EUROPEAN CHEMICALS AGENCY

Helsinki, 11 March 2020

Addressees
Registrants of JS_261-245-9 listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of this decision
12 April 2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: 3,5,5-trimethylhexyl acetate
EC number: 261-245-9
CAS number: 58430-94-7

Decision number: [Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/D)]

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***19 September 2022***.

A. Requirements applicable to all the Registrants subject to Annex IX of REACH

1. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2., column 2; test method OECD TG 414) in a second species (rabbit), oral route with the Substance;**

2. **Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: OECD TG 443) in rats, oral-gavage route, specified as follows:**
   - **At least two weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce systemic toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation which shall be followed to weaning;**
   - **Cohorts 2A and 2B (Developmental neurotoxicity).**

   You must report the study performed according to the above specifications. Any expansion of the study must be scientifically justified.

Conditions to comply with the requests

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.
To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annexes VII, VIII and IX of REACH, if you have registered a substance at 100-1000 tpa;

Registrants are only required to share the costs of information that they must submit to fulfil the information requirements for their registration.

The Appendices state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH.

The Appendix entitled Observations and technical guidance addresses the generic approach for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

You must submit the information requested in this decision by the deadline indicated above in an updated registration dossier and also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information. The timeline has been set to allow for sequential testing where relevant.

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[1] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[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 A: Reasons for the requests to comply with Annex IX of REACH

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

1. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2., column 2) in a second species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH. Annex IX, Section 8.7.2., column 2 provides that the decision on the need to perform a PNDT study on a second species at a tonnage level of 100 to 1000 tonnes per year should be based on the outcome of the PNDT study on a first species and all other relevant and available data.

You have provided the following study with the Substance:

Pre-natal developmental oral toxicity study (2016) according to OECD TG 414 in rats. GLP compliant.

We have assessed this information and identified the following issue(s):

There is a concern based on information from a first species and taking all the available information into account as required in column 2 at Annex IX, section 8.7.2.

You consider that no developmental toxicity was observed in the available study. You claim that the administration of the substance resulted in maternal mortality, and reduced food consumption, body weight, body weight gain, and corrected body weight, along with gross pathological observations primarily in the liver, for dams at 250 mg/kg bw/day.

Developmental toxicity was observed in the available PNDT study at dose levels which were not markedly toxic to dams. Malformations such as cheiloschisis, microstomia, mandibular micrognathia, missing snout, microphthalmia, fluid-filled thorax, hole in the heart, megaureter, and small renal papillas were recorded at the mid and high dose levels together with increased incidence of foetuses with skeletal variations.

Although, as aforementioned, you consider that no developmental toxicity was observed, there were incidents in the foetuses already at doses below 250 mg/kg bw/day, such as one foetus with cheiloschisis, missing snout and microphthalmia, and another foetus with fluid-filled thorax and hole in the heart.

In your comments to the draft decision you disagree with ECHA's assessment stating that the reported malformations are *"seen at rare incidences randomly in the mid or high dose, revealing neither a dose-response nor exceeding the historical range for spontaneous occurrence of those in this strain of rat".* You further claim that those findings are "accidental" and *"thus to conclude that these variations clearly indicate a potential for developmental toxicity that need to be further investigated in another study is an overinterpretation of the findings* [...].

ECHA reiterates that the dose-dependent increase in the number of foetuses with major facial malformations raises a concern for prenatal developmental toxicity. You have reported one

foetus with eye and facial malformations in the mid-dose group, and one with eye, the other with jaw and the third with mouth malformations in the high-dose group, when compared to the control (0). This cluster of various facial malformations cannot be explained with the historical control incidences of individual malformations.

Furthermore, the maternal toxicity at the highest dose level of 250 mg/kg bw/day seems to be only slight; corrected maternal body weight was reduced only by 6.9%. Maternal mortality observed at the highest dose (250 mg/kg bw/day) was only observed in one out of 24 animals which cannot be considered significant. Besides, this maternal mortality may be related to treatment-related complications during the last days of pregnancy similar to findings in the provided OECD TG 422 study at 125 and 400 mg/kg bw/day or to dosing errors (dark foci in the stomach) rather than to maternal toxicity.

In your comments to the draft decision you disagree with ECHA's conclusion on maternal toxicity. You highlight especially the reduction in the corrected body weight gain at the high-dose group and the mortality in two animals with associated clinical signs. You state that liver findings (pale discoloration/pale area, enlargement and/or prominent lobular architecture) are observed also in surviving animals and are consistent with possible hepatic lipidosis. You further consider that reduced foetal body weight and slightly delayed/incomplete ossification can be explained by reduced maternal food consumption and reduced maternal body weight gain and malnutrition of the pups.

ECHA agrees that observed increases in skeletal variations may reflect the reduced mean foetal body weight at the high-dose level. However, the reduction of the food consumption is minmal and statistically significant (16% reduction) only during one measurement point, between Days 18 and 21 in the high-dose animals. This cannot lead to malnutrition of the pups. Furthermore, the corrected maternal body weight showed only a slight reduction (6.9%). The hepatic lipidosis or the slight reduction in maternal body weight is not evidence of marked maternal toxicity and based on information you provided there is only one rat that was dying/euthanised during the study.

Therefore, in addition to the malformations at the mid dose, malformations and reduced foetal development observed at the high dose level are not considered secondary to maternal toxicity because the doses were not markedly toxic to dams. Effects related to developmental toxicity were also observed in the OECD TG 422 study at 125 mg/kg bw/day (increased post-implantation loss and consequently reduced litter size).

In your comments to the draft decision you state that no implantation loss and reduced litter size are observed in the PNDT study in rats, although seen in the OECD TG 422 study. You argue that if such effects are of concern, they will be investigated under the OECD TG 443 study, "[...] *as the OECD TG 414 study is not specifically designed to investigate such effects*".

ECHA wants to clarify that the above-mentioned effects in the OECD TG 422 study are specifically investigated in an OECD TG 414 study. The OECD TG 443 study will provide information on litter size, but it is not conclusive for postimplantation loss or birth litter size because there is possibility for cannibalisms and the malformed pups may be eaten by the dams.

These effects indicate a concern for prenatal developmental toxicity.

Based on the above, the information you provided do not fulfil the information requirement.

Information on study design

The test in the first species was carried out by using a rodent species (rat). A PNDT study according to the test method OECD TG 414 must be performed in rabbit as preferred non-rodent species.

The study shall be performed with oral[2] administration of the Substance.

In your comments to the draft decision you state that if ECHA considers a pre-natal developmental toxicity study in rabbits mandatory, "[...] *a sequential testing is mandatory as findings in this study could trigger a classification requirement that would no longer allow to marked the substance for consumer application and further testing would be of no value. Thus, the need to perform the also requested OECD TG 443 can only be decided when the results of a second OECD TG 414 are available and the potential consequences for C&L can be decided upon".*

According to Annex IX, Section 8.7., Column 2, third paragraph, if the substance is known to cause developmental toxicity meeting the criteria for classification as toxic for reproduction category 1A or 1B: May damage the unborn child (H360D), and the available data are adequate to support a robust risk assessment, then no further testing for developmental toxicity will be necessary. However, testing for effects on fertility must be considered.
Therefore, your statement that the OECD TG 443 study will not be needed if your Substance is classification as Repr. 1B for development is not in accordance with the provisions of Annex IX, Section 8.7., Column 2,

2. Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.)

The basic test design of an extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is a standard information requirement under Annex IX to REACH, if the available repeated dose toxicity studies indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. Furthermore column 2 defines the conditions under which the study design needs to be expanded.

You have provided a combined Repeated Dose 28-Day Oral Toxicity with the Reproduction / Developmental Toxicity Screening Test. Dettwiler M. 2013, according to OECD Guideline 422.

We have assessed this information and identified the following issue(s):

Adverse effects on reproductive organs or tissues or other concerns in relation with reproductive toxicity are observed in the available study. More specifically, eight females at the high-dose level (400 mg/kg bw/day) and two females at the mid-dose level (125 mg/kg bw/day) were found dead between days 18 and 23 post coitum due to dystocia (pregnancy complications and/or difficult parturition). Additionally, two non-pregnant females at the high dose level, survived without any indication of adverse toxicity which supports the assumption that the aforementioned mortalities were treatment related. Furthermore, in the OECD TG 408 study, changes in oestrous cycle were observed.

Besides, follicular hypertrophy of the thyroid glands was recorded in males at the dose levels of 400, 125 and 40 mg/kg bw/day and females at the dose level of 125 mg/kg bw/day (the highest dose without dystocia). Although, you claim that "*the finding is deemed to be*

[2] ECHA Guidance R.7a, Section R.7.6.2.3.2.

associated with increased hepatic turnover of thyroid hormones due to hepatocellular hypertrophy and, therefore, deemed to represent a secondary change", the effect cannot be disregarded because of the essential role thyroid hormones in reproduction and development, and taking into account the mild observed effects in liver.

Furthermore, post-implantation loss resulting in reduction of litter size at first litter check and consequent reduction in birth index was observed at the dose level of 125 mg/kg bw/day. Increased postnatal loss and reduced viability index were also noted at this dose level. These effects were considered to be test item-related.

An EOGRT study according to OECD TG 443 as specified in this decision is an information requirement for your registration, because Column 1 criteria at Annex IX, section 8.7.3 are met.

Based on the above, the information you provided does not fulfil the information requirement.

In your comments to the draft decision you agree to conduct the OECD TG 443 study to further investigate the observed effects: prolongation of the oestrous cycle (OECD TG 408) and dystocia (OECD TG 422). However, you disagree with the requested design of the study.

ECHA has provided its assessment of your comments under the relevant paragraphs below, describing the requested study design.

The specifications for the study design

Premating exposure duration and dose-level setting

The length of premating exposure period must be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

A 2-week premating exposure duration for P0 animals is sufficient for your Substance, because the F1 animals of Cohort 1B are mated to produce the F2 generation and, thus, the premating exposure duration will be 10 weeks for these Cohort 1B animals.

In your comments to the draft decision you propose "*a 10 week pre-treatment of the P0 generation*".

As stated above the premating exposure duration should be at least 2 weeks if the extension of Cohort 1B is needed. As this is the case for your Substance, the requested premating exposure duration is at least two weeks.

In order to be compliant and not to be rejected due to too low dose levels, the highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects. A descending sequence of dose levels should be selected in order to demonstrate any dose-related effect and to establish NOAELs.

If there is no relevant data to be used for dose level setting, it is recommended that range-finding results are reported with the main study.

You have to provide a justification with your study results that demonstrates that the dose level selection meets the conditions described above.

In your comments to the draft decision you acknowledge the criteria for the selection of the highest dose, however, you express your concern that it would be difficult to be fulfilled, due to the dystocia induced by the Substance. You state that for you, dystocia would be the effect to determine the high dose in the OECD TG 443 study. You further say that "*if ECHA sees here other options/path forward we would welcome that ECHA share those with us before initiation of the study*".

ECHA considers that the observed effects under the OECD TG 422 are relevant to be used for dose level setting and these effects must be followed. In practise this means that the aim should be to induce dystocia, but less frequently than at the highest dose in the OECD TG 422 (8/11 animals) in order to get enough pups for different F1 cohorts. To follow dystocia, the study should be conducted using the same rat strain and same exposure route of administration (oral gavage) as in the OECD TG 422 study.

Cohorts 1A and 1B

Cohorts 1A and 1B belong to the basic study design and must be included.

In your comments to the draft decision you agree to include these cohorts in the study design.

Extension of Cohort 1B

If the Column 2 conditions of 8.7.3., Annex IX are met, Cohort 1B must be extended.

The extension is inter alia required, if the use of the registered substance is leading to significant exposure of consumers and professionals (column 2, first paragraph, lit. (a) of Section 8.7.3., Annex IX) and there are indications of one or more relevant modes of action related to endocrine disruption from available *in vivo* studies or non-animal approaches (column 2, first paragraph, lit. (b), third indent of Section 8.7.3., Annex IX).

The use of the Substance is leading to significant exposure of consumers and professionals because the Substance is used by professionals as washing and cleaning products, polishes and wax blends (PROCs 1, 2, 4, 8a, 8b, 10, 11, 13) and consumers as washing and cleaning products, air care products, biocides, polishes and wax blends.

Furthermore, there are indications of one or more modes of action related to endocrine disruption because changes in organs/parameters sensitive to endocrine activity are observed. More specifically, changes in oestrous cycle in the OECD TG 408 study, dystocia and follicular hypertrophy of the thyroid glands in the OECD TG 422 study indicate endocrine effects. ECHA considers changes in thyroid gland as relevant signs of concern and indication of an endocrine effect, irrespective its potential relationship to liver enzyme induction. Furthermore, post-implantation loss resulting in reduction of litter size at first litter check and consequent reduction in birth index observed in the OECD TG 422 study may be also due to endocrine effects. These effects were observed at dose levels which are not markedly systemically toxic.

In your comments to the draft decision you agree that the Substance has consumer uses. However, you do not agree "*that the extention of cohort 1B is triggered based on the effects noted for the thyroid in the OECD TG 422 study*". You still consider that the effect on the thyroid gland observed in the OECD TG 422 is secondary and therefore not relevant for the testing design. You further state that such effect has not been seen in OECD TG 408 "*a significant longer duration study*".

ECHA reiterates that the effect to the thyroid gland cannot be disregarded. Furthermore, your statement that "*the findings are deemed to be associated with the increased trurnover of thyroid hormones due to the liver hyperthrophy*" is not supported by the reported results and you have not substantiated your claim by specific investigations. Firstly, under the OECD TG 422 study, no measurements of thyroid hormones have been performed. Secondly, no changes in the liver weights are observed in male rats, for which the dose-dependent changes in the thyroid are recorded. For the females, the liver weight was increased only at the highest dose group and macroscopical changes in the liver (thicked, tan discoloured) are reported only for two high-dosed and one mid-dosed animals. No liver histopathology is reported for both males and females.

Further, under the OECD TG 408 study, histopathological examination of the thyroid gland has not been performed. The reported liver hypertrophy does not result in any changes of the thyroid hormones concentration. Thus, there is no evidence that the follicular hypertrophy of the thyroid glands would be linked, totally or partly, to liver toxicity, and these effects are relevant to be included to indicators of one or more modes of action related to endocrine disruption.

Therefore, based on the above, Cohort 1B must be extended.

The F2 generation shall be followed to weaning allowing assessment of nursing and lactation of the F1 parents and postnatal development of F2 offspring. Investigations for F2 pups must be similar to those requested for F1 pups in OECD TG 443 and described in OECD GD 151[3]. It is recommended to aim to have similar statistical power for investigations than in P0 generation.

Cohorts 2A and 2B

The developmental neurotoxicity Cohorts 2A and 2B need to be conducted in case of a particular concern on (developmental) neurotoxicity.

Existing information on the Substance itself derived from the available OECD TG 422 study, show evidence of thyroid toxicity. More specifically, follicular hypertrophy of the thyroid glands was recorded in males at all dose levels and in females at mid dose. Although, you claim that "*the finding is deemed to be associated with increased hepatic turnover of thyroid hormones due to hepatocellular hypertrophy and, therefore, deemed to represent a secondary change*", the effect cannot be disregarded because of the essential role thyroid hormones in developmental neurotoxicity, and taking into account the mild observed effects in liver. Thyroid toxicity rises a particular concern on developmental neurotoxicity (ECHA Guidance R.7a).

In your comments to the draft decision you disagree with the requested cohorts 2A and 2B because according to your assessment, the basis for this requirement is not fulfilled and should be deleted. You further state that "*even at toxic doses no effects on neurotoxicity were noted in either pregnant (OECD 422) or non-pregnant (OECD 408) animals*".

ECHA reiterates that the observed thyroid toxicity rises a particular concern for developmental neurotoxicity. As explained above, ECHA does not consider the follicular hyperthrophy of the thyroid gland as secondary effect. Therefore, the developmental neurotoxicity Cohorts 2A and

[3]http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2013)10&doclanguage=en

2B need to be conducted.

Species and route selection

As explained under Section *Premating exposure duration and dose-level setting* above, the findings from the OECD TG 422 study must be followed in an EOGRTS study with the same rat strain and the same route of administration. Therefore, the study must be performed in Wistar rats with oral-gavage[4] administration.

Further expansion of the study design

No triggers for the inclusion of Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 3 if relevant information becomes available from other studies or during the conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Column 2, Section 8.7.3., Annex IX. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in ECHA Guidance[5].

[4] ECHA Guidance R.7a, Section R.7.6.2.3.2.
[5] ECHA Guidance R.7a, Section R.7.6.

Appendix B: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of REACH.

The compliance check was initiated on 9 April 2019.

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 within 30 days of the notification.

ECHA took into account your comments and amended the request for the Extended one-generation reproduction toxicity study by defining the administration route more specifically.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix C: Observations and technical guidance

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

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 the Member States.

3. Test guidelines, GLP requirements and reporting

   Under Article 13(3) of REACH, all new data generated as a result of this decision needs to be conducted according to the test methods laid down in a European Commission Regulation or according to international test methods recognised by the Commission or ECHA as being appropriate.

   Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses shall be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

   Under Article 10 (a) (vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide: 'How to report robust study summaries'[6].

4. Test material

   Selection of the test material(s)

   The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

   While selecting the test material you must take into account the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected test material must contain that constituent/ impurity.

   Technical reporting of the test material

   The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

   Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[7].

[6] https://echa.europa.eu/practical-guides
[7] https://echa.europa.eu/manuals

5. List of references of the ECHA Guidance and other guidance/ reference documents[8]

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 in this decision.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 in this decision.

ECHA Read-across assessment framework (RAAF, March 2017)[9]

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

OECD Guidance documents[10]
Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD23.
Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment – No 43, referred to as OECD GD43.

[8] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[9] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[10] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix D: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fufilled
[redacted]	[redacted]	[redacted]