EUROPEAN CHEMICALS AGENCY

Helsinki, 21 September 2016

Addressee: [redacted]

Decision number: CCH-D-2114345337-48-01/F
Substance name: 3,5,5-trimethylhexanoic acid
EC number: 221-975-0
CAS number: 3302-10-1
Registration number: [redacted]
Submission number: [redacted]
Submission date: 20.12.2013
Registered tonnage band: 1000+T

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. **Dissociation constant (Annex IX, Section 7.16.; test method: OECD TG 112) with the registered substance;**

2. **In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: EU B.10./OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;**

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

4. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;**

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **28 March 2019**. You shall also update the chemical safety report, where relevant.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.**]**

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

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

Appendix 1: Reasons

1. Dissociation constant (Annex IX, Section 7.16.)

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

"Dissociation constant" is a standard information requirement as laid down in Annex IX, Section 7.16 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.

The technical dossier does not contain relevant data to fulfil this standard information requirement. You reported that the outcome of an OECD TG 112 study was that the dissociation constant was "*not determinable due to low water solubility*". ECHA considers this an implicit adaptation under Annex XI, Section 2 of the REACH Regulation (testing is technically not possible).

However, at the water solubility endpoint, you stated: "*The water solubility of the test item was determined according to OECD guideline no. 105 [adopted on 27 July 1995] and EU test method A.6 [Directive 92/69/EEC, Official Journal L 383 A 1992] with the flask method. Water solubility (at 20°C): 0.7 g/L*". So the water solubility is not so low as to prevent a study for the dissociation constant. Indeed, the water solubility allows a study to be carried out at the concentration of 5 mM.

You sought to adapt the information requirement, but did not provide a sufficient justification. Therefore, you are requested to submit the information for this endpoint using an appropriate test method on the registered substance.

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.

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: Dissociation constants in water (test method: OECD TG 112).

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 more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

An "*In vitro* 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.

In the technical dossier you have provided a study record for a *Test substance MRD-03-780 in vitro chromosomal aberration assay in CHO cells* (Exxon, 2004, OECD TG 473). However, this study does not provide the information required by Annex VIII, Section 8.4.2., because the study is considered as being inadequate. In the endpoint study summary ([redacted] (+2004, key study, CA, RL1, OECD TG 473)) in IUCLID section 7.6.1 there are only observations and data reported for the 19 hour and 43 hour culture harvest time. According to OECD TG 473 (as adopted 21st July 1997), paragraph 25: "*cells should be exposed to the test substance both with and without metabolic activation for 3-6 hours, and sampled at a time equivalent to about 1.5 normal cell cycle length after the beginning of treatment (12). If this protocol gives negative results both with and without activation, an additional experiment without activation should be done with continuous treatment until sampling at a time equivalent to about 1.5 normal cell cycle lengths.*" OECD TG 473 (as adopted 26 September 2014) contains substantially the same request in paragraph 28. ECHA takes the view that you have not followed the dosing protocol set out in paragraph 25 of OECD TG 473 (as adopted 21 July 1997), or in paragraph 28 of OECD TG 473 (as adopted 26 September 2014). ECHA considers the dosing protocol to be a key parameter of the Test Guideline, and that your failure to follow the dosing protocol means that the study is not compliant with the guideline. It does not provide information that is sufficient to meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA considers that the *in vitro* mammalian 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. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.) in a second species

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

Pre-natal developmental toxicity studies (test method EU B.31./OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

The technical dossier contains information on a pre-natal developmental toxicity study in rats by the oral route using the registered substance as test material.

However, there is no information provided for a pre-natal developmental toxicity study in a second species.

The technical dossier does not contain an adaptation in accordance with column 2 of Annex X, Section 8.7.2. or with the general rules of Annex XI for this standard information requirement.

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.

The test in the first species was carried out by using a rodent species (rats). According to the test method EU B.31./OECD 414, the rabbit is the preferred non-rodent species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbits as a second 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 4.1, October 2015) R.7a, chapter 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 second species rabbit by the oral route.

4. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

The basic test design of an extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex X. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in the ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 4.1, October 2015).
Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information requirement

In the technical dossier you have provided a study record for a One generation reproduction range finding study in rats. However, this study does not provide the information required by Annex X, Section 8.7.3. because it is missing several key parameters of the EOGRTS, such as animal number per group and hence statistical power of the test (10 vs. 20), the histopathological or clinical chemistry examination of the F1 generation. Therefore, your adaptation of the information requirement is rejected.

While you have not explicitly claimed an adaptation, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex XI, Section 1.2. You provided the following information: *One generation reproduction range finding study in rats* (test method: OECD TG 415, [redacted]), *OECD Guideline 471 (Bacterial Reverse Mutation Assay)* [redacted] and [redacted], *OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)*, [redacted], *OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)*, [redacted].

You provided the following justification for the adaptation: *"As all of the genetic toxicity studies show negative results and there is also a 1 gen study available showing no adverse effects in the absence of maternal toxicity, a 2gen study is not necessary".* However, ECHA notes that your adaptation does not meet the general rules for adaptation of Annex XI, Section 1.2., because it is not possible to assume/conclude based on the information if the registered substance has not a hazardous property on sexual function and fertility. Especially information on the following relevant aspects in relation to sexual function and fertility has not been covered: histopathology of the reproductive organs of the parental and F1 generation and development during postnatal period up to the adulthood. Furthermore, the conducted range-finding one-generation reproductive toxicity study provides only limited information on sexual function and fertility of the parental generation due to low statistical power (similar to a screening study, OECD TG 421) not comparable to the statistical power of an comprehensive reproductive toxicity study such as an extended one-generation reproductive toxicity study.

The lack of the histopathological investigations of the reproductive organs further increases the concern for the lack of sensitivity to detect the reproductive effects. The study does not sufficiently address information on hazardous properties to the postnatal development of the offspring including information on endocrine disrupting properties (e.g. oestrus cycle, AGD, nipple/areolae retention) and histopathological integrity of the reproductive organs at adulthood. Moreover, the study shows that there is a concern for the offspring viability and sexual maturation which may not be easily explained by the maternal toxicity demonstrated in the dossier. Specifically, *"there were statistically significant decreases in 0.5% group offspring compared with the control offspring for the live birth index (6.0%), the Day 1 survival index (14%), and the Day 4 survival index (21%)."* You also reported that *"There was a statistically significant advance for preputial separation for the 0.06% group males (1.2 days) when compared with the controls.*

Due to the small size of this advance and the absence of a dose response, this difference was not considered biologically significant. There also was a statistically significant retardation of preputial separation for the 0.5% group males (2.1 days) compared with the control male offspring. In the females, the 0.5% group exhibited a statistically significant retardation (1.8 days) for vaginal patency compared with controls." While you claim that these are not biologically relevant, the effects raise a concern, which should be examined in more detail in an extended one-generation reproductive toxicity study.

Concerning your reference to negative genetic toxicity studies, ECHA notes that while genetic toxicity may correlate with reproductive toxicity in some cases, it is not possible to conclude based on results from genotoxicity studies directly on reproductive toxicity, except for germ cell mutagens.

Thus, the information from these studies do not allow to assume/conclude that the substance has not hazardous properties with regard to sexual function and fertility.

Therefore, 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. Thus, an extended one-generation reproductive toxicity study according Annex X, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) The specifications for the study design

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA Guidance, the starting point for deciding on the length of premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required because there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 4.1, October 2015).

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results of the main study.

Species and route selection

According to the test method EU B.56./ OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in 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 4.1, October 2015) R.7a, chapter 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.

c) Outcome

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: Extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;

Notes for your consideration

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if new information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the new information shows triggers which are described in column 2 of Section 8.7.3., Annex X and further elaborated in ECHA *Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.6 (version 4.1, October 2015). You may also expand the study to address a concern identified during the conduct of the extended one-generation reproduction toxicity study and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the expansion must be documented. The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/triggers must be documented.

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 31 March 2016.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments.

ECHA did not receive any comments by the end of the commenting period.

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

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

Appendix 3: Further information, observations and technical guidance

1. The substance subject to the present decision is provisionally listed in the draft Community rolling action plan (CoRAP) for start of substance evaluation in 2018.

2. This compliance check decision does not prevent ECHA from initiating further compliance checks on the present registration at a later stage.

3. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

4. In relation to the information required by the present decision, the sample of the substance used for the new test(s) must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants. It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new test(s) is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new test(s) must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the test(s) to be assessed.