~~Confidential~~

Helsinki, 14 May 2024

Addressee(s)
Registrant(s) of JS_Diisobutylene as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
19 April 2022

Registered substance subject to this decision ("the Substance")
Substance name: 2,4,4-trimethylpentene
EC/List number: 246-690-9

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

DECISION ON TESTING PROPOSAL(S)

Under Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***21 February 2028***.

Requested information must be generated using the Substance unless otherwise specified.

Information required from all the Registrants subject to Annex IX of REACH

1. Extended one-generation reproductive toxicity study also requested below (triggered by Annex IX, Section 8.7.3., column 1)

Information required from all the Registrants subject to Annex X of REACH

2. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) by oral route, in rats, specified as follows:
    - Ten weeks premating exposure duration for the parental (P0) generation;
    - The highest dose level in P0 animals must be determined based on clear evidence of an adverse effect on sexual function and fertility without severe suffering or deaths in P0 animals as specified further in Appendix 1, or follow the limit dose concept. The reporting of the study must provide the justification for the setting of the dose levels;
    - 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; and
    - 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.

The reasons for the decision(s) are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressee(s) of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

In the requests above, the same study has been requested under different Annexes. This is because some information requirements may be triggered at lower tonnage band(s). In such cases, only the reasons why the information requirement is triggered are provided for the lower tonnage band(s). For the highest tonnage band, the reasons why the standard information requirement is not met and the specification of the study design are provided. Only one study is to be conducted; all registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the others under Article 53 of REACH.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report, where** relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4.

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the decision
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[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 for the decision

Contents

Reasons for the decision(s) related to the information under Annex IX of REACH

1. Extended one-generation reproductive toxicity study

An extended one-generation reproductive toxicity study (EOGRTS; OECD TG 443) is an information requirement under Annex IX, Section 8.7.3. if the available repeated dose toxicity studies indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity.

Your dossier contains an OECD TG 408 study (2021) which indicates a concern in relation with reproductive toxicity. More specifically, the decrease of T3 observed in males and females at 1000 mg/kg/day (0.76x and 0.74x of controls, respectively; statistically significant for females), and the significant decrease of T4 concentration (0.55x of controls) observed in males at 1000 mg/kg/day indicate biologically relevant changes in hormone levels related to reproductive toxicity. Therefore, the concern for reproductive toxicity must be further investigated.

ECHA agrees that an EOGRTS is necessary to address the identified concerns in relation with reproductive toxicity.

For the assessment of the testing proposal, see Section 2.

Reasons for the decision(s) related to the information under Annex X of REACH

2. Extended one-generation reproductive toxicity study

The basic test design of an extended one-generation reproductive toxicity study (EOGRTS) is a standard information requirement under Annex X. Furthermore, Annex X, Section 8.7.3., Column 2 defines when the study design needs to be expanded.

2.1. Information provided to fulfil the information requirement

You have submitted a testing proposal for an EOGRTS according to OECD TG 443 with the Substance.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Toxicity to reproduction. You provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA agrees that an EOGRTS is necessary.

2.2. Specification of the study design

2.2.1. *Species and route selection*

You proposed testing by oral route in rats. ECHA agrees with your proposal.

2.2.2. *Pre-mating exposure duration*

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

You proposed ten weeks pre-mating exposure duration. ECHA agrees with your proposal.

Ten weeks pre-mating exposure duration is required to obtain results adequate for classification and labelling and/or risk assessment. There is no substance specific information in the dossier supporting shorter pre-mating exposure duration (Guidance on IRs & CSA, Appendix R.7.6-3).

In addition, the substance is lipophilic (log Kow > 4.5); therefore, ten weeks pre-mating is required to ensure that a steady state is reached in the parental animals before mating.

2.2.3. *Dose-level setting*

You propose to select the dose levels based on the existing OECD TG 408 study conducted with the Substance, subject to a maximum dose of 1000 mg/kg bw/day. ECHA agrees that all available and relevant information should be taken into account.

The aim of the requested test must be to demonstrate whether the classification criteria of the most severe hazard category for sexual function and fertility (Repr. 1B; H360F) and developmental toxicity (Repr. 1B; H360D) under the CLP Regulation apply for the Substance (OECD TG 443, para. 22; OECD GD 151, para. 28; Annex I Section 1.0.1. of REACH and Recital 7, Regulation 2015/282), and whether the Substance meets the criteria for a Substance of very high concern regarding endocrine disruption according to Art.57(f) of REACH as well as supporting the identification of appropriate risk management measures in the chemical safety assessment.

To investigate the properties of the Substance for these purposes, the highest dose level must be set on the basis of clear evidence of an adverse effect on sexual function and fertility, but no deaths (i.e., no more than 10% mortality; Annex I, Section 3.7.2.4.4. to the CLP Regulation) or severe suffering such as persistent pain and distress (OECD GD 19, para. 18) in the P0 animals.

In case there are no clear evidence of an adverse effect on sexual function and fertility, the limit dose of at least 1000 mg/kg bw/day or the highest possible dose level not causing severe suffering or deaths in P0 must be used as the highest dose level. A descending sequence of dose levels should be selected to demonstrate any dose-related effect and aiming to establish the lowest dose level as a NOAEL.

In summary: Unless limited by the physical/chemical nature of the Substance, the highest dose level in P0 animals must be as follows:

(1) in case of clear evidence of an adverse effect on sexual function and fertility without severe suffering or deaths in P0 animals, the highest dose level in P0 animals must be determined based on such clear evidence, or
(2) in the absence of such clear evidence, the highest dose level in P0 animals must be set to be the highest possible dose not causing severe suffering or death, or
(3) if there is such clear evidence but the highest dose level set on that basis would cause severe suffering or death, the highest dose level in P0 animals must be set to be the highest possible dose not causing severe suffering or death, or
(4) the highest dose level in P0 animals must follow the limit dose concept.

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

Numerical results (i.e. incidences and magnitudes) and description of the severity of effects at all dose levels from the dose range-finding study/ies must be reported to facilitate the assessment of the dose level section and interpretation of the results of the main study.

2.2.4. *Cohorts 1A and 1B*

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

2.2.4.1. Splenic lymphocyte subpopulation analysis

Splenic lymphocyte subpopulation analysis must be conducted in Cohort 1A (OECD TG 443, para. 66; OECD GD 151, Annex Table 1.3).

2.2.4.2. Investigations of sexual maturation

To improve the ability to detect rare or low-incidence effects, all F1 animals must be maintained until sexual maturation to ensure that sufficient animals (3/sex/litter/dose) are available for evaluation of balano-preputial separation or vaginal patency (OECD GD 151, para. 12 in conjunction with OECD TG 443, para. 47). For statistical analyses, data on sexual maturation from all evaluated animals/sex/dose must be combined to maximise the statistical power of the study.

2.2.5. *Extension of Cohort 1B*

If the conditions of Annex X, Section 8.7.3., Column 2 are met, Cohort 1B must be extended by mating the Cohort 1B animals to produce the F2 generation.

The extension is required, among others, if the use of the Substance is leading to significant exposure of consumers or professionals (column 2, first para., point (a) of

Section 8.7.3.) and if there are indications that the internal dose for the Substance will reach a steady state in the test animals only after an extended exposure (column 2, first para., point (b), second indent of Section 8.7.3.), or 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 para., point (b), third indent of Section 8.7.3.).

The use of the Substance reported in the joint submission is leading to significant exposure of consumers because the Substance is used by consumers as fuel.

Furthermore, there is indication that the internal dose for the Substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure. Specifically, the logKow for the Substance is above 4.5 indicating potential accumulation.

Finally, there are indications of one or more modes of action related to endocrine disruption because biologically relevant changes in thyroid hormone levels (T3 and T4) are observed in the available OECD TG 408 study (2021). More specifically, a decrease of T3 was observed in males and females at 1000 mg/kg/day (0.76x and 0.74x of controls, respectively; statistically significant for females), and a significant decrease of T4 concentration (0.55x of controls) was observed in males at 1000 mg/kg/day.

You proposed to include an extension of Cohort 1B.

ECHA agrees that an extension of Cohort 1B is necessary.

Organs and tissues of Cohort 1B animals processed to block stage, including those of identified target organs, must be subjected to histopathological investigations (according to OECD TG 443, para. 67 and 72) because there is a concern for reproductive toxicity/endocrine activity indicated by the toxicity-triggers to extend the Cohort 1B.

The F2 generation must 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.

2.2.6. *Cohorts 2A and 2B*

Annex IX/X, Section 8.7.3., Column 2 provides that 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 shows evidence of thyroid toxicity, with biologically relevant changes in thyroid hormone (T3 and T4) levels (see section 2.2.5 above). This is considered a specific mechanism/mode of action with an association to developmental neurotoxicity (OECD GD 150).

According to the ECHA/EFSA Guidance[2] for the identification of endocrine disruptors, "Substances that alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment."

In your comments to the draft decision, you advance numerous reasons for why these findings would not justify the DNT cohort inclusion, which cannot however remove or resolve the particular concern on (developmental) neurotoxicity, as further explained in the following.

First, you acknowledge that the 90-day study indicates a statistically significant decrease in total T3 in females and in total T4 in males at 1000 mg/bw/day. However, you consider those observed thyroid hormone (TH) level effects secondary to the increased liver weight reported in males and females. But ECHA notes that you have not provided substance-

[2] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5311.

specific proof[1] to support your assumption that the changes in thyroid hormones level would be secondary non-specific consequences to change of the liver weight.

You also report the absence of any compensatory upregulation of TSH or induction of follicular cell hypertrophy or hyperplasia in the thyroid. And based on this lack of effects on TSH or thyroid, you argue that the reported Thyroid Hormones (TH) effects are "*mild and are of no physiological relevance and do not trigger the addition of a DNT cohort 2A and 2B in an EOGRTS*". However, it is not known what the most sensitive adverse outcome mediated via the reduced thyroid hormone levels is. Even if there were no adverse effects in thyroid, this does not exclude adverse effects in the nervous system via the same mechanism (reduced thyroid hormone levels). With respect to unaltered TSH levels vis-à-vis decreased T3 and T4 concentrations, the Substance might influence the physiological feedback mechanisms at different levels thereby altering the normal TSH response, resembling 2° hypothyroidism evidenced by reduced or normal TSH, reduced T4 and reduced or normal T3 levels.

Second, you provide the laboratory Historical Control Data (HCD) for the 90-day study. You indicate that there is a statistically significant difference between the laboratory HCD and the level of T4 following exposure of males to the Substance at 1000 mg/kg/day. The observed effects of T4 in males are outside of the HCD range (27.25 ng/mL vs 31.3 and 62.14 ng/mL, P5-P95). However, you consider that the effect in T4 level in males is "*marginal"* and "*unlikely to have any physiological consequence*".

ECHA maintains that the reported effects in T4 in males outside of the HCD are still a concern. The primary reference point should be the concurrent control data (OECD GD 43, paragraph 67). The T4 level in males and T3 level in females at 1000 mg/kg bw/d are statistically significantly lower when compared to the concurrent control in the 90-day study.

Third, you also argue that "[*w]e know from the OECD 414 PNDT in rats that there are no maternal changes in total hormone concentrations up to day 20 post-coitum in rats*." You refer to the absence of effects on T3 and T4 plasma level and the small increase of the liver weight reported in females at 1000 mg/kg/day in the PNDT study.

As already explained under section 2.2.2, reaching the steady-state in parental animals may take time due to the lipophilicity of the substance (log Kow 5.00). Based on this, the shorter exposure duration in the PNDT study may explain the absence of effects on thyroids hormones.

Finally, you argue: *"LOA does not believe that cohorts 2A and 2B in an EOGRTS with 244 TMP can distinguish between developmental neurotoxicity and the CNS effects of exposure to high concentrations of 244TMP"*.

ECHA acknowledges that it is generally not possible to distinguish the precise origin or timing of the toxicological insult if adverse neuropathological, functional, or behavioural outcome is observed in cohort 2A. This is why the RAC Guidance Note[3] specifies that any effects investigated or detected in Cohorts 2A and 2B are relevant for developmental toxicity and the respective hazard classification.

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[1] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5311
ECHA emphasises that even though the ECHA/EFSA Guidance was developed for hazard identification for endocrine-disrupting properties for other regulatory purposes, the same scientific principles apply also under the REACH Regulation

[3] RAC Guidance Note 'Addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes':
https://www.echa.europa.eu/documents/10162/17090/rac_clh_guidance_note_neurotoxicity_en.pdf/96717ed9-55d3-10e0-785b-093d07e267f3?t=1665034511575

In this context, you propose to "[p]*repare a protocol amendment to the standard EOGRTS to use animals not required to study serum free and total T4 and T3 concentrations in offspring at selected time-points in post-natal pups up to PND 28*." As part of your argumentation, you state that *"After PND 28 all CNS development is complete in rats."* This statement is scientifically unfounded, and ECHA refers to the aforementioned RAC Guidance Note, which clarifies that the nervous system continues to develop even after sexual maturation through adolescence.

For these reasons, ECHA does not agree with your justification.

For the reasons stated above, the developmental neurotoxicity Cohorts 2A and 2B must be conducted.

2.3. Outcome

Under Article 40(3)(b) your testing proposal is accepted under modified conditions, and you are requested to conduct the test with the Substance, as specified above.

2.3.1. *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 Cohort 3 if relevant information becomes available from other studies or during 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/X. 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 Guidance on IRs & CSA, Section R.7.6.

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).

All guidance documents are available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

RAAF, 2017 Read-across assessment framework (RAAF); ECHA (2017)
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs); ECHA (2017).

The RAAF and related documents are available online:
https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 16 December 2021.

ECHA held a third-party consultation for the testing proposal(s) from 15 July 2022 until 29 August 2022. ECHA did not receive information from third parties.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

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

ECHA took into account your comments and did not amend the request but amended the deadline.

In your comments on the draft decision, you requested an extension of the deadline from the date of adoption of the decision. More specifically, you provided a document from a CRO indicating that they will have the capability to perform the requested study only around 18 months after the date of adoption of the decision.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

Having further regard of the document from the CRO, ECHA has extended the deadline to 42 months.

Following the Board of Appeal's decision in cases A-002-2022 and A-003-2022 ECHA removed the request to perform additional investigations in learning and memory function as part of the information requirement of the second column of Annex IX/X, section 8.7.3.

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 3: Addressee(s) of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

Registrant name	**Registration number**	**Highest REACH Annex applicable to you**
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third-party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. Test methods, GLP requirements and reporting

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

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

(3) 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 on How to report robust study summaries[4].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

(1) Selection of the Test material(s)

The Test Material used to generate the new data must be selected taking into account the following:

- the variation in compositions reported by all members of the joint submission,
- the boundary composition(s) of the Substance,
- 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.

(2) Information on the Test Material needed in the updated dossier

- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
- The reported composition must include the careful identification and description of the characteristics of the Tests Materials in accordance with OECD GLP (ENV/MC/CHEM(98)16) and EU Test Methods Regulation (EU) 440/2008 (Note,

[4] https://echa.europa.eu/practical-guides

Annex), namely all the constituents must be identified as far as possible as well as their concentration. Also any constituents that have harmonised classification and labelling according to the CLP Regulation must be identified and quantified using the appropriate analytical methods,

With that detailed information, ECHA can confirm whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers (https://echa.europa.eu/manuals).