EUROPEAN CHEMICALS AGENCY

Helsinki, 10 July 2018

Addressee: [redacted]

Decision number: CCH-D-2114436058-50-01/F
Substance name: Cinnamaldehyde
EC number: 203-213-9
CAS number: 104-55-2
Registration number: [redacted]
Submission number: [redacted]
Submission date: 07/08/2017
Registered tonnage band: 100-1000

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]

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* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or *in vitro* micronucleus study (Annex VIII, Section 8.4.2., test method: OECD TG 487) with the registered substance;**

3. ***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 both studies requested under 1. and 2. have negative results;**

4. **Screening study for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: OECD TG 421 or 422) in rats, oral route with the registered substance;**

5. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rat or rabbit), oral route with the registered substance;**

6. **Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: DOC die-away test, OECD TG 301A) or**

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: CO2 evolution test, OECD TG 301B) or

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: MITI test

[1] No testing for endpoints listed in Annexes IX or X to the REACH Regulation may be started or performed at this moment: A decision only becomes legally effective and binding for you after it has been adopted according to Article 51 of the REACH Regulation. ECHA will take the decision either after the date it has become clear that Member State competent authorities have not made any proposals to amend the draft decision or, where proposals to amend it have been made, after the date the Member State Committee reached a unanimous agreement on the draft decision.

(I), OECD TG 301C) or

Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Closed bottle test, OECD TG 301D) or

Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Modified OECD screening test, OECD TG 301E) or

Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Manometric respirometry test, OECD TG 301F) or

Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Ready biodegradability – CO2 in sealed vessels (headspace test), OECD TG 310) with the registered substance

7. **Identification of PNEC and risk characterisation (Annex I, Section 3.3.1. and 6.): revise PNECs and revise the risk characterisation by recalculating the RCRs for freshwater, marine water, intermittent releases (if applicable), microorganisms in sewage treatment plants, freshwater sediment, marine sediment and soil:**
- using the study giving rise to the highest concern according to Annex I, Section 3.1.5 and revise the risk characterisation accordingly *or* provide a full justification for not using the study giving rise to the highest concern;
- using the default assessment factors and other recommendations of ECHA Guidance R.10 and revise the risk characterisation accordingly *or* provide a detailed justification for not using the recommendations of ECHA Guidance R.10 for PNEC derivation;

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 to the REACH Regulation. 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 adequate and reliable documentation.

You have to submit the requested information in an updated registration dossier by **17 January 2020**. You also have to 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 and 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, has to 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[2] by **Claudio Carlon**, Head of Unit, Evaluation **E2**

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

Appendix 1: Reasons

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

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 the following study records:

i. A test from the year 1982, no test guideline followed and not GLP compliant, with an assigned reliability score of 2. The test used only one strain: *E. coli* WP2 uvrA.
ii. Another test from the year 1982, no test guideline followed and not GLP compliant, 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).
iii. A test from the year 1986, no test guideline followed and not GLP compliant, 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).
iv. A test from the year 1998 (publication data), no test guideline followed and not GLP compliant, with an assigned reliability score of 2. The test used five different strains of *S. typhimurium* TA 1538, TA 97a, TA 98, TA 100 and TA 104, and it also included the test with strain *S. typhimurium* TA 102; and
v. A review paper published in 2005: "*A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients*". In the review of assays there is reference to the study mentioned above under (i.).

ECHA notes that none of the above studies follow test guidelines and they are not GLP compliant. In studies (i.) to (iii.) all the required strains were tested (in different tests) and they all resulted negative for mutagenicity. However, with reference to study (iv.) where all the required strains, including the fifth strain, were tested, a weakly positive response was produced in the strain *S. typhimurium* TA 100. From the available study records provided in the technical dossiers it seems that this weakly positive result was not followed up. ECHA also notes that no adequate and reliable documentation has been provided for the study records hence the validity of the studies cannot be assessed. Therefore, the provided study records do not meet the current guidelines, nor can they be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

In your comment on the draft decision you refer to the "*multiple in vitro-based bacterial gene mutation studies*" which were provided in the technical dossier. ECHA notes that, as mentioned above, the study records do not provide information equivalent to the data generated by the corresponding test method (OECD TG 471) because you fail to provide adequate and reliable documentation. More specifically, in your comments you mention "*the review of Bickers et al. (2005)*". ECHA notes that the study records provided for this particular endpoint, in particular the Bicker's review, have limited information. In your comments you claim that the Bickers' review includes the "*results of 15 published Ames tests performed with cinnamaldehyde [...] (including e.g. Mortelmans, 1986 and Sekizawa, 1992 that used only 4 Salmonella strains)*" and you also provide some further details. ECHA notes that in the current study record you only indicate that in the review (2005) "*the results of 6 published bacterial mutagenicity and genotoxicity tests [...] on cinnamaldehyde were shown. Mutation assays [...] were negative for cinnamaldehyde (Sekizawa and Shibamoto, 1982; Yoo, 1986)*" however, you fail to provide further details on the various studies referred to in this review. Hence, you fail to address the issue raised by ECHA where you provided study records with "*no adequate and reliable documentation*". ECHA notes that a robust study summary is required under Article 10(a)(vii). Hence, ECHA considers that the information provided in the endpoint study records do not meet the requirements of a robust study summary[3], as defined in Article 3(28).

Moreover, as already noted, in study (iv.) above there was a weakly positive result in the strain *S. typhimurium* TA 100, which was not further investigated. In your comments you refer to "*some weakly positive to positive results [...] reported for cinnamaldehyde in S. typhimurium strain TA100 using the pre-incubation method [...] However, the majority of similar studies in strain TA100, including a recent study using a prolonged pre-incubation time (120 min), [...] did not find any evidence of mutagenicity in strain TA100*". ECHA notes that it is not clear which is the "*recent study*" referred in your comments. The most *recent in vitro* gene mutation study in bacterial cells study available in the current dossier is from 1998 and it is the one with the positive result for *S. typhimurium* TA 100.

You also state that "*the presented in vitro-based bacterial gene mutation studies are also superseded by available data presented in several in vivo-based gene mutation studies*". The *in vivo* genetic toxicity study records provided in the technical dossier to further justify the weight of evidence adaptation have a number of shortcomings as highlighted in sections 2 and 3 of the decision. Moreover, ECHA notes that the *in vivo* study records provided do not address gene mutation. Hence, the studies are not relevant for the assessment of the standard information requirement according to Annex VII Section 8.4.1.

[3] ECHA's practical guide for "*How to report robust study summaries*", available at: http://echa.europa.eu/documents/10162/13643/pg_report_robust_study_summaries_en.pdf.

In your comments you also state that "*Additional testing of E.coli WP2 or S. typhimurium TA102 is not considered necessary*" since "*Cinnamaldehyde has no oxidizing or cross-linking activity and is not a hydrazine derivative*". ECHA notes that according to the OECD TG 471 at least five strains of bacteria should be used, including *E.coli* WP2 strains or *S. typhimurium* TA102. As stated above, the information provided for this endpoint does not meet the standard information requirement. Finally, ECHA notes that on the basis of the provided information in the technical dossier, you cannot conclude that the information is "*reliable and sufficient*" since as discussed above, you failed to provide adequate and reliable documentation of the study records.

Following the referral of the draft decision to the Member States Competent Authority (MSCAs) ECHA received a proposal for amendment (PfA) indicating that the registered substance has already been part of an in-depth genotoxicity evaluation by the EFSA Panel on the group of cinnamyl derivatives (EFSA, 2009)[4]. According to the EFSA Panel, based on the available data, it was concluded that the registered substance should not be regarded as genotoxic. Moreover, according to the MSCA, there is no new data in the technical dossier, compared to the Panel's data set that would change this conclusion. It was also indicated that ECHA did not consider the studies as being valid because they are non-GLP and non-guidelines. The MSCA asked ECHA to reconsider this request taking into account the EFSA Panel's opinion so as to also ensure consistency between the different European agencies with regard to scientific conclusions on the toxic effects of a substance.

In your comments on the PfA you agreed with the MSCA and requested ECHA to remove this request because of the number of available studies available and due to the EFSA Panel's conclusion. However, with reference to your comments on the PfA, ECHA notes the following:

(i.) EFSA provided an opinion on the evaluation of flavouring substances using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The information required (Article 3), format of the information (Annex IV), and the nature of the assessment of that information as part of a risk assessment under Commission Regulation (EC) No 1565/2000 are markedly different from under the REACH regulation. During compliance check, ECHA must ensure that information is present (in a specific format, a (robust) study summary) in the registration dossier that corresponds to specific information requirements (Annex VII, 8.4.1; Annex VIII, 8.4.2 and 8.4.3), and so it is entirely possible that EFSA and ECHA would come to different actions as a result of the different tasks they perform.

(ii.) The non-GLP and non-test guideline studies were not considered as invalid because they were non-GLP and non-guideline studies. Rather studies which are non-GLP and non-guideline must meet the requirements of Annex XI, 1.1.2 (as set out above), and for many of these studies, you did not provide robust study summaries.

(iii.) More specifically, as already indicated above, for the endpoint study records provided in the technical dossier, "*no adequate and reliable documentation has been provided for the study records hence the validity of the studies cannot be assessed.*" Hence, ECHA notes that there is insufficient information in the technical dossier to make an independent assessment of the studies.

In conclusion, the issues (i.) to (iii.) above are not adequately addressed in your comments on the MSCA's PfA, and as your technical dossier addresses none of the issues above, it is

[4] EFSA (2009) Scientific opinion: Flavouring Group Evaluation 214:alpha,beta-Unsaturated aldehydes and precursors from chemical subgroup 3.1of FGE.19: Cinnamyl derivatives. Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Adopted on 27 November 2008. The EFSA Journal (2009) 880, 1-27. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/880.pdf

not possible to conclude whether the information submitted in the proposal for amendment and supported by your comments allows this end-point to be fulfilled.

Therefore, currently 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.

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* cytogenicity study in mammalian cells or *in vitro* micronucleus study (Annex VIII, Section 8.4.2.)

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* cytogenicity study in mammalian cells or an *in vitro* micronucleus study" is a standard information requirement as laid down in Annex VIII, Section 8.4.2. 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.

a) Information provided

You have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this provision.

You indicate that "*by applying weight of evidence approach it can be concluded that cinnamaldehyde has no relevant mutagenic potential in vitro and in vivo".* In the IUCLID endpoint summary for genetic toxicity, you only provided the following information as a justification for the weight of evidence adaptation:

"*Chromosomal aberrations in Chinese hamster ovary (CHO) cells were not induced at concentrations up to and including 100 µg/ml, both with and without metabolic activation (Galloway et al., 1987). Tests for the induction of sister chromatid exchange (SCE) in CHO cells produced negative results at low concentrations and weakly positive results at concentrations approaching cytotoxic levels (Galloway et al., 1987).*

[...]A Mouse Peripheral Blood Micronucleus Test was performed in the course of a subchronic feeding study with cinnamaldehyde initiated by NTP (US Department of Health, 2004 and Hooth et al., 2004) [...] The frequency of micronucleated erythrocytes was not enhanced at any dose. As result, dietary concentrations of 4100 to 33000 ppm of trans-cinnamaldehyde administered by feeding for 3 months did not increase the frequency of micronucleated erythrocytes in the peripheral blood of male or female B6C3F1 mice (US Department of Health, 2004 and Hooth et al., 2004).

[...] No increase in the number of micronucleated erythrocytes was seen in these tests for cinnamaldehyde (Hayashi, 1988).

A Mouse micronucleus test was conducted to evaluate the antimutagenic effects of cinnamaldehyde in vivo in X-ray irradiated male ddYmice [...] X-ray-induced chromosome aberrations were suppressed when cinnamaldehyde was given orally to mice after X-ray irradiation. The frequency of micronuclei was depressed about 55-60%.

In a review of Bickers et al. (2005) micronucleus assay results of different tissues after oral treatment with cinnamaldehyde were reported (Mereto et al., 1994; Martelli et al., 1993) [...] The frequency of micronuclei in polychromatic erythrocytes was not increased when rats or mice were given up to 1100mg/kg bw or 1700mg/kg bw, respectively, of cinnamaldehyde by oral gavage. However, a dose dependent increase of micronucleated hepatocytes was observed in mice (850 and 1700mg/kg bw) [...] Bickers et al interpret the results as 'The induction of micronuclei in hepatocytes and forestomach mucosal cells most likely relates to the method of dosing with cinnamaldehyde. Positive findings were detected in these tissues following gavage administration of large bolus doses of the reactive aldehyde with high exposure to the stomach and liver. These same doses did not cause an increased frequency of micronuclei in erythrocytes presumably because of the first pass extraction and metabolism of cinnamaldehyde by intestinal and hepatic tissue. Induction of micronuclei was dose-dependent and demonstrated a threshold. At highly exaggerated doses cinnamaldehyde would affect cellular defense mechanisms (i.e. glutathione), which could explain the threshold phenomenon and dose dependency that were observed. The authors (Mereto et al., 1994) acknowledged these facts and concluded that the data did not justify classification of cinnamaldehyde as clastogenic for gastric mucosa.'

[...] Cinnamaldehyde failed to induce UDS in rats, thus, the test item was shown not to induce DNA damage after exposure to oral doses of up to and including 1000 mg/kg bw."

To support your weight of evidence adaptation for the *in vitro* cytogenicity endpoint, you have provided the following sources of information with the registered substance:

i. "*Chromosome Aberrations and Sister Chromatid Exchanges in Chinese Hamster Ovary Cells: Evaluations of 108 Chemicals*" (Galloway *et al.*, 1987). Publication. No test guideline followed. Non-GLP. Reliability 2.
ii. "*A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients*" (Bickers *et al.*, 2005). [Review of two sister chromatic exchange tests and four chromosomal aberration test] Publication. No test guideline followed. Non-GLP. Reliability 2.

Moreover, you provided the following sources of individual information with the registered substance, concerning *in vivo* genetic toxicity, to further justify the weight of evidence adaptation:

iii. "*NTP Technical Report on the Toxicology and Carcinogenesis: Studies of Trans-Cinnamaldehyde (Microencapsulated) (CAS No. 14371-10-9) In F344/N Rats and B6C3F1 Mice (Feed Studies)*" (U.S. Department Of Health And Human Services, 2004). [Mouse Peripheral Blood Micronucleus Test following administration of trans-cinnamaldehyde in feed for 3 months] Publication. No test guideline followed. Non-GLP. Reliability 2.
iv. "*Micronucleus Tests in Mice on 39 Food Additives and Eight Miscellaneous Chemicals*" (Hayashi *et al.*, 1988). Publication. No test guideline followed. Non-GLP. Reliability 2.
v. "*Suppressing effects of vanillin, cinnamaldehyde, and anisaldehyde on chromosome aberrations induced by X-rays in mice*" (Sasaki *et al.*, 1990). [Mouse bone marrow micronucleus test] Publication. No test guideline followed. Non-GLP. Reliability 2.
vi. "*A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and*

cinnamic acid when used as fragrance ingredients" (Bickers *et al.*, 2005). [Review of micronucleus assay in bone marrow and liver] Publication. No test guideline followed. Non-GLP. Reliability 2.

vii. "*Measurement of Unscheduled DNA Synthesis and S-Phase Synthesis in rodent hepatocytes following in vivo treatment: testing of 24 compounds*" (Mirsalis et al., 1998). [*in vivo* liver UDS test: DNA damage and/or repair] Publication. No test guideline followed. Non-GLP. Reliability 2.

viii. "*Chemical Mutagenesis Testing in Drosophila. V. Results of 53 Coded Compounds Tested for the National Toxicology Program*" (Woodruff *et al.*, 1985). [*in vivo* insect germ cell study: gene mutation & Drosophila sex-linked recessive lethal (SLRL) assay] Publication. No test guideline followed. Non-GLP. Reliability 2.

b) ECHA's evaluation and conclusion of the information provided

Evaluation approach/criteria

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property with respect to the information requirement in question including an adequate and reliable documentation while the information from each single source alone is regarded insufficient to support this notion.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance with respect to the information requirement of Annex VIII, Section 8.4.2. for an *in vitro* cytogenicity study in mammalian cells. ECHA examined whether the set of information presented addresses the properties of the substance by covering, as a minimum, the most relevant elements investigated in the *in vitro* mammalian chromosome aberration test (test method OECD TG 473) or the *in vitro* mammalian cell micronucleus test (OECD TG 487).

All the study records provided are non-GLP and do not follow any test guidelines. ECHA notes that a robust study summary[5] is required under Article 10(a)(vii), and ECHA considers that the information provided in the endpoint study records do not meet the requirements of a robust study summary, as defined in Article 3(28). Limited information has been provided for each study record. Hence, ECHA cannot fully assess the data provided for this endpoint.

ECHA also notes that Annex XI, section 1.1.2., provides that test data from experiments not carried out according to GLP shall be considered equivalent to data generated in accordance with the relevant test methods referred to in Article 13(3) REACH if the four conditions set out in Annex XI, section 1.1.2. are met.

More specifically, in the endpoint study record for study (i.) above, the chromosome aberration assay reported in the publication does not provide adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods OECD TG 473 and/or OECD TG 487 (Annex XI, Section 1.1.2.(2)). The exposure duration with metabolic activation lasted only two hours while for the treated groups without the metabolic activation there was only a long-term exposure (8-12 hours). According to OECD TG 473, a short-term treatment (3 to 6 hours) should be conducted for treated groups with and without metabolic activation; and cells without metabolic activation should also be continuously exposed until sampling at a time equivalent 1.5 normal cell

[5] ECHA's Practical Guide 3: "*How to report robust study summaries*", (Version 2.0, November 2012), http://echa.europa.eu/documents/10162/13643/pg_report_robust_study_summaries_en.pdf

cycle lengths. Hence, in the groups with the metabolic activation there was a shorter exposure duration while in the cells without metabolic activation there was no short treatment and the continuous treatment. Additionally only 100 cells were evaluated; according to OECD TG 473 "*at least 300 well-spread metaphases should be scored per concentration and control*" and "*should be equally divided among the replicates, when replicate cultures are used. When single cultures are used per concentration*". The number of cells can be reduced if there is a high number of cells that show chromosome aberrations (a clear positive result), however in the study provided this was not the case.

Furthermore, ECHA notes that study record (i.) fails to provide adequate and reliable documentation (Annex XI, Section 1.1.2.(4)); in particular, tabulated data in the results' section from the different trials performed is missing, along with other information, such as number of replicates used and comparison with the historical control data.

With reference to study record (ii.) above, ECHA notes that there is no information on the study design, including missing information on the number of and values of test concentrations used. Hence the data provided in study record (ii.) is not considered as being adequate and reliable (Annex XI, Section 1.1.2.(4)).

As an additional point, ECHA notes that, according to ECHA's guidance document[6], the sister chromatid exchange assays referred to in study records (i.) and (ii.) do not provide the information required by Annex VIII, Section 8.4.2., because the *in vitro* DNA damage and repair study provides only an indication of induced damage to DNA (but not direct evidence of mutation) via effects of sister chromatid exchange (SCE). As a consequence, these SCE assays fail to provide adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods OECD TG 473 and/or OECD TG 487 (Annex XI, Section 1.1.2.(2)).

In the technical dossier you have also provided six study records (studies (iii.) to (viii.) above) under the *in vivo* genotoxicity endpoint.

ECHA notes that the *in vivo* micronucleus study records (iii.) to (v.) above, do not provide adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test method OECD TG 474 (Annex XI, Section 1.1.2. (2)). According to OECD TG 474, "*at least 4000 immature erythrocytes per animal should be scored for the incidence of micronucleated immature erythrocytes*". However, in these study records the frequency of micronuclei was only observed in 1000 polychromatic erythrocytes (PCEs).

Moreover for study record (iv.), ECHA notes that the route of administration used is intraperitoneal. According to OECD TG 474, "*intraperitoneal injection is generally not recommended since it is not an intended route of human exposure, and should only be used with specific scientific justification*". ECHA notes that you fail to provide a justification on the chosen route for administrating the test substance.

Additionally for studies (iv.) and (v) there is no information on the criteria for dose selection. In the study summaries there is no explanation why the highest dose used was only 500 mg/kg bw and there is no data on whether a range-finding study has been performed.

With reference to the study record (vi.) above, ECHA notes that it fails to provide adequate and reliable documentation (Annex XI, Section 1.1.2.(4)). The study record is a review of the following studies with the registered substance:

[6] According to ECHA's *Guidance document on Information Requirements and Chemical Safety Assessment, (Chapter R.7a: Endpoint specific guidance* (version 6.0, July 2017), p551.

1. Cinnamaldehyde-induced micronuclei in rodent liver (Mereto *et al.*, 1994); and
2. Evaluation of the carcinogenic potential of cinnamaldehyde in a battery of *in vivo* short-term tests (Martelli *et al.*, 1993).

However, you fail to provide adequate information on each study, such as information on study design, the number of animals tested. Hence, ECHA cannot fully assess the review provided by Bickers *et al.* (2005).

As regards study record (vii.) above, according to ECHA's guidance document[7], the liver unscheduled DNA synthesis (UDS) assay provides only an indication of induced DNA damage followed by DNA repair (but not direct evidence of mutation). Hence, the UDS assay fails to provide adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods OECD TG 473 and/or OECD TG 487 (Annex XI, Section 1.1.2.(2)).

The other study record used in the weight of evidence adaptation (study (viii.) above) does not provide information on *in vitro* cytogenicity in mammalian cells. Hence, it is not relevant for the assessment of this standard information requirement as per Annex VIII, Section 8.4.2.

Conclusion

ECHA considers that the individual lines of evidence you provided are not sufficient on their own to fulfil the information requirement for an *in vitro* cytogenicity endpoint. For those studies with deficiencies in documentation, ECHA is unable to independently assess the individual sources of information, and subsequently to examine in what way the individual studies may together form an adequate weight of evidence. ECHA considers that these individual lines of evidence taken together and with your justification for the adaptation do not provide sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that the registered substance, has or has not a particular dangerous property, with respect to the information requirement stated in Annex VIII, Section 8.4.2.

Hence, the sources of information you provided, together with your justification for the adaptation, do not allow to assume/conclude on the dangerous (hazardous) property of the registered substance with respect to the information requirement for Annex VIII, Section 8.4.2.

Therefore, the general rules for adaptation laid down in Annex XI, Section 1.2. of the REACH Regulation are not met and your adaptation of the information requirement is rejected.

In your comments to the draft decision you state that the "*presented in vitro-based gene mutation studies are superseded by available data presented in several in vivo-based gene mutation studies included in the current dossier.*" ECHA notes that the study records provided in the technical dossier have a number of shortcomings, as highlighted above under this section. Specifically, the *in vivo* micronucleus study records do not provide adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test method OECD TG 474 (Annex XI, Section 1.1.2. (2)), while the liver unscheduled DNA synthesis (UDS) assay provides only an indication of induced DNA damage followed by DNA repair (but not direct evidence of mutation).

ECHA also notes that, as already explained above, the various study records provided under

[7] *Guidance document on Information Requirements and Chemical Safety Assessment, (Chapter R.7a: Endpoint specific guidance* (version 6.0, July 2017), p 551, 558.

this specific endpoint (including the *in vitro* and *in vivo* studies), when taken together and with the justification provided for adaptation, do not provide sufficient weight of evidence.

Following the referral of the draft decision to the Member States Competent Authority (MSCAs) ECHA received a proposal for amendment (PfA) indicating that the registered substance has already been part of an in-depth genotoxicity evaluation by the EFSA Panel on the group of cinnamyl derivatives (EFSA, 2009)[8]. According to the EFSA Panel, based on the available data, it was concluded that the registered substance should not be regarded as genotoxic. Moreover, according to the MSCA, there is no new data in the technical dossier, compared to the Panel's data set that would change this conclusion. It was also indicated that ECHA did not consider the studies as being valid because they are non-GLP and non-guidelines. The MSCA asked ECHA to reconsider this request taking into account the EFSA Panel's opinion so as to also ensure consistency between the different European agencies with regard to scientific conclusions on the toxic effects of a substance.

In your comments on the PfA you agreed with the MSCA and requested ECHA to remove this request because of the number of available studies available and due to the EFSA Panel's conclusion. Furthermore, once again, you indicated that there are already available supporting *in vivo* studies which have confirmed that the substance is "*not genetically toxic substance*". However, with reference to your comments on the PfA, ECHA notes the following:

(i.) EFSA provided an opinion on the evaluation of flavouring substances using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The information required (Article 3), format of the information (Annex IV), and the nature of the assessment of that information under Commission Regulation (EC) No 1565/2000 are markedly different from under the REACH regulation. During compliance check, ECHA must ensure that information is present (in a specific format, a (robust) study summary) in the registration dossier that corresponds to specific information requirements (Annex VII, 8.4.1; Annex VIII, 8.4.2 and 8.4.3), and so it is entirely possible that EFSA and ECHA would come to different actions as a result of the different tasks they perform.

(ii.) The non-GLP and non-test guideline studies were not considered as invalid because they were non-GLP and non-guideline studies. Rather studies which are non-GLP and non-guideline must meet the requirements of Annex XI, 1.1.2, and for many of these studies, you did not provide robust study summaries.

(iii.) More specifically, according to the Bickers *et al.* review (2005) provided in the technical dossier: "*Cinnamaldehyde was reported to induce chromosome aberrations at low concentrations (i.e., <15lg/ml) in Chinese hamster fibroblasts and B241 cells tested with and without metabolic activation (Ishidate et al., 1984; Kasamaki et al., 1982, 1987; Kasamaki and Urasawa, 1983, 1985; JECFA, 2000).* As already indicated above in this section, you failed to provide information on the different studies performed, hence ECHA could not assess the validity of the studies.

(iv.) With reference to the negative *in vivo* intraperitoneal micronucleus test of Hayashi *et al.* (1984 and 1988), ECHA notes that in the technical dossier you only provided the 1988 study. As explained above, ECHA considers that this study fails to provide adequate and reliable coverage of key parameters (sufficient cells counted) or justification for the use of the route (i.p.).

[8] EFSA (2009) Scientific opinion: Flavouring Group Evaluation 214:alpha,beta-Unsaturated aldehydes and precursors from chemical subgroup 3.1of FGE.19: Cinnamyl derivatives. Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Adopted on 27 November 2008. The EFSA Journal (2009) 880, 1-27. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/880.pdf

In conclusion, the issues (i.) to (iv.) above are not adequately addressed in your comments on the MSCA's PfA, and as your technical dossier addresses none of the issues above, it is not possible to conclude whether the information submitted in the proposal for amendment and supported by your comments allows this end-point to be fulfilled.

Therefore, currently 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 chromosome aberration test (test method OECD TG 473) and the *in vitro* mammalian cell micronucleus test (OECD TG 487) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.2. of the REACH Regulation.

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 chromosome aberration test (test method: OECD TG 473) or *in vitro* mammalian cell micronucleus study (test method: OECD TG 487).

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to 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.

a) Information provided

You have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this provision.

You indicate that "*by applying weight of evidence approach it can be concluded that cinnamaldehyde has no relevant mutagenic potential in vitro and in vivo*". In the IUCLID endpoint summary for genetic toxicity, you only provided the following information as a justification for the weight of evidence adaptation:

"[...] *Tests for the induction of gene mutations in Chinese hamster V79 cells (HPRT Test; Fiorino and Bronzetti, 1994) exposed to cinnamaldehyde produced negative results. [...] Cinnamaldehyde failed to induce UDS in rats, thus, the test item was shown not to induce DNA damage after exposure to oral doses of up to and including 1000 mg/kg bw*".

To support your weight of evidence adaptation for the *in vitro* gene mutation in mammalian cells endpoint, you have provided the following sources of information with the registered substance:

i. "*Effects of cinnamaldehyde on survival and formation of HGPRT- mutants in V79 cells treated with methyl methanesulfonate, N-nitroso-N-methylurea, ethyl methanesulfonate and UV light*" (Fiorio and Bronzetti, 1994). [In vitro mammalian cell

gene mutation assay - Target gene HGPRT] Publication. No test guideline followed. Non-GLP. Reliability 2.

ii. *"p53 induction as a genotoxic test for twenty-five chemicals undergoing in vivo carcinogenicity testing"* (Duerksen-Hughes *et al.*, 1999). Publication. No test guideline followed. Non GLP. Reliability 2.

Moreover, you provided the study records (iii.) to (viii.) referred to under Appendix 1, section 2, of this decision, for *in vivo* genetic toxicity with the registered substance, to further justify the weight of evidence adaptation.

b) ECHA's evaluation and conclusion of the information provided

Evaluation approach/criteria

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property with respect to the information requirement in question including an adequate and reliable documentation while the information from each single source alone is regarded insufficient to support this notion.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance with respect to the information requirement of Annex VIII, Section 8.4.3. for an *in vitro* gene mutation study in mammalian cells. ECHA examined whether the set of information presented addresses the properties of the substance by covering, as a minimum, the most relevant elements investigated in 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).

ECHA notes that Annex XI, section 1.1.2., provides that test data from experiments not carried out according to GLP shall be considered equivalent to data generated in accordance with the relevant test methods referred to in Article 13(3) REACH if the four conditions set out in Annex XI, section 1.1.2. are met.

Study record (i.) provided in the dossier does not provide adequate and reliable coverage of key parameters foreseen to be investigated in the corresponding OECD test guidelines 476 and 490 (Annex XI, Section 1.1.2.(2)), since the number of test concentrations used in the study is less than four (only one test concentration was tested). Moreover, this study fails to provide adequate and reliable documentation as information related to metabolic activation analysis is not present in the dossier (Annex XI, Section 1.1.2.(4)). Hence, the conditions set out for allowing ECHA to consider the data as equivalent to data generated by the corresponding test method referred to in Article 13(3) are not met.

As regards study record (ii.), ECHA notes that the p53-induction is an *in vitro* assay for genotoxicity based on the ability of cells to increase their level of the tumor-suppressor protein p53 in response to DNA damage and is used to predict carcinogenicity in rodents, however it does not provide direct evidence of mutation. As a consequence, this assay fails to provide adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods OECD TG 476 and/or OECD TG 490 (Annex XI, Section 1.1.2.(2)).

ECHA notes that the shortcomings of the six study records provided for the *in vivo* genotoxicity endpoint have already been addressed under Appendix 1, section 2, of this decision.

Conclusion

ECHA considers that the individual lines of evidence you provided are not sufficient on their own to fulfil the information requirement for an *in vitro* gene mutation in mammalian cells endpoint. For those studies with deficiencies in documentation, ECHA is unable to independently assess the individual sources of information, and subsequently to examine in what way the individual studies may together form an adequate weight of evidence. ECHA considers that these individual lines of evidence taken together and with your justification for the adaptation do not provide sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that the registered substance, has or has not a particular dangerous property, with respect to the information requirement stated in Annex VIII, Section 8.4.2.

Hence, the sources of information you provided, together with your justification for the adaptation, do not allow to assume/conclude on the dangerous (hazardous) property of the registered substance with respect to the information requirement for Annex VIII, Section 8.4.3.

Therefore, the general rules for adaptation laid down in Annex XI, Section 1.2. of the REACH Regulation are not met and your adaptation of the information requirement is rejected.

In your comments on the draft decision you again refer to the "*several in vivo-based gene mutation studies included in the current dossier*"; you also provide the details of the individual study records, which were already available in the technical dossier. ECHA notes that the *in vivo* micronucleus study records provided in the dossier do not address gene mutation (but chromosome aberration) while the liver unscheduled DNA synthesis (UDS) assay provides only an indication of induced DNA damage followed by DNA repair (but not direct evidence of mutation). Hence, none of the i*n vivo* study records provided in the dossier are relevant for the assessment of this standard information requirement as per Annex VIII Section 8.4.3. Additionally, there are a number of shortcomings in the individual study records.

As already explained above, the various study records provided, including the *in vitro* and *in vivo* studies, under this specific endpoint, when taken together and with the justification provided for adaptation do not provide sufficient weight of evidence. As a final note ECHA notes that in your comments on the draft decision you state that if "*insisted*" you agree to perform the test.

Following the referral of the draft decision to the Member States Competent Authority (MSCAs) ECHA received a proposal for amendment (PfA) indicating that the registered substance has already been part of an in-depth genotoxicity evaluation by the EFSA Panel on the group of cinnamyl derivatives (EFSA, 2009)[9]. According to the EFSA Panel, based on the available data, it was concluded that the registered substance should not be regarded as genotoxic. Moreover, according to the MSCA, there is no new data in the technical dossier, compared to the Panel's data set that would change this conclusion. It was also indicated that ECHA did not consider the studies as being valid because they are non-GLP and non-guidelines. The MSCA asked ECHA to reconsider this request taking into account the EFSA

[9] EFSA (2009) Scientific opinion: Flavouring Group Evaluation 214:alpha,beta-Unsaturated aldehydes and precursors from chemical subgroup 3.1of FGE.19: Cinnamyl derivatives. Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Adopted on 27 November 2008. The EFSA Journal (2009) 880, 1-27. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/880.pdf

Panel's opinion so as to also ensure consistency between the different European agencies with regard to scientific conclusions on the toxic effects of a substance.

In your comments on the PfA you agreed with the MSCA and requested ECHA to remove this request because of the number of available studies available and due to the EFSA Panel's conclusion. Furthermore, once again, you indicated that there are already available supporting *in vivo* studies which have confirmed that the substance is "*not genetically toxic substance*". However, with reference to your comments on the PfA, ECHA notes the following:

(i.) EFSA provided an opinion on the evaluation of flavouring substances using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The information required (Article 3), format of the information (Annex IV), and the nature of the assessment of that information under Commission Regulation (EC) No 1565/2000 are markedly different from under the REACH regulation. During compliance check, ECHA must ensure that information is present (in a specific format, a (robust) study summary) in the registration dossier that corresponds to specific information requirements (Annex VII, 8.4.1; Annex VIII, 8.4.2 and 8.4.3), and so it is entirely possible that EFSA and ECHA would come to different actions as a result of the different tasks they perform.

(ii.) The non-GLP and non-test guideline studies were not considered as invalid because they were non-GLP and non-guideline studies. Rather studies which are non-GLP and non-guideline must meet the requirements of Annex XI, 1.1.2, and for many of these studies, you did not provide robust study summaries.

(iii.) More specifically, with reference to the *in vitro* gene mutation study in mammalian cells (Fiorio and Bronzetti, 1994) provided in the technical dossier, as explained above this study cannot be considered as a valid study according to Annex XI, 1.1.2. Also, according to the EFSA Scientific opinion (2009), this study has been considered as being invalid.

(iv.) With reference to the comment on already available supporting *in vivo* studies, as indicated above, ECHA notes that none of the i*n vivo* study records provided in the dossier are relevant for the assessment of this standard information requirement as per Annex VIII Section 8.4.3. Although there is an *in vivo* UDS study present in the dossier, ECHA's Guidance[10] states that a negative result in a UDS assay alone is not a proof that a substance does not induce gene mutation, and so this *in vivo* study does not provide an adaptation for the lack of an *in vitro* gene mutation study.

In conclusion, the issues (i.) to (iv.) above are not adequately addressed in your comments on the MSCA's PfA, and as your technical dossier addresses none of the issues above, it is not possible to conclude whether the information submitted in the proposal for amendment and supported by your comments allows this end-point to be fulfilled.

Therefore, currently 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* 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

10 ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance, Section R.7.7.6.3, Version 6.0

present decision: *In vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490) provided that both studies requested under 1. and 2. have negative results.

4. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

"Screening for reproductive/developmental toxicity" (test method OECD TG 421 or 422) is a standard information requirement as laid down in Annex VIII, Section 8.7.1. of the REACH Regulation if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from *in vitro* methods that the substance may be a developmental toxicant. No such evidence is presented in the dossier. Therefore, adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have not provided any study record of a screening for reproductive/developmental toxicity in the dossier that would meet the information requirement of Annex VIII, Section 8.7.1.

c) Information provided

You have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this provision.

You have provided the following weight of evidence adaptation with respect to reproductive toxicity:

"As confirmed by literature[11] in rodents histopathological examinations in repeated dose toxicity studies of reproductive tissues are of high value and high sensitivity for evaluation of reproductive toxicity in males and females. Histopathological changes in reproductive and/or endocrine tissues/organs observed in repeated dose toxicity studies are indicative of effects on fertility. With this respect repeated dose toxicity studies should be considered as sensitive and sufficient information to evaluate toxicity on fertility can be obtained if histopathological examination of the reproductive organs is covered. The available information on reproductive parameters for the substance are subchronic and chronic repeated dose toxicity studies on rats and mice (NTP, 2004) that give no indication of adverse effects on organs/tissues of the male and female reproductive and endocrine system at any dose level.

Moreover, the available toxicity studies of the substance show that cinnamaldehyde in general has a low systemic toxicity potential, i.e. NOAEL in chronic studies on rats and mice

[11] *Mangelsdorf. et al., 2003: Some aspects relating to the evaluation of the effects of chemicals on male fertility. Regulatory toxicology and Pharmacology 37, 356-369; Ulbrich & Palmer, 1995: Detection of effects on male reproduction – a literature survey. J Am. College of Toxicology 14, 293-327; Janer et al., 2007: A retrospective analysis of the added value of the rat two-generation reproductive toxicity study versus the rat subchronic toxicity study. Reproductive Toxicology 24, 103-113; Dent, 2007: Strength and limitations of using repeated dose toxicity studies to predict effects on fertility. Regulatory Toxicology and Pharmacology 48, 241-258; Sanbuissho et al., 2009: Collaborative work on evaluation of ovarian toxicity by repeated dose and fertility studies in female rats. J Tox. Sci. 34: Special Issue SP1-SP22*

≥ 100 mg/kg bw, NOAEL in subchronic studies on rats and mice ≥ 275 mg/kg bw, LD50 oral and dermal > 2000 mg/kg bw, no genotoxic potential in vivo and no relevant potential for developmental toxicity.

Cinnamaldehyde is rapidly absorbed and almost completely eliminated within 24 hours. Cinnamaldehyde has no relevant bioaccumulation potential. Cinnamaldehyde is a skin and mucosal membrane irritant and thus, local effects as nonneoplastic forestomach lesions (inflammati on, hyperplasia) and olfactory degeneration in the nasal cavity (mice) are the most sensitive adverse effects after repeated oral dosing. Due to reduced palatability also body weight gain reduction is seen at higher doses. With regard to repeated dermal exposure the moderate skin sensitizing activity of cinnamaldehyde is expected to be the leading health effect.

Taken together, although studies on fertility, respectively multi-generation studies are not available for the substance further testing is considered to be of low priority. In accordance to REACH Annex XI, 1.2., there is sufficient weight of evidence to conclude that the substance is not a reproductive toxicant, and further testing on vertebrate animals for that endpoint shall be omitted."

To support your weight of evidence adaptation you have provided the following sources of information:

i. Two-year carcinogenicity study in rats, oral, (GLP compliant) with trans-cinnamaldehyde (CAS No. 14371-10- 9) [registered substance contains >97% of trans-cinnamaldehyde], NTP, 2014, (study report), rel. 2
ii. Two-year carcinogenicity study in mice, oral, (GLP compliant) with trans-cinnamaldehyde (CAS No. 14371-10- 9) [registered substance contains >97% of trans-cinnamaldehyde], NTP, 2014, (study report), rel. 2
iii. Sub-chronic 14-week toxicity study in rats, oral, (GLP compliant) with trans-cinnamaldehyde (CAS No. 14371-10- 9) [registered substance contains >97% of trans-cinnamaldehyde], NTP, 2014, (study report), rel. 2
iv. Sub-chronic 14-week toxicity study in mice, oral, (GLP compliant) with trans-cinnamaldehyde (CAS No. 14371-10- 9) [registered substance contains >97% of trans-cinnamaldehyde], NTP, 2014, (study report), rel. 2
v. Evaluation of 60 Chemicals in a Preliminary Developmental Toxicity Test in mice, oral (gavage) route (no test guideline; non-GLP) with the registered substance (Hardin *et al.*, 1987), rel. 2.
vi. Pre-Natal (Segment II) Toxicity Study of Cinnamic Aldehyde in the Sprague-Dawley Rats, oral (gavage) route (no test guideline followed; non-GLP) with the registered substance (Mantovani *et al.*, 1989), rel. 2.
vii. Oestrogenic activity test in rats, oral, (no test guideline followed; non-GLP) with the registered substance, (Bernhard *et al.*, 1938), rel. 2

Other studies under Toxicity to Reproduction section:

viii. Combined Repeated, repro and developmental toxicity study in 1 generation of *A. Obtectus insects,* (no test guideline; non-GLP) with the registered substance (Regnault-Roger and Hamraoui, 1995), rel. 2.
ix. Molecular structure - teratogenicity relationships of some fragrance additives, (no test guideline; non-GLP) with the registered substance, (Abramovici and Rachmuth-Roizman, 1983), rel. 4.

d) ECHA's evaluation and conclusion of the information provided

Evaluation approach/criteria

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property with respect to the information requirement in question including an adequate and reliable documentation while the information from each single source alone is regarded insufficient to support this notion.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance with respect to a screening study (OECD TG 421/422). Relevant elements are in particular exposure route, duration and levels, investigations of the effects on male and female reproductive performance, histopathological information on reproductive organs, initial information on the offspring and additional parameters for endocrine disrupting modes of action.

Evaluation of the provided information

In the technical dossier, under this endpoint, you have provided two chronic studies (studies i. and ii. above) and two sub-chronic studies (studies iii. and iv. above). However, ECHA notes that these studies do not provide the information as required by Annex VIII, Section 8.7.1., because these studies provide only an indication on reproductive effects. Key elements, including the effects of the test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition, are missing.

ECHA notes that studies v. to ix. above are not performed according to GLP and do not follow any test guidelines. Annex XI, section 1.1.2. provides that test data from experiments not carried out according to GLP shall be considered equivalent to data generated in accordance with the relevant test methods referred to in Article 13(3) REACH if the conditions set out in Annex XI , section 1.1.2. are met. The second condition requires that there are adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3) REACH. The third condition requires an exposure duration comparable to or longer than the exposure duration than the corresponding test methods referred to in Article 13(3).

In the assessment of each individual source of information you have provided, ECHA has found that in regard of quality and relevance, the studies have a number of shortcomings.

ECHA notes that both pre-natal developmental toxicity studies in mice (study v.) and rats (study vi.) do not provide adequate and reliable coverage of key parameters foreseen to be investigated in the corresponding OECD test guideline 421 or 422, since they do not include information on mating or post-natal effects.

Following a proposal for amendment (PfA) submitted by one of the Member States it was also noted that in both studies (v. and vi.) there was a short exposure duration: gestation days 6 to 13 in the mouse study and days 7 to 17 in the rat study. In the mouse study (v.) only two doses were tested while in the rat study (vi.) only 14 to 16 pregnant females were tested. ECHA notes that the maternal exposure should at least last from implantation to one or two days before the expected delivery of both rodent species. In addition, according to OECD TG 414: "*At least three dose levels and a concurrent control should be used*" and each group "*should contain a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy. Groups with fewer than 16 animals with implantation sites may be inappropriate.*" Consequently, both studies fail to meet the second and third conditions set out in Annex XI, Section 1.1.2. since they do not provide adequate and reliable coverage of key parameters foreseen to be investigated in the

corresponding OECD test guideline 414 and the exposure duration is shorter than the exposure duration in the corresponding test methods referred to in Article 13(3). Hence, the pre-natal developmental study records provided in the technical dossier are both incompliant in respect of the information requirement of Annex IX, 8.7.2 (pre-natal developmental toxicity). Since these studies are incompliant in respect of the pre-natal developmental toxicity information requirement, they cannot be used to adapt the information requirement of Annex VIII, 8.7.1, according to column 2 (i.e. that a pre-natal developmental toxicity study is available).

With reference to oestrogenic activity study (vii.) key elements are missing, such as the pre-mating exposure duration and histopathological examination of reproductive organs. The exposure duration in this study (vii.) lasted only for 24 hours. Hence, the second and third conditions set out in Annex XI, section 1.1.2. are not met.

As additional toxicity studies, you have also provided a combined reproductive and developmental toxicity study (study viii. above). ECHA notes that this study was performed on insects and not on rats or any other rodent species, as specified in the corresponding OECD TG 414. You have also provided a study (xi.) with an assigned reliability score of 4 (not assignable). In view of the reliability you assigned, this information cannot be used as reliable source of information within your weight of evidence adaptation. The very limited level of information reported prevents ECHA from assessing the reliability of this data.

You have argued that information from repeated-dose toxicity studies provides sufficient information on reproductive toxicity or fertility. This is not a column 2 adaptation, and ECHA does not accept that repeated-dose toxicity studies provide sufficient information on either fertility, reproductive toxicity or on the processes of development or post-natal growth which are measured in this information requirement.

You have argued that the registered substance has a "*low systemic toxicity potential*" that it is eliminated within 24 hours and has no relevant bioaccumulation potential. This argument does not correspond to a column 2 or Annex XI adaptation. Moreover, this argument does not directly provide information on the specific information requirement of Annex VIII, 8.7.1 and in this case ECHA considers that this arguments does not provide a reason for considering that the information requirement for Annex VIII, 8.7.1 can be assumed or concluded on the basis of Annex XI, 1.2.

You have not taken into account the defects and lack of coverage of key parameters of the individual studies, and shown how these together are remedied in the overall weight of evidence.

Conclusion

Hence, the sources of information you provided, together with your justification for the adaptation, do not allow to assume/conclude on the dangerous (hazardous) property of the registered substance with respect to the information requirement for Annex VIII, Section 8.7.1.

Therefore, the general rules for adaptation laid down in Annex XI, Section 1.2. of the REACH Regulation are not met and your adaptation of the information requirement is rejected.

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 the test methods OECD TG 421/422, the test is designed for use with rats. On the basis of this default assumption ECHA considers testing should be performed with rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

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: Reproductive/developmental toxicity screening test (test method: OECD TG 421) *or* Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (test method: OECD TG 422) in rats by the oral route.

In your comments on the draft decision and on the proposal for amendment (PfA) submitted by one of the Member State Competent authorities (MSCAs) you indicated that the screening study requested in this decision can be waived if the pre-natal developmental toxicity study is performed. Additionally, in your comments on the PfA you indicated that you already have "*the data available for the reproductive toxicity of the substance and which is already presented in the dossier*".

ECHA notes that indeed according to Annex VIII Section 8.7.1., the screening for reproductive/developmental toxicity study (OECD TG 421 or 422) does not need to be conducted if "*a pre-natal developmental toxicity study (Annex IX, 8.7.2) [...] is available.*" However, as explained above in this section, currently in the technical dossier there is no compliant study available that can be used to adapt this information requirement according to Annex VIII Section 8.7.1, column 2 .

Furthermore, ECHA notes that according to ECHA's *Guidance on information requirements and chemical safety assessment*, Chapter R.7a (version 6, July 2017) that "*where information from a reproductive toxicity study addressing a fertility endpoint is not available, it is strongly recommended that a screening study is considered to fulfil this endpoint.*"

Notes for your considerations
For the selection of the appropriate test, please consult ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.5 and 7.6 (version 6.0, July 2017).

You should also carefully consider the order of testing especially the requested screening (OECD TG 421/422) and the developmental toxicity study (OECD TG 414) to ensure unnecessary animal testing is avoided, paying particular attention to ECHA's *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.5 and 7.6 (version 6.0, July 2017).

5. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information

specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.
A "pre-natal developmental toxicity study" (test method EU B.31./OECD TG 414) for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. 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.

a) Information provided

You have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this provision.

You have provided the following explanation on how the sources of information/studies, which you have provided enable an assumption or conclusion that the registered substance does or does not have a dangerous property with respect to developmental toxicity:

"[...] the study of Mantovani et al [...] is mentioned by BfR with 'A significantly lower increase in weight coupled with non-impaired feed intake at the higher doses could be interpreted as possible maternal toxicity. The foetal findings showed significant effects particularly in the incidence of skeletal anomalies in the axial skeleton and skull, in ossification and in the incidence of variants and anomalies in the kidneys and excretory urinary tract. However, these findings did not reveal the expected dose dependency. At the lowest tested dose of 5 mg/kg body weight significant increases in the incidence of ossification defects of the cranial bones, kidney changes and dilated ureter were observed [...] BfR concludes that 'overall these results justify the suspicion that cinnamaldehyde may have teratogenic potential'.

[...] The study [...] was assessed by FEMA and RIFM [...] The authors of the study assume that some effects seen at 5 mg/kg bw may suggest that 'the foetus might be slightly more sensitive than the adult' to cinnamaldehyde. However, since no historical control data of the laboratory is available and no evidence of a dose related trend was seen for any of the findings we consider the observed effects as not biologically relevant. Since maternal toxicity occurred throughout all dose levels as reduced body weight gain of the dams, the findings do not indicate primary developmental toxicity.

[...] The study of Mantovani et al. (1989) was also assessed by the Expert Panels of the Flavor and Extract Manufacturers Association (FEMA) in 2004 (Adams et al., 2004) and the Research Institute for Fragrance Materials (RIFM) in 2005 (Bickers et al., 2005). Both panels did not judge cinnamaldehyde as developmental toxicant

[...] In a preliminary developmental toxicity assay of Hardin et al. (1987) the effects of cinnamaldehyde were evaluated in CD-1 mice [...] The results show no effects on maternal survival or body weight development and all 34 litters were viable. The number of lifeborns per litter, the survival and birthweight of pups and their weight gain was not affected by treatment. Therefore, NOAEL was considered to be 1200 mg/kg/day in dams and pups when exposed to cinnamaldehyde by gavage on gestation days 6-13 in this preliminary developmental toxicity test. After oral uptake cinnamic aldehyde is oxidized to cinnamic acid and then further metabolized mainly to hippuric acid and extreted via urine. Therefore, cinnamic acid (CAS-No. 621-82-9) can be used via read-across to accomplish the toxicological data base for cinnamic aldehyde

[...] Based on the available data on developmental toxicity of cinnamaldehyde in rats and

mice and the data on its oxidation product cinnamic acid in rats, although not fully in compliance with today's scientific requirements, cinnamaldehyde can be regarded as not owing a significant potential for developmental toxicity. This conclusion is in line with the RIFM (Bickers et al., 2005) expert panel for cinnamaldehyde, cinnamyl alcohol and cinnamic acid in that 'these materials do not possess any significant potential for developmental effects under the current conditions of use as fragrance ingredient'. The FEMA (Adams et al., 2004) expert panel reaffirmed the group of cinnamyl derivatives (including cinnamaldehyde) as GRAS (Generally Recognized As Safe)."

ECHA understands that you conclude that the registered substance does not have a dangerous (hazardous) property with respect to developmental toxicity.

To support your weight of evidence adaptation you have provided the following sources of information:

i. Evaluation of 60 Chemicals in a Preliminary Developmental Toxicity Test in mice, oral (gavage) route (no test guideline; non-GLP) with the registered substance (Hardin *et al.*, 1987), rel. 2.
ii. Pre-Natal (Segment II) Toxicity Study of Cinnamic Aldehyde in the Sprague-Dawley Rats, oral (gavage) route (no test guideline followed; non-GLP) with the registered substance (Mantovani *et al.*, 1989), rel. 2.
iii. Developmental toxicity in 1 generation of Wistar rats, oral route (no test guideline; non-GLP) with the registered substance (Akihiro *et al.*, 1990), rel. 3.
iv. Secondary literature: Publications from Bickers *et al.* (2005) and Zaitsev *et al.*, (1975) (no test guideline; non-GLP) with cinnamic acid, rel 4.

Other studies under Toxicity to Reproduction section:

v. Combined Repeated, repro and developmental toxicity study in 1 generation of *A. Obtectus* insects, (no test guideline; non-GLP) with the registered substance (Regnault-Roger and Hamraoui, 1995), rel. 2.
vi. Molecular structure - teratogenicity relationships of some fragrance additives, (no test guideline; non-GLP) with the registered substance, (Abramovici and Rachmuth-Roizman, 1983), rel. 4.

b) ECHA's evaluation and conclusion of the information provided

Evaluation approach/criteria

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property with respect to the information requirement in question including an adequate and reliable documentation while the information from each single source alone is regarded insufficient to support this notion.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance with respect to a pre-natal developmental toxicity study (EU B.31/OECD TG 414). Relevant elements are in particular, exposure route, duration and levels, sensitivity and depth of investigations to detect pre-natal developmental toxicity (including growth, survival, external, skeletal and visceral alterations) and maternal toxicity.

Furthermore, the relative values/weights of different pieces of the provided information needs to be assessed as indicated in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.4., Section 4.4 (version 1.1, December 2011). In particular relevance, reliability and consistency of results/data and coverage (completeness) need to be considered.

Evaluation of the provided information

ECHA notes that all the studies provided in the technical dossier are not performed according to GLP and do not follow any test guidelines. Annex XI , section 1.1.2. provides that test data from experiments not carried out according to GLP shall be considered equivalent to data generated in accordance with the relevant test methods referred to in Article 13(3) REACH if the conditions set out in Annex XI , section 1.1.2. are met. The second condition requires that there are adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3) REACH. The third condition requires an exposure duration comparable to or longer than the exposure duration than the corresponding test methods referred to in Article 13(3).

In the assessment of each individual source of information you have provided, ECHA has found that in regard of quality and relevance, the studies have a number of shortcomings.

In the mouse study (i. above) there was a short exposure duration (gestation days 6-13) and only 2 doses were tested. In the rat study (ii. above) there was also a short exposure duration (gestation days 7 to 17) and only 14 to 16 pregnant females were tested. ECHA notes that the maternal exposure should at least last from implantation to one or two days before the expected delivery of both rodent species. In addition, according to OECD TG 414: "*At least three dose levels and a concurrent control should be used*" and each group "*should contain a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy. Groups with fewer than 16 animals with implantation sites may be inappropriate.*" Consequently, both studies fail to meet the second and third conditions set out in Annex XI, Section 1.1.2. since they do not provide adequate and reliable coverage of key parameters foreseen to be investigated in the corresponding OECD test guideline 414 and the exposure duration is shorter than the exposure duration in the corresponding test methods referred to in Article 13(3). Therefore the studies specified above (studies i. and ii.) provide only limited evidence in regard of the information requirement.

As regards the other studies, ECHA notes that you assigned a reliability score of 3 (not reliable) to study iii. and reliability 4 (not assignable) to studies iv. and vi. (above). In view of the reliability you assigned, this information cannot be used as reliable source of information within your weight of evidence adaptation. The very limited level of information reported prevents ECHA from assessing the reliability of this data.

Moreover, in study iii. (rel. 3) there are a number of deviations from the corresponding test method OECD TG 414. More specifically, only one dose was tested, instead of three test concentrations. Thirteen pregnant females were used in the study, whilst according to the OECD TG 414, at least 20 pregnant females should be used. Hence the study also fails to meet the second condition set out in Annex XI, Section 1.1.2. since it does not provide adequate and reliable coverage of key parameters foreseen to be investigated in the corresponding OECD test guideline 414.

Additionally, as regards study iv. (rel. 4) with the analogue substance, cinnamic acid (EC no. 210-708-3), in your explanation above you claim that this analogue "*can be used via read-across to accomplish the toxicological data base for cinnamic aldehyde*". However, ECHA notes that you failed to provide documentation for the read-across. Therefore, your dossier is lacking a basis for predicting relevant human health properties of the registered substance from data for the source substance. In the absence of this information, ECHA

cannot verify that the properties of the registered substance can be predicted from the data on the source substance. Hence, the general rules of adaptation as set out in Annex XI, Section 1.5., are not met.

As additional toxicity studies, you have also provided a combined reproductive and developmental toxicity study (study v. above). ECHA notes that this study was performed on insects, and not on rats or any other rodent species, as specified in the corresponding OECD TG 414. Moreover, this study does not provide the information required by Annex IX, Section 8.7.2. because it does not cover key parameters of a pre-natal developmental toxicity study like examinations of foetuses for skeletal and visceral alterations.

Finally, ECHA notes that in your explanation you highlight the diverging views on the potential developmental toxicity effects of the registered substance: "*BfR concludes that 'overall these results justify the suspicion that cinnamaldehyde may have teratogenic potential'*" and both "*the Expert Panels of the Flavor and Extract Manufacturers Association (FEMA) in 2004 (Adams et al., 2004) and the Research Institute for Fragrance Materials (RIFM) in 2005 (Bickers et al., 2005) [...] did not judge cinnamaldehyde as developmental toxicant*". In your explanation you also show the uncertainty related to the effects observed in the available studies with the registered substance, as you only conclude that "*cinnamaldehyde can be regarded as not owing a significant potential for developmental toxicity.*" However, the currently provided data do not provide a sufficient basis to conclude on the potential for the developmental toxicity of the registered substance.

Conclusion

ECHA concludes that the relevant parameters and observations, which are needed to meet the information requirement, have not been adequately covered by the data provided in the dossier, which you have provided in a Weight of Evidence adaptation.

Hence, the sources of information you provided, together with your justification for the adaptation, do not allow to assume/conclude that the substance does not have a particular dangerous (hazardous) property with respect to the information requirement for Annex IX, Section 8.7.2.

Therefore, the general rules for adaptation laid down in Annex XI, Section 1.2. of the REACH Regulation are not met and your adaptation of the information requirement is rejected.

In your comments on the draft decision you state that "*there is sufficient evidence from several sources of information leading to the conclusion that cinnamaldehyde has no particular dangerous property with regard to pre-natal developmental toxicity.*" Furthermore, you provided details of the study records (same information provided in the technical dossier) that support the weight of evidence adaptation. The Registrant also informed ECHA that if "*insisted*" they "*can consider performing the OECD 414 study which will further used to waiver*" the screening study.

In your final comment you claim that "*cinnamaldehyde can be regarded as not owing a significant potential for developmental toxicity*", however, ECHA notes that there are concerns with the available data presented in the dossier. More specifically, as already mentioned above, the study records provided for this endpoint have a number of shortcomings in terms of quality and relevance. In your comments you refer again to the data on the oxidation product of the registered substance, that is cinnamic acid, however, you fail to provide documentation to support the read-across approach. Additionally, you did not provide any clarification and/or further information on the issues raised in the draft

decision. As a consequence, the issues and concerns raised above still hold.

Hence, 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 the test method EU B.31./OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default assumption ECHA considers testing should be performed with rats or rabbits as a first species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

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: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a first species (rat or rabbit) by the oral route.

Notes for your consideration

ECHA notes that a revised version of OECD TG 414 was adopted this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. You should test in accordance with the revised version of the guideline as published on the OECD website for adopted test guidelines (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).

6. Ready biodegradability (Annex VII, Section 9.2.1.1.)

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.

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. Test guidelines specify the domain of applicability depending on the substance profile. If a test is conducted outside the domain of applicability of a given test guideline, then that test may not be considered to be appropriate for the substance.

In addition, specifications for the interpretation of the ready biodegradability results are provided in ECHA Guidance on information requirements and chemical safety assessment, Volume 5: Endpoint specific guidance for environment R.7b (Chapter R.7.4). ECHA Guidance gives the following guidance for acceptable pass levels for ready biodegradability: 70% DOC removal; 60% theoretical carbon dioxide: 60% theoretical oxygen demand to be reached in a 10-day window within 28 days.

In the present case, the technical dossier contains an endpoint study record for *"DETERMINATION OF BOD-5 and % DEGRADATION"*, (key study, non-GLP, reliability score 1, conducted in 2015 by owned by [redacted]). You state that the study has been performed using standard OECD Guideline OECD 301 D. However, in describing the principle of the method, you clarify that *"the objective of the study was to measure Biochemical Oxygen Demand of Cinnamaldehyde over a 5-day period"* and that only *"The experimental set up was adopted from OECD guideline 301 D – Closed Bottle Test."* Therefore, the study is not conducted according to OECD 301 D as such, i.e. it is not run over a 28-day period and there is no information on degradation in the 10-day window. While the value provided indicates some degree of degradation (24.98% over 5 days), it does not fulfil the requirement for the use of BOD-5 test as indicator of ready biodegradability. In fact, according to the Guidance on the application of CLP criteria (v 5.0, July 2017), *"In those cases where only BOD5 and COD data are available, the ratio of BOD5/COD is greater than or equal to 0.5."* This means that when transforming this value in percentage, a 50% cut-off value needs to be met or passed. Therefore, the value of 24.98% which you provided cannot be used to indicate that the substance is readily biodegradable. ECHA also notes that the study you provided was not performed according to GLP, thus you cannot assign reliability score 1, but only 2.

In addition to the key study, you have also submitted two supporting studies that provide results estimated by calculation (BIOWIN v4.10 by US-EPA (EPIWIN) and PBT profiler US EPA v1.301), one suggesting fast degradability of the substance and the other indicating a 32% degradation in water after 15 days.

You also submitted results from secondary source: the Flavor and Fragrance High Production Volume Consortia Revised Robust Summaries for Cinnamyl Derivatives, Klimish.4, in which Cinnamaldehyde was found to be readily biodegradable in water with at 89% degradation at 7 days, 94% degradation at 14 days and 100% degradation at 21, 27, and 28 days. You also provided literature data: Haarmann and Reimer (2001) Ready Biodegradability of Cinnamic Aldehyde according to OECD Guideline No. 301B, Klimish.4. CO2 Evolution Test; where test substance cinnamaldehyde showed 100% biodegradability in contact time 28 days. However, the endpoint study records do not contain any other information than the results and it is thus impossible to assess the quality and reliability of the information.

In your comments to the draft decision, you indicated that you will update your dossier with two available studies (OECD 301F and 301E) that indicate that the substance is readily biodegradable. ECHA will assess these studies at the follow up stage.

Based on the above considerations, the information provided in the registration dossier is not appropriate to conclude that the registered substance is readily biodegradable. As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirements. Consequently, there is an information gap and it is necessary to provide information for this endpoint.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information using one of the indicated test methods and the registered substance subject to the present decision:

Ready biodegradability (test method: CO_2 evolution test, OECD TG 301B).
or
Ready biodegradability (test method: Ready biodegradability – CO_2 in sealed vessels (headspace test), OECD TG 310).
or

Ready biodegradability (test method: MITI test (I), OECD TG 301C).
or
Ready biodegradability (test method: Closed bottle test, OECD TG 301D).
or
Ready biodegradability (test method: Manometric respirometry test, OECD TG 301F).

Notes for your consideration:

Once the re-evaluation of the biodegradation test, as required by this decision, has been done, you shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation. If the revised chemical safety assessment indicates the need to submit further information in order to fulfil the REACH information requirements depending on the interpretation of biodegradability, you should do so. Indeed, ECHA reminds you that the information requirements of Annex IX, Section 9.2., regarding additional testing on degradation (simulation tests), will have to be addressed if it would be determined that the substance is not readily biodegradable. In this scenario, ECHA notes that you would need to consider submitting (a) testing proposal(s) to cover the information requirements of Annex IX, Section 9.2.

7. Identification of PNEC and risk characterisation (Annex I, Sections 3.3.1. and 6.)

Pursuant to Articles 10(b) and 14(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.

Annex I, Section 3.1.5. of the REACH Regulation requires that the study or studies giving rise to the highest concern shall normally be used to draw a conclusion and a robust study summary shall be prepared for that study or studies and included in the technical dossier. In addition, Annex I, Section 3.1.5. requires that if a study giving rise to the highest concern is not used, then this shall be fully justified.

You have calculated the risk characterisation ratios (RCRs) based on the results from the short aquatic tests, using an assessment factor of 200. ECHA notes that an assessment factor of 1000 shall be used when deriving the PNEC from short-term results. However, in your registration dossier you have also provided results for long-term endpoints, but you have not used them in your risk assessment and you have not updated your CSR.

In your comments to the draft decision you indicated that the PNEC will be updated within the dossier and will be calculated using the new IUCLID 6 version with the tool provided by ECHA and dossier will be accordingly updated. ECHA will assess this at the follow up stage.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to revise PNECs and revise the risk characterisation by recalculating the RCRs for freshwater, marine water, intermittent releases (if applicable), microorganisms in sewage treatment plants, freshwater sediment, marine sediment and soil:
- using the study giving rise to the highest concern according to Annex I, Section 3.1.5 and revise the risk characterisation accordingly *or* provide a full justification for not using the study giving rise to the highest concern;
- using the default assessment factors and other recommendations of ECHA Guidance R.10 and revise the risk characterisation accordingly *or* provide a detailed justification on how the chosen approach meets the general requirements for PNEC derivation as described in

Section 3.3. of Annex I, if not using the recommendations of ECHA Guidance R.10 for PNEC derivation.

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 15 September 2017.

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 request(s).

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

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

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 during its MSC-60 meeting 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 requests in this decision, or to otherwise fulfil the information requirements 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 tests 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 tests 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 tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.

4. ECHA reminds you of steps 1, 2 and 3 of Annex VI; if there is adequate information for a particular endpoint, you may achieve compliance by updating your registration dossier with a robust study summary for that endpoint, as set out in Article 10. ECHA notes that the information in the later updated dossier(s) will be assessed for compliance in the follow-up evaluation pursuant to Article 42 of REACH (after ECHA has sent the final decision).