~~Confidential~~

Helsinki, 22 February 2024

Addressee(s)
Registrant(s) of JS_5384-21-4_[redacted] as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
09 February 2023

Registered substance subject to this decision ("the Substance")
Substance name: 4,4'-methylenedi-2,6-xylenol
EC/List number: 226-378-9

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 ***31 May 2027.***

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

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

1. 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 (section 2.1.3), or follow the limit dose concept. The reporting of the study must provide the justification for the setting of the dose levels;
- Cohorts 1A and 1B (Reproductive toxicity);
- Cohorts 2A and 2B (Developmental neurotoxicity); and
- Cohort 3 (Developmental immunotoxicity).

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.

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 related to the information under Annex X of REACH

1. Extended one-generation reproductive toxicity study

1 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.

1.1 Information provided to fulfil the information requirement

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

3 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.

4 ECHA agrees that an EOGRTS is necessary.

1.2 Specification of the study design

2.1.1 Species and route selection

5 You proposed testing in the rat. ECHA agrees with your proposal because the rat is the species preferred by OECD TG 443.

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

2.1.2 Pre-mating exposure duration

7 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.

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

9 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).

2.1.3 Dose-level setting

10 You propose using 1000 mg/kg bw/day as the highest dose level for the EOGRT study. You also state that *'A dose range finding study will also be conducted to confirm the final dose selection for the study'*. ECHA agrees with your intentions.

11 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.

12 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.

13 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.

14 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.

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

16 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.

2.1.4 Cohorts 1A and 1B

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

2.1.4.1 Histopathological investigations in Cohorts 1A and 1B

18 In addition to histopathological investigations of cohorts 1A, 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) if

- the results from Cohort 1A are equivocal,
- the test substance is a suspected reproductive toxicant or
- the test substance is a suspected endocrine toxicant.

2.1.4.2 Splenic lymphocyte subpopulation analysis

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

2.1.4.3 Investigations of sexual maturation

20 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.

2.1.5 Cohorts 2A and 2B

21 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.

22 Existing data on the Substance[2] (Column 2 of Annex X, Section 8.7.3.) shows a mode of action which is considered a specific mechanism/mode of action with an association to developmental neurotoxicity (OECD GD 150). More specifically, the Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats Assay (2015) and Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats Assay (2015) show thyroid toxicity (changes in thyroid hormone levels, changes in thyroid organ weight as well as histopathology).

23 In females, the following findings were reported:

- Test substance-related decreases in thyroxine (moderate; no numerical data given). This finding correlated with statistically significant increases of thyroid stimulating hormone (TSH).
- Dose-dependent increases in thyroid gland weights (up to 14%, statistically significant at high dose). These organ weight increases correlated to a dose-dependent increase in overall follicular cell height in these groups compared to controls (control average score 1.2 vs. high dose average score 1.9).
- Dose-dependent, minimal to slight increase in the average score of follicular cell height with a corresponding decrease in colloid area when compared to the controls (control average score 4.7 vs. high dose average score 4.2).

24 In males, the following findings were reported:

- Test substance-related decreases in thyroxine (moderate; no numerical data given). There were no statistically significant differences in mean thyroid stimulating hormone (TSH) values between control and test substance exposed F1 males.
- Dose-dependent increases in thyroid gland weights (up to 29%, statistically significant at high dose). These organ weight increases correlated to a dose-dependent minimal to slight increase in overall follicular cell height in these groups compared to controls (control average score 2.2 vs. high dose average score 3.1).
- Dose-dependent, minimal to slight increase in the average score of follicular cell height with a corresponding decrease in colloid area (control average score 3.4 vs. high dose average score 3.1).

25 Disturbance of the thyroid hormonal system is considered a specific mechanism/mode of action with an association to developmental neurotoxicity (OECD GD 150). ECHA notes that development of the nervous system involves developmental processes that occur both pre-natally and post-natally, continuing to develop even after sexual maturation though adolescence[3]. Therefore, disturbance of the thyroid hormonal system observed in juvenile animals are a particular concern on developmental neurotoxicity.

[2] https://echa.europa.eu/registration-dossier/-/registered-dossier/5565/7/10/3
[3] RAC Guidance Note Addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes

26 You proposed not to include Cohort 2A and 2B, indicating that "the substance is not known to have any mode of action associated with neurotoxicity such as cholinesterase inhibition and thyroid toxicity'.

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

2.1.6 Cohort 3

28 The developmental immunotoxicity Cohort 3 needs to be conducted in case of a particular concern on (developmental) immunotoxicity, including evidence of adverse effects on the immune system in studies on adult animals or animals exposed prenatally.

29 Existing information (OECD TG 408[4]) on the Substance shows evidence of immunotoxicity. Specifically, the following histopathological effect is reported in the animals exposed to the Substance:

a dose-related increase in thymic epithelial cell proliferation was observed in females at 300 and 750 mg/kg bw/day; the dose-dependent effect was both in terms of the incidence and severity.

30 The thymus is an organ of the immune system, and thymic epithelial cells play a key role in the function of the immune system; they are essential during the immune adaptive period for evolution of self versus non-self recognition (Taub and Longo[5]).

31 Histopathological effects in adult animals on such a critical part of the thymus responsible for proper functioning of adaptive immunity are substance-specific findings which are "One severe statistically and/or biologically significant organ weight or histopathological finding related to an immunology organ" (ECHA R.7a, p.531).

32 The above changes in histopathological parameters indicate that the Substance may cause toxicity to the immune system. Therefore, there is a particular concern on (developmental) immunotoxicity.

33 Triggers can also come from specific mode of action information on the Substance which is associated with developmental immunotoxicity.

34 The Substance itself shows estrogenic/ androgenic/ steroidogenic (EAS) activity which are considered specific mechanisms/modes of action with an association to (developmental) immunotoxicity. The EAS activity is demonstrated by *in vitro* effects, effects in fish and effects in the OECD TG 408 study in rats. Specifically, *in vitro* findings of EAS activity include increased production of testosterone and 17β-oestradiol in a steroidogenic assay according to TG 456[6], and antagonistic activity on estrogen receptors (ER) and androgen receptor (AR)[7]. In an OECD TG 229 study in fathead minnows, the Substance showed effects on EAS-mediated and EAS-sensitive parameters including ovarian atresia, interstitial cell hyperplasia in testes, and increased plasma vitellogenin in males[8]. In the OECD TG 408 study in rats, referenced above, the dose-related finding of follicular ovarian cysts shows EAS activity *in vivo*.

35 The ECHA Guidance R.7a, p. 531 specifies that the DIT cohort could be triggered based on "information on hormonal mechanisms/modes of action with clear association with the

[4] As described in the IUCLID dossier; in Maffini and Canatsey (2020) Food Chem Toxicol. https://doi.org/10.1016/j.fct.2019.110889; and in the full study report https://sherwin-williams.app.box.com/s/yf7t38ohj95zfonbtojddwgblbduw4dw/file/1183353548036

[5] Taub, D. D., & Longo, D. L. (2005). Insights into thymic aging and regeneration. Immunological reviews, 205(1), 72-93

[6] Maffini and Canatsey (2020), https://doi.org/10.1016/j.fct.2019.110889

[7] Szafran et al. (2017), https://doi.org/10.1371/journal.pone.0180141

[8] https://sherwin-williams.app.box.com/s/yf7t38ohj95zfonbtojddwgblbduw4dw/file/647377099397

immune system, such as oestrogenicity (Adori et al., 2010) and androgenicity (Trigunaite et al. 2015)". As indicated above, *in vitro* and *in vivo* data are available showing that the Substance has EAS activity, which is a mode of action associated with immunotoxicity. Therefore, for this basis also, there is a particular concern on developmental immunotoxicity.

36 For the reasons stated above, the developmental immunotoxicity Cohort 3 must be conducted.

2.1.7 Outcome

37 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.

2.1.8 Further expansion of the study design

38 The conditions to include the extension of Cohort 1B are currently not met. However, you may expand the study by including the extension of Cohort 1B 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 (2023).
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 3 May 2023, following the necessary clarifications concerning your registration, namely the provision of the Sub-chronic toxicity study requested in a previous CCH decision[9].

ECHA held a third-party consultation for the testing proposal(s) from 3 April 2023 until 22 May 2023. ECHA did not receive information from third parties.

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

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.

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

ECHA did not receive any comments within the commenting period.

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

ECHA received proposal(s) for amendment and did not modify the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee unanimously agreed on the draft decision as modified during its MSC-85 meeting. ECHA adopted the decision under Article 51(6) of REACH.

[9] https://echa.europa.eu/documents/10162/e668c8c4-5e0b-9eb3-7ca0-7a033ada2b25

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 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]

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[10].

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

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

This information is needed to assess 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[11].

2. General recommendations for conducting and reporting new tests

References to Guidance on REACH and other supporting documents can be found in Appendix 1.

[11] https://echa.europa.eu/manuals