation for the testing proposals from 17 June 2014 until 2 August 2014. ECHA received information from third parties (see section III below).

On 30 March 2015 ECHA sent the draft decision to the Registrant and invited him to provide comments within 30 days of the receipt of the draft decision.

By 6 June 2015 the Registrant did not provide any comments on the draft decision to ECHA.

On 23 July 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 28 August 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 amended the draft decision.

On 7 September 2015 ECHA referred the draft decision to the Member State Committee.

By 28 September 2015 the Registrant did not provide any comments on the proposals for amendment.

A unanimous agreement of the Member State Committee on the draft decision was reached on 13 October 2015 in a written procedure launched on 1 October 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. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26/OECD 408) in rats;
2. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method: OECD 489) in rats, oral route, on the following tissues: liver and either glandular stomach or duodenum/jejunum;
3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31/OECD 414) in rats or rabbits, oral route.

while the originally proposed test(s) for a Two-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: EU B.35/OECD 416) in rats, oral route proposed to be carried out using the registered substance is rejected pursuant to Article 40(3)(d) of the REACH Regulation.

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 request(s) in this decision, or to fulfil otherwise the information requirement(s) 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 **17 December 2018** 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.

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.

A. Tests required pursuant to Article 40(3)

1. 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 rats via the oral route (EU B.26/OECD 408).

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.6.2. of the REACH Regulation. The proposed route is the most appropriate route of administration having regard to the likely route of human exposure due to the following reasons.

In light of the physico-chemical properties of the substance (liquid with low vapour pressure classified as irritating to the skin 2 and skin sensitising 1) and the information provided on the uses and human exposure i.e., no uses with spray application, ECHA considers that testing by the oral route is most appropriate.

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.

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 [p-tert-butylphenyl 1-(2,3-epoxy)propyl ether]: Sub-chronic toxicity study (90-day) in rats, oral route (test method: EU B.26/OECD 408).

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".

The technical dossier contains an *in vitro* Ames GLP study equivalent to OECD 471 that shows a positive result (just above the biological significance threshold) in one strain, TA 100 ([redacted]). Another newer ([redacted]) GLP and test guideline Ames test result shows negative test results. The bacterial mutagenicity data show that there is no clear concern for gene mutation. ECHA also notes that the *in vitro* mammalian chromosome aberration test according to OECD 473 with the registered substance shows positive test result: *"The test substance induced a statistically significant increase of the percent CHO cells with structural chromosome aberrations without liver derived S9 fraction metabolic activation. These data suggest that the test substance is an ultimate clastogen not requiring mammalian metabolism to be genotoxic"*.

An appropriate *in vivo* genotoxicity study to follow up the concern on chromosomal aberrations 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.

ECHA notes that this test is an appropriate test to investigate further effects on chromosomal aberrations *in vitro* as described in the ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.1. and figure R.7.7-1 (February 2014).

ECHA notes that paragraph 42 of the draft OECD 489 test guideline states "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". Therefore ECHA considers that the comet assay should be performed in liver and either in glandular stomach or duodenum/jejunum.

The Registrant proposed to extend the comet assay by including additional examinations; *"the frequency of micronuclei in the rat bone marrow will be accessed to evaluate chromosome damaging potential".* The Registrant may consider integrating an *in vivo* micronucleus test (OECD 474) in the proposed *in vivo* comet assay. 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.

The Registrant proposed testing in rats by the oral route. In line with test method OECD 489, ECHA considers that testing in the rat by the oral route is appropriate.

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 (Annex IX, Section 8.4., column 2; test method: OECD 489) in rats, oral route, on the following tissues: liver and either glandular stomach or duodenum/jejunum). ECHA notes, that it is at the Registrant's discretion to integrate an in vivo micronucleus test (OECD 474) in the proposed *in vivo* comet assay (i.e. to assess in the testing program the frequency of micronuclei in the rat bone marrow) and use the results to ensure the safe use of the substance.

Notes 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".

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

The Registrant is reminded that the comet assay test guideline OECD 489 gives the possibility for it to be integrated with the 90 day repeated dose toxicty study (OECD 408 or 413) (see point 7 of the guideline). In order to ensure that the generated data will be acceptable to cover the data requirement for the *in vivo* mammalian alkaline comet assay the issues referred to below need to be considered by the Registrant in case he decides to combine the two studies:

- The maximum tolerated dose (MTD) in the 90-day sub-chronic toxicity study may be lower than the MTD in a standard (2-day) comet assay. Negative results for a comet assay obtained in a combined sub-chronic /comet assay study where the maximum tolerated dose is significantly lower than would be expected in a comet assay performed as stand-alone study may lead to an underestimation of the potential of the test substance to cause genotoxicity and thus be considered inadequate to fulfil the information requirement of Annex IX, Section 8.4., column 2.
- The age of the animals and the corresponding historical controls: the laboratory performing the study should have historical control data for the comet assay 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 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 hours after the last treatment (see OECD 489, paragraph 33).

3. 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 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) 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.

The third party has indicated *"The studies do not need to be conducted if: the substance is known to be a genotoxic carcinogen and appropriate risk management measures are implemented, or the substance is known to be a germ cell mutagen and appropriate risk management measures are implemented, ...Thus it is recommended to start testing with the in vivo mammalian alkaline Comet assay which will be combined with the mammalian bone marrow chromosome aberration test (Test Guideline 475) according to the registrant's proposal"*.

ECHA notes that it is the Registrant's responsibility to consider and justify in the registration dossier any adaptation of the information requirements in accordance with the REACH Regulation. The adaptation according to Annex IX, Section 8.7., column 2, second indent of the REACH Regulation that the third party refers to specifies that in case the substance is known to be a germ cell mutagen (which correspond to a classification as germ cell mutagen category 1A or 1B) or a genotoxic carcinogen (carcinogenic categoy 1A or 1B) and appropriate risk management measures are implemented, the pre-natal developmental toxicity study does not need to be conducted. However, ECHA notes 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.

The third party also referred to *"registration data of the structurally related substance 2,3-epoxypropyl phenyl ether (EC No. 204-557-2, CAS No. 122-60-1, dossier submitted by* [redacted]*. An inhalation developmental toxicity study which meets generally accepted scientific principles has been published by* [redacted]*. Exposure to 1.7 ± 0.2 ppm, 5.7 ± 0.6 ppm or 11.5 ± 3.0 ppm did not result in signs of developmental or maternal toxicity"*.

ECHA again notes that it is the Registrant's responsibility to consider and justify any adaptation of the information requirements. With regard to the reference to a read-across, the Registrant should therefore assess whether he can justify a read-across in accordance with the relevant conditions as established in Annex XI, Section 1.5 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 1.5. in the registration dossier. 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. Therefore, the information provided by the third party in itself would not be sufficient to adapt the standard information requirement.

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 [p-tert-butylphenyl 1-(2,3-epoxy)propyl ether]: Pre-natal developmental toxicity study in rats or rabbits, oral route (test method: EU B.31/OECD 414).

d) Notes for consideration by the Registrant

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. Annex IX, Section 8.7.2., column 2 provides that the decision on the need to perform a pre-natal developmental toxicity study at a tonnage level of 100 to 1000 tonnes per annum on a second species should be based on the outcome of the first test and all other relevant and available data.

4. Two-generation reproductive toxicity study (Annex IX, Section 8.7.3.)

a) Examination of the testing proposal

Pursuant to Article 40(3)(d) of the REACH Regulation, ECHA may reject a proposed test.

The Registrant has submitted a testing proposal for a two-generation reproductive toxicity study according to [EU B.35/OECD 416] with the following justification: *"Two-Generation Reproduction Toxicity Study will be conducted in the rat by the oral route only as needed. Study conduct is dependent upon the outcome of the subchronic 90-day oral study in the rat. If the reproductive study is needed, it will be conducted according to O.E.C.D. test guideline 416 with GLP compliance.include justification".*

ECHA notes that the two-generation reproductive toxicity study originally foreseen in Annex IX, Section 8.7.3 has been replaced with the extended one-generation reproductive toxicity study through Regulation (EU) 2015/282. According to Annex IX, Section 8.7.3. as amended, an extended one-generation reproductive toxicity study is an information requirement, if adverse effects on reproductive organs or tissues are indicated in a 28-day or 90-day repeated dose toxicity study or if these studies reveal other concerns in relation with reproductive toxicity.

ECHA notes that there is no 28-day or 90-day repeated dose toxicity study available in the registration dossier, while the Registrant has proposed to perform a 90-day study.

ECHA notes further that the Registrant has not included any justification why he proposes to perform a two-generation reproductive toxicity study at tonnage level 100 – 1000 tonnes per year. ECHA considers that neither the proposed study nor an extended one-generation reproductive toxicity study is at this stage necessary to fulfil the information requirement of Annex IX, Section 8.7.3. of the REACH Regulation, because no repeated dose toxicity study is currently available to evaluate if performance of such a study is required at this tonnage level.

b) Consideration of the information received during third party consultation

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

A third party has indicated that the tonnage level of the registered substance only requires the conduct of a two-generation reproduction toxicity study if the 28-day or 90-day study indicates adverse effects on reproductive organs or tissues.

As already stated under section III.4.a) above, ECHA notes that the two-generation reproductive toxicity study originally foreseen in Annex IX, Section 8.7.3 has been replaced with the extended one-generation reproductive toxicity study through Regulation (EU) 2015/282. According to Annex IX, Section 8.7.3. as amended, an extended one-generation reproductive toxicity study is an information requirement, if adverse effects on reproductive organs or tissues are indicated in a 28-day or 90-day repeated dose toxicity study or if these studies reveal other concerns in relation with reproductive toxicity. For the substance subject to the present decision there is no 28-day or 90-day repeated dose toxicity study available in the registration dossier.

Therefore, ECHA has rejected the testing proposal for a two-generation reproductive toxicity study.

c) Outcome

ECHA concludes that there is at this stage no information gap for the standard information requirement of Annex IX, Section 8.7.3. Therefore, pursuant to Article 40(3)(d) of the REACH Regulation, the proposed test for a two-generation reproduction toxicity study (OECD 416) is rejected.

IV. 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 has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

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

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.

V. 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[1] by Guilhem de Seze, Head of Unit, Evaluation E1

[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.