EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 2 June 2021

Addressees
Registrant listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
15/04/2020

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: p-tert-butylstyrene
EC number: 217-126-9
CAS number: 1746-23-2

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)

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***11 September 2023***.

The requested information must be generated using the Substance unless otherwise specified.

A. Information required from the Registrants subject to Annex IX of REACH

1. Extended one-generation reproductive toxicity study (triggered by Annex IX, Section 8.7.3., column 1; 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;
   - Dose level setting shall aim to induce systemic 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;
   - Cohorts 2A and 2B (Developmental neurotoxicity).

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

Reasons for the request(s) are explained in the following appendix:

- Appendix entitled "Reasons to request information required under Annex IX of REACH".

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(b) and 12(1) of REACH:

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

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 testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

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.

Approved[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 to request information required under Annex IX of REACH

This decision is based on the examination of the testing proposals you submitted.

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

The basic test design of an extended one-generation reproductive toxicity study (EOGRTS) is a standard information requirement under Annex IX to the REACH Regulation, 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 when the study design needs to be expanded.

ECHA considers that adverse effects on reproductive organs or tissues and other concerns in relation with reproductive toxicity are observed in available studies. More specifically, in the sub-chronic study substance-related degeneration/atrophy of seminiferous tubules was observed at terminal necropsy in 9 of 10 males at 50 mg/kg bw/day and was considered adverse. Decreases in testes weight and macroscopic observations of small testes correlated with these changes. In addition, in the OECD TG 422 study, treatment-related degeneration/atrophy of seminiferous tubules in all high dose males resulted in complete loss of fertility. Although degeneration of spermatids was only identified in one animal administered 30 mg/kg bw/day, this change was associated with decreased fertility and fecundity; therefore, testicular changes were considered adverse at or above 30 mg/kg bw/day.

As the condition of Annex IX, Section 8.7.3. column 1 is fulfilled, an EOGRTS is an information requirement for your registration.

You have submitted a testing proposal for an EOGRTS according to OECD TG 443. You have provided the following justification and specification of the study design according to the criteria described in Column 2 of Section 8.7.3, Annex IX, and detailed in ECHA Guidance R.7a: *"Classification of the substance as category 1 for toxicity on reproduction was considered. However, the effects are not sufficient for classification as category 1. Only the highest doses produced effects, which were absent in the mid- and low dose groups. - Observations in the reproductive organs and effects on reproductive parameters indicate the need for testing."* You proposed testing with the basic test design (Cohorts 1A, and 1B without extension), with the registered substance.

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.

The proposed study design requires modification to fulfil the information requirement.

Premating exposure duration and dose-level setting

You did not propose the premating exposure duration. ECHA considers that 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 R.7a

Therefore, the requested premating exposure duration is ten weeks.

In order to be compliant and not to be rejected due to too low dose levels, the highest dose

level must 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 with the other cohorts being tested at the same dose levels. 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 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.

You must provide a justification with your study report that demonstrate that the dose level selection meets the conditions described above.

Cohorts 1A and 1B

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

Cohorts 2A and 2B

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

ECHA considers that the criteria to include Cohorts 2A and 2B are met, because existing information of the Substance itself, derived from available *in vivo* studies show the following evidence: In the TG 422 study with the registered substance, toxicity was observed at doses of 30 and 60 mg/kg bw/day primarily due to reduced body weight and microscopic findings in liver, kidney and thyroid, including thyroid follicular cell hypertrophy in males from 10 mg/kg bw/day. Furthermore, in the sub-chronic toxicity study with the Substance, thyroid follicular cell hypertrophy was observed in females administered ≥10 mg/kg bw/day.

The developmental neurotoxicity cohorts 2A and 2B must be conducted because there is a particular concern on (developmental) neurotoxicity.

In your comments, you acknowledge the presence of effects on the thyroid of test animals of one species in two independent studies (OECD TG 422 and OECD TG 408). No Information is present on thyroidal hormone levels in test animals and no adverse effects on F1-Generation animals are reported by the Laboratory in TG 422 or TG 414 which are related to effects of the thyroid of the parent animals. Therefore, you question the need for this cohort (DNT). You refer to Annex IX, 8.7.3. column 2, according to which, inclusion of cohorts 2A/2B may be justified by "*Specific mechanisms/modes of action of the substance with an association to (developmental) neurotoxicity (e.g. cholinesterase inhibition or relevant changes in thyroidal hormone levels associated to adverse effects)*". You claim that because there is no information on thyroidal hormone levels, this justification does not apply. You further refer to Guidance given by EFSA indicating that "*Substances inducing histopathological changes (i.e. follicular cell hypertrophy and/or hyperplasia and/or neoplasia) in the thyroid, with or without changes in the circulating levels of THs, would pose a hazard for human thyroid hormone insufficiency in adults as well as pre- and post-natal neurological development of offspring*" and ask ECHA to give the reasoning for inclusion of cohorts 2A/2B and give appropriate arguments, which lead to the particular concern.

ECHA notes that in the ECHA Guidance, R.7a, Appendix R.7.6-2 lists examples of findings which may indicate a particular concern justifying inclusion of the DNT Cohort. Specifically, mechanism/mode of action that has been closely linked to (developmental) neurotoxic effects may be "relevant changes in thyroid hormone levels *or* signs of thyroid toxicity indicating such changes". Therefore, signs of thyroid toxicity, such as findings in weight or histopathology,

would merit as a particular concern. Furthermore, changes in thyroid histopathology are considered as sensitive, or more sensitive, than changes in thyroid hormone levels.

ECHA notes that it is not possible to conclude on the lack of thyroid related developmental neurotoxicity based on the information gained from F1-generation in OECD TG 422 and in OECD TG 414. The results from the OECD TG 422 study support the concern from the OECD TG 408 study, namely the histopathological changes in thyroid. OECD TG 414 does not have comparable exposure duration to OECD TG 443, where information is gained after exposure during all sensitive life stages.

Based on the above, the described changes in thyroid histopathology are considered as triggers for the inclusion of cohorts 2A/2B.

Species and route selection

You did not specify either the route or the species to be tested. ECHA considers that the oral route is the most appropriate route of administration, since the substance to be tested is a liquid, and according to the test method OECD TG 443, the rat is the preferred species.

Outcome

Under Article 40(3)(b) of REACH, you are requested to carry out the proposed test under modified conditions, as explained above with the Substance.

Further expansion of the study design

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B or 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. 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[2].

[2] ECHA Guidance R.7a, Section R.7.6.

Appendix B: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. 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[3].

B. Test material

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 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 all constituents of each Test material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test material is relevant for the Substance.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[4].

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

Appendix C: Procedure

ECHA held a third party consultation for the testing proposal(s) from 25 May 2020 until 9 July 2020. 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.

In your comments on the draft decision, you have requested a prolongation of the deadline, requesting a deadline of at least 28 months. You have provided a letter from your CRO, which specifies certain timelines in the overall execution of the study (21 months in total).

Furthermore, you justify your request by referring to the time needed for

i) the formation of contract between you and the CRO (1 month)
ii) the review of the reports of the Dose Range Finding (DRF) study and the main study (1 month)
iii) the review of draft study report of main study and discussion with CRO (1 month)
iv) the preparation of the study record in IUCLID (1 month)
v) the update of CSA (3 months).

ECHA has assessed your arguments. The time required for DRF is not included in the EOGRTS deadline, as you may under your own responsibility, but are not required to, perform the DRF before initiating the EOGRTS. In addition, the letter from the CRO indicates this time is included in the 21 months estimated for the study.

In conclusion, ECHA has considered your arguments and has only partially granted the request based on the indication on study duration by the CRO and set the deadline to 27 months.

ECHA took into account your comments and amended the deadline.

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 D: List of references - ECHA Guidance[5] and other supporting documents

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 where relevant.

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 where relevant.

Read-across assessment framework (RAAF, March 2017)[6]

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)[6]

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.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

[5] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[6] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

OECD Guidance documents[7]

Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

[7] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix E: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[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.