EUROPEAN CHEMICALS AGENCY

Helsinki, 22 November 2017

Addressee: [redacted]

Decision number: CCH-D-2114375574-40-01/F
Substance name: ISOBUTYL VINYL ETHER
EC number: 203-678-8
CAS number: 109-53-5
Registration number: [redacted]
Submission number: [redacted]
Submission date: 08-04-2016
Registered tonnage band: [redacted]

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 using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102;**

2. ***In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490)*** **with the registered substance provided that the study requested under 1 has negative results;**

3. **Robust study summary (RSS) for key study, [redacted].Biodegradation in water: screening tests (2004) according to OECD TG 310 and GLP. Biodegradation in water; screening tests (Annex VII, Section 9.2.1.1 in conjunction with Annex 1, Section 3.1.5)**

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 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 and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **29 November 2018**. You shall also 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. 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, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Authorised[1] by Ofelia Bercaru, Head of Unit, Evaluation E3

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

Appendix 1: Reasons

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

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at [redacted] per year shall contain as a minimum the information specified in Annexes VII to X of 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.

According to Article 13(3) of the REACH Regulation, tests required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods recognised by the Commission or ECHA.

Other tests may be used if the conditions of Annex XI are met. More specifically, Section 1.1.2 of Annex XI provides that existing data on human health properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3) may be used if the following conditions are met:

(1) Adequacy for the purpose of classification and labelling and/or risk assessment;
(2) Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3);
(3) Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3) if exposure duration is a relevant parameter; and
(4) Adequate and reliable documentation of the study is provided.

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.

You have provided a test from the year 1989 according to OECD TG 471 and GLP with an assigned reliability score of 2. The test used four different strains of *S. typhimurium* TA 1535, TA 1537, 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.

In your comments on the draft decision, you request a deadline extension to 12 months. ECHA has amended the draft decision by setting the deadline to 12 months.

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.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to complete 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) using one of the following strains: *E. coli WP2* uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.

2. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at [redacted] per year shall contain as a minimum the information specified in Annexes VII to X of 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 mammalian cells" is an information requirement as laid down in Annex VIII, Section 8.4.3. of the REACH Regulation, "if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2." is obtained. ECHA notes that the registration dossier contains negative results for both of these latter information requirements. Therefore, adequate information on *in vitro* gene mutation in mammalian cells needs to be present in the technical dossier for the registered substance to meet this information requirement, provided that the study data for the bacterial reverse mutation assay (Annex VII, Section 8.4.1) requested under 1 also has negative results.

In the technical dossier you have provided a study record for an *in vitro* HPRT gene mutation assay ([redacted], 1993). However, Article 13(3) of the REACH Regulation requires that tests be conducted in accordance with the tests methods laid down in Regulation 440/2008 or with other international test methods recognized by the Commission or the Agency as being appropriate, while this study has not been conducted in accordance with OECD TG 476 (ver. 1984). Specifically, ECHA observes the following limitations in this study:

i. In the Robust Study Summary (RSS), you indicate that the test was conducted according to OECD TG 476. ECHA notes that the OECD TG 476 (ver. 1984) recommends that "the highest concentration should produce a low level of survival". In experiment 1 of the present study, in the absence of S9, no apparent cytotoxicity was observed at the highest tested concentration, and in the presence of S9 no high cytotoxicity was observed either. When the level of cytotoxicity in the highest concentration is compared to the one of the vehicle control no substantial increase is observed. In contrast, in the 2nd experiment, the test article was toxic at the same highest tested concentration. Therefore, ECHA concludes that the study has not been conducted in accordance with the OECD TG 476 and that the results of the study are inconsistent.

ii. The mutation rates reported for a certain number of sporadic concentrations and vehicle control in both experiments of the study are zero either in the presence or absence of S9. ECHA understands that the spontaneous mutant frequency for these concentrations and the vehicle control was equal to zero. ECHA notes that CHO cells used for the HPRT tests should have a stable spontaneous mutant frequency. For this reason the study design of the gene mutation assay requires a large bulk population of cells to be maintained in such a way that a defined range of frequency of spontaneous mutants may be anticipated at the time of treatment. This spontaneous or negative control mutant frequency is crucial to ensure the stability of the test system. Overall, ECHA considers that stable spontaneous mutant frequency was not attained in the test system of the study.

Therefore, ECHA concludes that the study does not meet the requirements of Article 13(3) of the REACH Regulation and, so, it does not provide the information required by Annex VIII, Section 8.4.3.

As explained 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 *in vitro* mammalian cell gene mutation tests using the Hprt and xprt test genes (OECD TG 476) and the *in vitro* mammalian cell gene mutation tests using the thymidine kinase gene (OECD TG 490) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.3.

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: *In vitro* mammalian cell gene mutation test (test method: OECD TG 476 or OECD TG 490) provided that the study requested under point 1 above has negative results.

In your comments on the draft decision, you request a deadline extension to 12 months. ECHA has amended the draft decision by setting the deadline to 12 months.

3. Robust study summary (RSS) for key study, [redacted].Biodegradation in water: screening tests (2004) according to OECD TG 310 and GLP. Biodegradation in water; screening tests (Annex VII, Section 9.2.1.1 in conjunction with Annex 1, Section 3.1.5)

Pursuant to Articles 10(a) and 12(1)(e) of the REACH Regulation, a technical dossier registered at [redacted] per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Pursuant to Articles 10(a)(vii) of the REACH Regulation, the information set out in Annex VII to XI must be provided in the form of robust study summary, if required under Annex I. Article 3(28) defines a robust study summary as a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report. Guidance on the preparation of the robust study summaries is provided in the ECHA Practical Guide 3: 'How to report robust study summaries'.

"A Ready biodegradability test" is a standard information requirement as laid down in Annex VII, Section 9.2.1.1 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. Furthermore, pursuant to Article 10 (a)(vii) and Annex I, Section 3.1.5. if there are several studies addressing the same effect, then, the study or studies giving rise to the highest concern shall be used to draw the conclusion and a robust study summary shall be prepared for that study or studies and included as part of the technical dossier. Robust summaries will be required of all key data used in the hazard assessment.

In the technical dossier you have provided a study record for a biodegradation in water: screening test (Key, reliability 1, GLP, [redacted] (2004), test method: Ready biodegradability – CO2 in sealed vessels (headspace test), "*Guideline study (ISO standard)*" equivalent or similar to OECD TG 310) with the registered substance, to meet the standard information requirement of Annex VII, Section 9.2.1.1.

According to OECD TG 310 (2014), paragraph 66, a test is valid when: (a) the mean percentage degradation in vessels F_C containing the reference substance is >60% by the 14th day of incubation; and (b) the mean amount of TIC present in the blank controls F_B at the end of the test is <3mg C/L. You have not indicated in the technical dossier whether these validity criteria have been fulfilled, i.e. you have not populated the "Validity criteria fulfilled" in the relevant IUCLID field under this endpoint. The results you have provided show a degradation of 88% TIC/ThIC at day 14 for the reference substance, but no information was provided by you on the second criteria listed above. The mean amount of total inorganic carbon (TIC) present in the blank controls at the end of the test is not in the technical dossier. Therefore, ECHA cannot verify whether the validity criteria have been fulfilled for this study.

According to OECD TG 310, paragraph 11, this test method is applicable to volatile substance with a Henry's law constant of up to 50 $Pa \cdot m^3 \cdot mol^{-1}$ when the recommended headspace to liquid volume ratio of 1:2 is used, as the proportion of test substance in the headspace will not exceed 1%. For substances that are more volatile, a smaller headspace volume may be used. The registered substance has a reported Henry's law constant of 1248 $Pa \cdot m^3 \cdot mol^{-1}$ at 20°C, thus it has a high potential to be lost from solution by volatilisation. However, it appears from the dossiers that no allowances have been made for the high volatility of the substance in order to minimise volatile losses. The registered substance has been tested using a headspace to liquid volume ratio of about 1:2 (60 mL to 100 mL), although OECD 310 suggests to use a smaller headspace. Given how it was designed, the test is not applicable to the highly volatile registered substance, thus there is no level of confidence in the results on ready biodegradability.

ECHA also notes that you have reported contradicting information on the biodegradation of the test substance. Table "Kinetic of test substance" shows that in the inhibition controls and in the vessels containing the reference substance, the percentage biodegradation increased consistently with time. However, the percentage biodegradation of the test substance did not reflect a constant pattern: it increased up to 79% at day 17, then it went down to 40% at day 21 and finally reached 63% at day 28. You have not provided a scientific explanation on why the biodegradation of the registered substance alone did not increase consistently with time until reaching a plateau phase. ECHA considers that this undermines the reliability of the test results on ready biodegradability.

According to the reported test results, the substance has been assessed as readily biodegradable (TOC removal: 60-70% after 28-d) but failing the 10-d window. However, the limitations listed above undermine the reliability of the test results for the registered substance. In addition, according to OECD TG 310 (2014), paragraph 68, a substance is readily biodegradable when biodegradation > 60% ThIC is reached within the 10-d window. As the 10-d window has not been met in the test, the substance cannot be assessed as readily biodegradable. ECHA notes contradictions in your interpretation of this test result. For C&L, you self-classified the substance as Aquatic Chronic 3, using acute aquatic results and considering the substance as non-rapidly biodegradable, thus assuming worst-case scenario. However, in the PBT assessment and in the risk assessment, you considered the substance as readily biodegradable. Therefore, clarifications are needed on the interpretation of the results and the consequences in the CSA if the substance cannot be considered readily biodegradable.

Furthermore, ECHA notes that in the study summary, information is missing on the analytical method used to measure the degradation. Paragraph 50 of OECD TG 310 (2014) clearly indicates the possibility to use two recommended methods to measure the amount of IC produced in the test. However, you have not reported nor described in the technical dossier the analytical method used in the test.

Therefore, ECHA notes that, contrary to Article 3 (28) of the REACH Regulation the documentation of this study is insufficient and does not allow an independent assessment of the adequacy of this study, its results and its use for hazard assessment. In particular, the following elements are missing:

a. Specific chemical analytical data, if available;
b. The graph of percentage degradation against time for the test and reference substances to include lag phase, degradation phase, the 10-d window and slope percentage removal at plateau, at end of test, and/or after 10-d window;
c. Discussion of results and explanation of the deviations;
d. Detailed information on the validity criteria specified in OECD TG 310 (2014);
e. Information on environmental conditions such as inoculum adaptation and pH;
f. Information on how the volatility of the registered substance was taken into account in the design, calculations and expression of the result of the test and whether OECD TG 310 (2014) was considered applicable.

In addition, the outcome of the ready biodegradability test was used to adapt the information requirements for simulation testing in surface water, soil and sediment (Annex IX Sections 9.2.1.2, 9.2.1.3 and 9.2.1.4); these adaptations are thus dependent on the validity of the ready biodegradability test. However, as currently the validity of the ready biodegradability test cannot be established, you may have to re-assess these other information requirements in light of the requested complete robust study summary.

In your comments on the draft decision you have provided as an attachment additional information regarding the ready biodegradability study "[redacted]*Biodegradation in water: screening tests*", whose robust study summary (RSS) was provided in the technical dossier (IUCLID section 5.2.1) submission number: [redacted], submitted 08-04-2016, used to prepare the draft decision. In addition, you have indicated your intention to revise Section 5.2.1 of your IUCLID dossier addressing the information requirement in an update of the registration. ECHA will examine such information in the updated dossier only after the deadline set in the adopted decision has passed and all the information requested in this decision has been submitted.

However, regarding your submitted additional information provided in the comments on the draft decision, ECHA considers that this information is not sufficient to verify the results of the ready biodegradability test for the following reasons:

ECHA acknowledges that the study has been performed with ISO guideline 14593 (1999), which is considered equivalent to the standard OECD TG 310 (2014), as given in Appendix R.7.9-1 of *ECHA's Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017)*. ECHA notes that in your comments on the draft decision you have provided the mean amount of total inorganic carbon (TIC) present in the blank controls at the end of the test, which is 1.6 mg C/L. Based on this information and on the value for the degradation of the reference substance provided in the RSS (88% TIC/ThIC at day 14), ECHA agrees with your statement in the comments on the draft decision that the validity criteria of OECD TG 310 (2014) par. 66 and ISO guideline 14593 par. 11 have been fulfilled for this study.

In addition, ECHA acknowledges that in your comments on the draft decision you have provided detailed information on the analytical method used and on environmental conditions (pH, inoculum adaptation), as requested in the draft decision.

Furthermore, in response to your comments on the draft decision concerning C&L and PBT assessment, ECHA acknowledges that, based on the results of this study, the substance has been adequately considered as "*non rapidly degradable*" for the purposes of C&L and as "*readily biodegradable without fulfilling the 10-day window criterion*" for the purposes of the CSA including PBT Assessment.

However, ECHA notes that there is still some level of uncertainty regarding the reliability of the results, since you have not provided specific chemical analytical data nor the graph of percentage degradation against time for the test substance.

In response to your comments on the draft decision concerning the headspace and liquid ratio, ECHA agrees that "*this ratio is crucial in providing enough oxygen to the activated sludge over the full testing period of 28 days.*" However, ECHA does not agree with your statement in your comments on the draft decision that "*according to OECD 310, paragraph 11, a smaller ratio may be used when the bioavailability may be limited due to limited low water solubility.*" ECHA considers that what this paragraph suggests is to use a smaller headspace volume in order to reduce losses of volatile substances because high volatility may lower the bioavailability especially of poorly water soluble substances that tend not to stay in solution. The registered substance is water soluble (720 mg/L) but has also a high vapour pressure (89.7 hPA at 20°C).

Hence, losses due to volatilisation cannot be totally excluded and may thus have an influence on the test outcome. Furthermore, regarding the percentage biodegradation of the test substance, ECHA notes that it did not reflect a constant pattern: it increased up to 79% at day 17, then it went down to 40% at day 21 and finally reached 63% at day 28. You have not provided a scientific explanation on why the biodegradation of the registered substance alone did not increase consistently with time until reaching a plateau phase. However, you do state in your comments on the draft decision regarding the variation of the test results that "*this variation is only slightly*". ECHA does not agree with this statement, since the degradation percentage is almost reduced by half from day 17 (75%) to day 21 (40%) and it increases again at day 28 (63%). While ECHA agrees with your statement in your comments on the draft decision that "*the controls (curve of the reference substance and of the inhibitory control) are all within range*", the variability of the results in the test vessels may undermine the reliability of this study.

While ECHA agrees that the ISO guideline 14593 (1999) can potentially be used to fulfil the current information requirement, in this case the volatility of the test substance may undermine the reliability of this study since it was conducted without applying measures to reduce losses, as indicated in the more recent OECD TG 310 (2014). Therefore, ECHA concludes that in your comments on the draft decision you have not adequately addressed whether the results may be influenced by losses of the test substance due to volatilisation and the variability of the results. ECHA considers that the latter undermines the reliability of the test results on ready biodegradability.

Therefore, ECHA considers that the following aspects have not been sufficiently addressed in your comments: the influence of the volatility of the test substance on the test results, as well the variation of the test results at the different sampling points that might undermine the reliability of the test results.

In order to allow an independent assessment of the study submitted, pursuant to Article 41(1) and (3) of the REACH Regulation you are requested to provide a complete robust study summary with the above missing elements for this study.

Deadline to submit the requested information in this decision

In the draft decision communicated to you, the time indicated to provide and submit the requested information to ECHA in a dossier update was 6 months from the date of adoption of the decision. In your comments on the draft decision, you requested an extension of this timeline as you consider the current 6 month timeline will be highly challenging and most probably not possible. ECHA acknowledges due to an administarive error, 6 months was indicated instead of the standard deadline of 12 months. Based on the registrant's comments on the draft decsion, ECHA has granted the request and set the deadline for providing the requested information and to submit it to ECHA in a dossier update to 12 months from the date of adoption of the decision.

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 30 September 2016.

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.

ECHA took into account your comments and amended the deadline.

In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the request(s).

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

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s).

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-55 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

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 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 your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new test(s) 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 test(s) 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 test(s) 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 test(s) to be assessed.