

Helsinki, 30 March 2017

Addressee: [REDACTED]

Decision number: CCH-D-2114355521-55-01/F

Substance name: (E)-anethole

EC number: 224-052-0

CAS number: 4180-23-8

Registration number: [REDACTED]

Submission number: [REDACTED]

Submission date: 11.03.2016

Registered tonnage band: 100-1000T

### **DECISION ON A COMPLIANCE CHECK**

Based on Article 41 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA requests you to submit information on

- 1. *In vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, EU B.13/14. / OECD TG 471) with the registered substance;**
- 2. *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or OECD TG 490) with the registered substance, provided that the study requested under 1. has a negative result;**
- 3. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: Daphnia magna reproduction test, EU C.20./OECD TG 211) with the registered substance;**
- 4. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) with the registered substance;**

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 **8 October 2018**. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

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<sup>1</sup> by Claudio Carlon, Head of Unit, Evaluation E2

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<sup>1</sup> 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 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX 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 reference to a publication from the year 1995 similar to OECD TG 471 and non-GLP with an assigned reliability score of 2. The test used two different strains of *S. typhimurium* TA 1535 and TA 100 and it did not include tests with strains *S. typhimurium* TA 1537, TA 98, TA102 or *E. coli* WP2 *uvrA* or *E. coli* WP2 *uvrA* (pKM101). You have also provided references to two publications from the years 1979 and 1982 both assigned with a reliability score of 4 with the strains *S. typhimurium* TA 1535, TA 98 and TA 100 included.

The study from 1979 has a positive result and the study from 1982 a negative result. However, since the tests were conducted, significant changes has 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 studies do not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

ECHA concludes that a test using at least five strains of bacteria including *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.

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

In your comments on the draft decision you put forward a question whether there is a need to classify the substance in case the Bacterial reverse mutation test (Annex VII, Section 8.4.1) gives a positive result and in that event whether the *in vitro* gene mutation study (Annex VIII, Section 8.4.3) should be performed anyway. You also asked whether the Bacterial reverse mutation test could be avoided by performing directly the *in vitro* gene mutation study in mammalian cells. ECHA's responses are as follows: if the Bacterial reverse mutation test (Annex VII, Section 8.4.1) gives a positive result, the *in vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3) does not need to be performed, but the performance of an appropriate *in vivo* test (i.e. TGR assay or comet assay) shall be considered. The need to classify a substance for mutagenicity is usually assessed only when *in vivo* mutagenicity data are available. Finally, the performance of the Bacterial reverse mutation test (Annex VII, Section 8.4.1) cannot be avoided by performing directly the *in vitro* gene mutation study in mammalian cells (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: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471).

## **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 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX 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 a negative result for the information requirement *in vitro* mammalian chromosome aberration test (test method: OECD TG 473) corresponding to Annex VIII, Section 8.4.2. 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 requested under 1. (Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471) has a negative result.

ECHA considers that the *in vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* 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 1. has a negative result.

### **3. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)**

"Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex IX, Section 9.1.5. 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.

You have sought to adapt this information requirement. You provided the following justification for the adaptation: "Data waiving: *study scientifically not necessary / other information available*" and "Justification for data waiving: *Chemical Safety assessment does not indicate the need to investigate further the effects on aquatic organisms*".

While you have not explicitly claimed an adaptation, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex IX, Section 9.1.5., column 2.

However, ECHA notes that your adaptation does not meet the specific rules for adaptation of Annex IX, Section 9.1.5., column 2 because you have not correctly taken into account the results of the short-term toxicity test to aquatic invertebrates in the Chemical Safety Assessment (CSA).

In fact, ECHA notes that the  $PNEC_{\text{water}}$  used in the risk assessment has been calculated starting from the LC50 value for short-term toxicity to aquatic invertebrates (this being the lowest LC50) and applying an assessment factor (AF) of 1000. ECHA notes that as prescribed by the ECHA Guidance, R.10 - Characterisation of dose-response for the environment (May 2008), Section R.10.3.1.2., the EC50 value should be used for invertebrates. ECHA notes when using the EC50 value for the short-term toxicity to aquatic invertebrates (4.25 mg/l), with the AF of 1000, the new PNEC leads to RCRs for freshwater above 1 for several uses, i.e. Section 9.1.1.3 Manufacture; Section 9.3.1.3 Formulation; Section 9.4.1.3 Industrial end-use of washing and cleaning products; as well as Section 10.2.2 Local exposure due to all wide dispersive uses. Thus, the CSA indicates a concern for the environment that needs to be followed up with long-term testing.

Therefore, your adaptation of the information requirement cannot be accepted.

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.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 3.0, February 2016) *Daphnia magna* reproduction test (test method EU C.20. / OECD TG 211) is the preferred test to cover the standard information requirement of Annex IX, Section 9.1.5.

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: *Daphnia magna* reproduction test (test method: EU C.20./OECD TG 211).

#### *Notes for your consideration*

According to ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, February 2016), Chapter R7b (Section R.7.8.5., including Figure R.7.8-4) if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both. In such case, according to the integrated testing strategy, the *Daphnia* study is to be conducted first. If based on the results of the long-term *Daphnia* study and the application of a relevant assessment factor, no risks are observed (PEC/PNEC<1), no long-term fish testing may need to be conducted. However, if a risk is indicated, the long-term fish study needs to be conducted.

#### **4. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)**

“Long-term toxicity testing on fish” is a standard information requirement as laid down in Annex IX, Section 9.1.6. of the REACH Regulation. Adequate information on Fish, early-life stage (FELS) toxicity test (Annex IX, 9.1.6.1.), or Fish, short-term toxicity test on embryo and sac-fry stages (Annex IX, 9.1.6.2.), or Fish, juvenile growth test (Annex IX, 9.1.6.3.) needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement by providing the same justification for adaptation as for the long-term toxicity to aquatic invertebrates endpoint. Hence, for the same reasons and considerations outlined under the request for long-term toxicity to aquatic invertebrates, your adaptation of the information requirement cannot be accepted.

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.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 3.0, February 2016) fish early-life stage (FELS) toxicity test (test method OECD TG 210), fish short-term toxicity test on embryo and sac-fry stages (test method EU C.15. / OECD TG 212) and fish juvenile growth test (test method EU C.14. / OECD TG 215) are the preferred tests to cover the standard information requirement of Annex IX, Section 9.1.6.

However, the FELS toxicity test according to OECD TG 210 is more sensitive than the fish, short-term toxicity test on embryo and sac-fry stages (test method EU C.15 / OECD TG 212), or the fish, juvenile growth test (test method EU C.14. / OECD TG 215), as it covers several life stages of the fish from the newly fertilized egg, through hatch to early stages of growth (see ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, February 2016), *Chapter R7b, Figure R.7.8-4*).

Moreover, the FELS toxicity test is preferable for examining the potential toxic effects of substances which are expected to cause effects over a longer exposure period, or which require a longer exposure period of time to reach steady state (ECHA *Guidance Chapter R7b*, version 3.0, February 2016).

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: Fish, early-life stage (FELS) toxicity test (test method: OECD TG 210).

*Notes for your consideration*

Before conducting any of the tests mentioned above in points 3-4 you shall consult the ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, February 2016), Chapter R7b, Section R.7.8.5 to determine the sequence in which the aquatic long-term toxicity tests are to be conducted and the necessity to conduct long-term toxicity testing on fish.

According to ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, February 2016), Chapter R7b (Section R.7.8.5., including Figure R.7.8-4), if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both. In such case, according to the integrated testing strategy, the *Daphnia* study is to be conducted first. If based on the results of the long-term *Daphnia* study and the application of a relevant assessment factor, no risks are observed (PEC/PNEC<1), no long-term fish testing may need to be conducted. However, if a risk is indicated, the long-term fish study needs to be conducted.

## **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 28 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 did not amend the request(s).

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

As no amendments were proposed, ECHA took the decision according to Article 51(3) 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.