~~Confidential~~

Helsinki, 11 June 2021

Addressees
Registrant(s) of JS_202-461-5 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
09/04/2018

Registered substance subject to this decision ("the Substance")
Substance name: 2,5-xylenol
EC number: 202-461-5
CAS number: 95-87-4

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***20 June 2022***.

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

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

1. In vivo mammalian erythrocyte micronucleus test, oral route; or In vivo mammalian bone marrow chromosomal aberration test, oral route; or In vivo mammalian alkaline comet assay, oral route, on the following tissues: liver, glandular stomach and duodenum (triggered by Annex VIII, Section 8.4., column 2).

2. Short-term repeated dose toxicity (28 days; Annex VIII, Section 8.6.1.) to be combined with the Screening for reproductive/developmental toxicity below

3. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.64/OECD TG 422) by oral route, in rats

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

- Appendix entitled "Reasons common to several requests";
- Appendix entitled "Reasons to request information required under Annexes VIII of REACH", respectively.

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(a) and 12(1) of REACH:

- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa.

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 testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations 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, 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 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 on Reasons common to several requests

1. **Assessment of your weight of evidence adaptation under Annex XI, Section 1.2**

You have adapted the following standard information requirements by applying weight of evidence approaches in accordance with Annex XI, Section 1.2:

- In vivo mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2)
- Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)

ECHA has considered the scientific and regulatory validity of your weight of evidence approach in general before assessing the specific standard information requirements in the following appendices.

Annex XI, Section 1.2 states that there may be sufficient weight of evidence weight of evidence from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude that the Substance has or has not the (dangerous) property investigated by the required study.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence adaptation.

You have summarised the sources of information for each endpoint in relation to the relevance, reliability, and consistency of the results and conclude that as a weight of evidence based on the available sources of information, no further studies are needed.

ECHA has assessed the validity of your adaptation and identified the following issues:

Your weight of evidence adaptation has deficiencies that are common to all information requirements under consideration and deficiencies that are specific for these information requirements individually. The common deficiencies are set out here, while the specific ones are set out under the information requirement concerned in the Appendix A below.

Reliability of the provided information with analogue substances

Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

Additional information on what is necessary when justifying a read-across approach can be

found in the ECHA Guidance[2].

You have provided a read-across justification document in IUCLID Section 13.

You read-across between the structurally similar substances:
- 2,4-xylenol (EC No. 203-321-6),
- 2,6-xylenol (EC No. 209-400-1),
- 3,5-xylenol (EC No. 203-606-5), and
- mixed xylenols (numerical identifier not assigned)

as source substances and the Substance as target substance.

You have provided the following reasoning for the prediction of toxicological properties: *"The analogue approach is based on the very close structural similarity of source and target substances (isomers). The common structural elements are the phenol moiety and the two methyl groups",* and that *"the OECD QSAR Toolbox profiling in combination with similar hazard profiles shows that there is a common mode of action in the case of source and target chemicals, and therefore the read-across strategy described in this document is justified.".*

Furthermore, you have indicated that "*Quantitative variations in biological effects within the chemical category are considered to be associated with differing steric hindrance of the reactive phenolic hydroxyl group, dependent on the relative position of the methyl groups on the benzene ring. The quantitative variation in biological effects of members of the chemical category is generally not considered to form a regular pattern. Therefore, the prediction is based on a worst-case approach for specific endpoints. This means that the source chemical for the specific endpoint is the xylenol isomer with the maximum strength of toxicity effects observed."*

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.mes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assu The properties of your Substance are predicted based on a based on a worst-case approach.

ECHA notes the following shortcoming with regards to predictions of toxicological properties.

Supporting information to substantiate your worst-case considerations

Annex XI, Section 1.5 of the REACH Regulation states that "*physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)*". For this purpose, "*it is important to provide supporting information to strengthen the rationale for the read-across*"[3]. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

As indicated above, your read-across hypothesis is based on the assumption that the source substances constitutes a worst-case for the prediction of the property under consideration of the Substance. In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to

[2] ECHA Guidance R.6
[3] ECHA Guidance R.6: QSARs and grouping of Chemicals, Section R.6.2.2.1.f

confirm a conservative prediction of the properties of the Substance from the data on the source substance(s). Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

With regard to your predictions for *in vivo* mammalian erythrocyte micronucleus test and for short-term repeated-dose toxicity (28 day), you have provided certain information to support your hypothesis, as described under the relevant information requirement sections under Appendix A, sections 1 and 2. However, this information provided in your dossier and the justification document does not support your hypothesis of worst-case predictions. The specific reasons are explained under Appendix A, sections 1 and 2.

As there is no supporting information that would substantiate your hypothesis of the worst-case, the information from the analogue substances submitted under your weight of evidence adaptation is not considered reliable.

Additional issues related to weight of evidence are addressed under the corresponding endpoints.

Information from your comments to the draft decision

In your comments to the draft decision you have commented on the deficiencies noted by ECHA in the draft decision.
You stated that you agree with ECHA that the database provided was insufficient for a sound weight of evidence and that you intend to extend the weight of evidence and extensively broaden the database. In addition to the weight of evidence initially proposed for the *in vivo* mammalian erythrocyte micronucleus test and short-term repeated dose toxicity (28-day), you also intend to adapt the information requirement of the screening for reproductive/developmental toxicity study by this new weight-of-evidence approach. You have extended the weight of evidence for these information requirements by providing information on the studies conducted with the additional analogue substances not included in the initial submission or in the registration dossier.

In the comments you have stated that you consider that the presentation of the initial read-across data lead to the discussion being focussed on the worst-case hypothesis, aspects of structural hindrance and QSAR profiles and this was considered misleading. To avoid further confusion, you intend to present "*substantial amendments and adjustments to the previous justification*". You provided a list of new analogue substances and corresponding studies for the endpoints genotoxicity, repeated dose toxicity and toxicity to reproduction/developmental toxicity in a high-level data matrix. The reporting of all studies is generally limited to a study name and the results.

We have identified the following issues with regard to the information provided in your comments:

Your comments on the deficiencies noted in the draft decision on adaptations present in your dossier are addressed in the relevant sections below (Appendix A, sections 1 and 2).

Regarding your intention to extend your weight of evidence to other analogues and the information requirement of the screening for reproductive/developmental toxicity study, ECHA notes the following:

Firstly, you did not provide information on how you intend to integrate the additional information in the weight of evidence approach and why the sources of information provide sufficient weight of evidence leading to the conclusion that the Substance has or has not the

particular dangerous properties as investigated by the required studies to fulfil the information requirements.

Secondly, you did not provide the necessary information for the read-across approach of the additional analogue substances, including no hypothesis and no explanation of the rationale for prediction of properties. Therefore, ECHA cannot verify that the properties of your Substance can be predicted from the data on the additional source substance(s).
Furthermore, you did not provide robust study summaries containing information on the study design of the new studies referred to in your comments and described in a very limited manner in the high-level data matrix. Therefore ECHA cannot assess the reliability of the studies.

Due to the above, ECHA cannot assess your intended extension of the weight of evidence approach. With the limited information provided in your comments you have not demonstrated that the information provided with the new analogue substances could reliably contribute to the weight of evidence approach.

Finally, although you expressed the intention to present substantial amendments to the original read-across justification contained in your dossier, including your worst-case hypothesis, you have not done so.

The information you provide in your comments is not affecting the current assessment of the information in your dossier.

Appendix A: Reasons to request information required under Annex VIII of REACH

1. **In vivo mammalian erythrocyte micronucleus test; or In vivo mammalian bone marrow chromosomal aberration test or In vivo mammalian alkaline comet assay**

Under Annex VIII, Section 8.4, column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

Your dossier contains positive results for the *in vitro* cytogenicity test conducted with the test substance mixed xylenols (numerical identifier not provided). Based on this study, as a worst-case prediction, you consider that the Substance is also positive for *in vitro* cytogenicity and concluded that "*2,5-Xylenol (CAS 95-87-4) is considered to induce chromosome aberrations in vitro*". This information raises the concern for chromosomal aberration for the Substance.

You have adapted this information requirement by using a Weight of evidence approach under Annex XI, Section 1.2.

Your dossier contains the following sources of information on analogue substances:

i. Mammalian Erythrocyte Micronucleus Test (OECD TG 474; [redacted], 1998) conducted with conducted with 2,4-xylenol (EC No. 203-321-6)

ii. Mammalian Erythrocyte Micronucleus Test (OECD TG 474; [redacted] 1998) conducted with conducted with 3,5-xylenol (EC No. 203-606-5)

iii. Mammalian Erythrocyte Micronucleus Test (OECD TG 474; [redacted] 1998) conducted with conducted with 2,6-xylenol (EC No. 209-400-1)

As explained under Appendix on Reasons common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

In case of cytogenicity concern, relevant information that can be used to support weight of evidence adaptation for information requirement of column 2, Section 8.4, at Annex VIII includes similar information that is produced by a test performed according to the OECD TG 489, OECD TG 474 or OECD TG 475. This includes:

- the detection and quantification of cytogenicity, i.e. the determination of the frequency of cells with micronuclei or chromosomal aberrations in cells isolated from blood or tissues in mammals exposed *in vivo* to the test material (OECD TG 474 or 475, respectively).
- the detection and quantification of DNA strand breaks in cells or nuclei isolated from different tissues in mammals exposed *in vivo* to the test material (OECD TG 489).

The sources of information (i-iii) provide relevant information on detection and quantification of micronuclei in mammalian cells (*in vivo*).

However, as indicated under the 'Appendix on Reasons common to several requests', the information from the analogue substances (sources of information (i-iii)) is not considered reliable. The specific reasons are explained below.

Supporting information to substantiate your worst-case considerations

As indicated under the 'Appendix on Reasons common to several requests' your read-across hypothesis is based on the assumption that the source substances constitute a worst-case for the prediction of the property under consideration of the Substance. In this context, relevant,

reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm a conservative prediction of the properties of the Substance from the data on the source substance(s).

You have provided information from the OECD Toolbox structural profilers for the genetic toxicity that indicate structural features with concern for cytogenicity (protein binding alerts for Chromosomal aberration by OASIS).

You have further supported your worst-case predictions with the following experimental data:

In the registration dossier, you have provided positive *in vitro* cytogenicity study with the mixed xylenol containing [redacted]% of the target substance. In addition to the Substance, the test material contains [redacted]% of the other five xylenol isomers (2,3-, 2,4-, 2,6-, 3,4- and 3,5-xylenol). Based on this study, as a worst-case prediction, you consider that the Substance is also positive for *in vitro* cytogenicity.

To follow up this positive *in vitro* prediction, you have provided three negative *in vivo* studies conducted with the source substances 2,4-, 2,6- and 3,5-xylenol.

Based on the information above, you conclude that "*2,5-xylenol was therefore not considered to be genotoxic due to the consistently negative in vivo results of the source substances which omit the positive results from the in vitro mammalian chromosome aberration*".

According to your read-across hypothesis the position of the substituents and the related steric hindrance can affect the properties of the substance.

The hypothesis related to the position of the substituents is corroborated by the information provided from the *in vitro* chromosome aberration studies. The *in vitro* chromosome aberration study conducted with mixed xylenol was positive with and without metabolic activation (S9). On the other hand, the *in vitro* chromosome aberration study on the source substance 2,6-xylenol – as corrected in your comments to the draft decision – was positive with S9 but negative without S9. This indicates that some of the isomers present in the source substance mixed xylenol will induce chromosome aberrations without metabolic activation, while others are active only with S9, and that there are differences between the xylenol isomers on the potential to be converted into the active intermediates that cause cytogenicity.

It is not possible to say which constituents(s) tested in the *in vitro* cytogenicity study conducted with the mixed xylenols (source) cause positive results with and without S9.

No information (*in vitro* or *in vivo*) is available on the potential of phenols with the steric hindrance originating from the position 2 and 5 in phenolic ring present in the target substance, to induce chromosome aberrations. Furthermore, the provided structural alerts, while indicating structural features of concern for cytogenicity, do not inform on potential quantitative differences between the isomers. Therefore, it cannot be ruled out that the target substance with the steric hindrance at positions 2 and 5 have different properties than the source substances 2,4-, 3,5- or 2,6-xylenol.

In conclusion, the provided structural profilers, *in vitro* and the *in vivo* data do not allow to conclude that the source substances would represent the worst-case for the target substance.

In the absence of information addressing the impact of the position of the hydroxyl groups on the claimed steric hindrance affecting the cytogenicity of the substances, your worst-case prediction for the in vivo genotoxicity is not supported.

Therefore, the information from the analogue substances submitted under your weight of evidence adaptation is not considered reliable.

Taken together, the sources of information as indicated above provide information on detection and quantification of cytotoxicity and the frequency of cells with chromosomal aberrations or micronuclei in mammalian cells following *in vivo* exposure. However, due to significant reliability issues, they cannot contribute to the conclusion on the potential of the Substance to cause chromosomal aberrations or micronuclei in mammalian cells following *in vivo* exposure.

Conclusion

It is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in *in vivo* mammalian erythrocyte micronucleus test; or *in vivo* mammalian bone marrow chromosomal aberration test or *in vivo* mammalian alkaline comet assay.

Therefore, your adaptation is rejected, and the information requirement is not fulfilled.

Information on the study design

i. Test selection

According to the ECHA Guidance Chapter R.7a, Section R.7.7.6.3, the mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) or the mammalian bone marrow chromosomal aberration test ("CA test", OECD TG 475) are suitable to follow up a positive *in vitro* result on chromosomal aberration if the Substance or its metabolite(s) will reach the target tissue. Alternatively, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is a suitable test to be performed. Therefore, the MN test, the CA test and the comet assay are suitable tests to follow up the chromosomal aberration concern identified for the Substance.

ii. Test design

In case you decide to perform a MN or CA test, according to the test method OECD TG 474 / OECD TG 475, the test must be performed in mice or rats. Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

Regarding the exposure of the target tissue, the applicable test guideline (OECD TG 474 / OECD TG 475) states "If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test". Additionally, a negative test result can be considered reliable if "Bone marrow exposure to the test substance(s) occurred". Accordingly, if the Substance is negative in this test, but it is not possible to demonstrate that bone marrow exposure to the Substance occurred, then ECHA will consider any remaining uncertainty concerning the mutagenic potential of the Substance and whether to request any further information.

In case you decide to perform the comet assay according to the test method OECD TG 489, the test must be performed in rats. Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as

sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

iii. Germ cells

In case you decide to perform the comet assay, you may consider to collect the male gonadal cells collected from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay you should consider analysing the slides prepared with gonadal cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

2. Short-term repeated dose toxicity (28 days)

A Short-term repeated dose toxicity study (28 days) is a standard information requirement in Annex VIII to REACH.

You have adapted this information requirement by using a Weight of evidence approach under Annex XI, Section 1.2.

Your dossier contains the following sources of information:

i. Repeated dose 28-day oral toxicity study in rodents (OECD TG 407; [redacted] 1993) conducted with 3,5-xylenol (EC No. 203-606-5);

ii. Combined repeated dose and reproduction / developmental screening study (OECD TG 422; [redacted] 2005) conducted with mixed xylenols (numerical identifier not provided).

In addition, your justification document for the use of data on the analogue substances contain a summary of:

iii. Repeated dose 28-day oral toxicity study in rodents (OECD TG 407; [redacted] 2005) conducted with 2,4-xylenol (EC No. 203-321-6).

As explained under Appendix on Reasons common to several requests, the weight of evidence adaptation must fulfil the information requirement based on relevant and reliable sources of information. These sources of information must provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

Relevant information that can be used to support weight of evidence adaptation for information requirement of Section 8.6.1 at Annex VIII includes, at general level, information on systemic toxicity in intact, non-pregnant and young adult males and females from:

1) in-life observations,
2) blood chemistry,
3) organ and tissue toxicity. Information should address effects on the following physiological systems: circulatory system, digestive/excretory system, endocrine system, immune system, integumentary system, musculoskeletal system, nervous system, renal/urinary system, reproductive system, and respiratory system.

This information is covered by information similar to the information obtained from OECD TG 407/422.

The sources of information in the dossier provide relevant information on all aspects of in-life observations, blood chemistry as well as organ and tissue toxicity. The source of information (iii) describes a study conducted according to an OECD TG 407 and therefore, it is also considered to provide relevant information for this weight of evidence approach.

However, as indicated under the 'Appendix on Reasons common to several requests', the information from the analogue substances (sources of information (i-iii)) is not considered reliable in the context of this weight of evidence approach. The specific reasons are explained below.

Supporting information to substantiate your worst-case considerations

As indicated under the 'Appendix on Reasons common to several requests' your read-across hypothesis is based on the assumption that the source substances constitute a worst-case for the prediction of the property under consideration of the Substance. In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm a conservative prediction of the properties of the Substance from the data on the source substance(s).

You have provided information from the OECD Toolbox structural profiler for the repeated-dose toxicity which indicates systemic toxicity (hepatotoxicity and irritation) concerns for the Substance and the source substances.

You further support your worst-case predictions with the following experimental data:

In the registration dossier, you have provided a combined repeated dose and reproduction / developmental screening (OECD TG 422) conducted with mixed xylenol containing [redacted]% of the target substance. In addition to the Substance itself, the test material contains [redacted]% of the other five xylenol isomers (2,3-, 2,4-, 2,6-, 3,4- and 3,5-xylenol). In this study, the general toxicological no observable adverse effect level (NOAEL) was reported at 100 mg/kg bw/day based on increased kidney, liver and ovarian relative weight at 245 mg/kg bw/day (appr 40 mg/kg bw/day of the target). You indicated that "*The lowest NOAEL, found from all repeated dose studies, of 100 mg/kg bw/day was taken as basis for a worst-case risk assessment*."

In addition, in your registration dossier and in the read-across justification document, you have provided short-term repeat dose toxicity studies (OECD TG 407) with the source substances 3,5-xylenol and 2,4-xylenol, respectively. In these studies, changes in testis and epididymis weights were observed at the highest dose of 300 mg/kg bw/day. However, no effects were reported on ovaries shown to be affected in the OECD TG 422 study conducted with the source substance mixed xylenol.

Different target organs were identified in the above described studies conducted with the source substances. This indicates that the types of toxicity within the complex endpoints of systemic toxicity and thereby the toxicity profile may vary between the xylenol isomers.

Effects on ovaries were reported in the study conducted with source substance mixed xylenols containing [redacted]% of the Substance. However, effects on ovaries were not seen in the studies conducted with source substances 3,5-xylenol and 2,4-xylenol. Therefore, it can be assumed that the constituent(s) of the mixed xylenols other than the 3,5-xylenol and 2,4-xylenol have effects on ovaries. It is not possible to say which constituent(s) from the mixed xylenols tested in the OECD 422 study cause these effects. There are no substance specific data available on the Substance following repeated exposure to allow comparison. Furthermore, the complexity

of the systemic interactions and the large number of targets/mechanisms associated with the broad area of systemic toxicity following repeated exposure is not covered by computational tools (structural profilers) to allow reliable comparisons. Therefore, it cannot be ruled out that the effects observed with the source substance mixed xylenol could be caused by exposure to the Substance.

In your comments to the draft decision you argue that the duration of dosing period for the OECD TG 422 study (ii) was longer than for the OECD TG 407 studies (i) and (iii), and that therefore "*the increase in ovary weight may be due to the prolonged exposure time for females in the OECD 422-screening study. Moreover, as the quantitative degree on the organ-weights is not known from the data available, since histological changes were not present, the toxicological relevance at the high doses (≥ 245 –300 mg/kg bw/d) can be questioned*".

ECHA acknowledges that the exposure duration is different in females between the OECD TG 407 and 422 and that this difference may contribute to the differences in findings reported in the OECD TG 407 and the OECD TG 422 studies. However this does not remove the concern that the potential effects observed in the ovaries could be due to the exposure to the Substance present in the composition of the mixed xylenols. The dose levels of the Substance that the experimental animals were exposed in the OECD TG 422 study conducted with the mixed xylenol were only approximately 40 mg/kg bw/day. Therefore, it cannot be excluded that the effects on ovaries could be evident in a 28-day study if the animals were treated with the Substance up to the dose levelssimilar to those used for 3,5-xylenol and 2,4-xylenol, i.e. 300 mg/kg bw/day.

In conclusion, you have not provided supporting data to demonstrate that the source substance mixed xylenol will present a worst case for the Substance. Most importantly, based on the provided information, it is not possible to determine if other and/or more severe effects would be observed when the Substance is tested at the higher doses than when present only at 16% in the test substance.

Therefore, the information from the analogue substances submitted under your weight of evidence adaptation is not considered reliable.

Taken together, the sources of information as indicated above provide information on 1) in-life observations, 2) blood chemistry, 3) organ and tissue toxicity. However, due to significant reliability issues, they cannot contribute to the conclusion on the potential of the Substance to cause short-term repeated dose toxicity (28 days).

Conclusion

It is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated short-term repeated dose toxicity (28 days).

Therefore, your adaptation is rejected, and the information requirement is not fulfilled.

When there is no information available neither for the 28-day repeated dose toxicity endpoint (EU B.7, OECD TG 407), nor for the screening study for reproductive/developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided. Such an approach offers the possibility to avoid carrying out a 28-day study according to OECD TG 407, because the OECD TG 422 can at the

same time fulfil the information requirement of REACH Annex VIII, 8.6.1 and that of REACH Annex VIII, 8.7.1.[4]

Information on the study design
Referring to the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity, because the Substance is handled and marketed only in a molten form and therefore, the exposure via the inhalation route is unlikely. Furthermore, no repeated dose toxicity study by the oral route is available.

Therefore, the study according to the test method EU B.64/OECD TG 422 must be performed in rats and with oral administration of the Substance.

3. Screening for reproductive/developmental toxicity

A Screening for reproductive/developmental toxicity study (test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) is a standard information requirement under Annex VIII to REACH, if there is no evidence from analogue substances, QSAR or *in vitro* methods that the Substance may be a developmental toxicant. There is no information available in your dossier indicating that your Substance may be a developmental toxicant.

You have adapted this information requirement by using a read-across approach under Annex XI, Section 1.5.

Your dossier contains the following study:

i. Combined repeated dose and reproduction / developmental screening study (OECD TG 422; [redacted] 2005) conducted with mixed xylenols (numerical identifier not provided)

We have assessed this information and identified the following issue:

Grouping of substances and read-across approach

Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (addressed under 'Predictions for toxicological properties').

Additional information on what is necessary when justifying a read-across approach can be found in the ECHA Guidance R.6. and related documents[5,6].

A. Predictions for toxicological properties

You have provided a read-across justification document in IUCLID Section 13.

You read-across between the structurally similar substance mixed xylenols (numerical identifier not assigned) as source substance and the Substance as target substance.

[4] ECHA Guidance R.7a., Section R.7.6.2.3.2.
[5] Read-Across Assessment Framework (RAAF). 2017. Available online: Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across)
[6] Read-across assessment framework (RAAF) - considerations on multi-constituent substances and UVCBs. 2017. Available online: https://doi.org/10.2823/794394

You have provided the following reasoning for the prediction of toxicological properties: *"The analogue approach is based on the very close structural similarity of source and target substances (isomers). The common structural elements are the phenol moiety and the two methyl groups",* and that *"the OECD QSAR Toolbox profiling in combination with similar hazard profiles shows that there is a common mode of action in the case of source and target chemicals, and therefore the read-across strategy described in this document is justified.".*

Furthermore, you have indicated that "*Quantitative variations in biological effects within the chemical category are considered to be associated with differing steric hindrance of the reactive phenolic hydroxyl group, dependent on the relative position of the methyl groups on the benzene ring. The quantitative variation in biological effects of members of the chemical category is generally not considered to form a regular pattern. Therefore, the prediction is based on a worst-case approach for specific endpoints. This means that the source chemical for the specific endpoint is the xylenol isomer with the maximum strength of toxicity effects observed.".*

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.mes that different compounds have the same type of effects.assumes that different compounds have the same type of effects.assu The properties of your Substance are predicted based on a worst-case approach.

ECHA notes the following shortcoming with regards to predictions of toxicological properties.

Supporting information to substantiate your worst-case considerations

Annex XI, Section 1.5 of the REACH Regulation states that "*physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)*". For this purpose, "*it is important to provide supporting information to strengthen the rationale for the read-across*"[7]. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

As indicated above, your read-across hypothesis is based on the assumption that the source substance constitutes a worst-case for the prediction of the property under consideration of the Substance. In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm a conservative prediction of the properties of the Substance from the data on the source substance(s). Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

You have provided information from the OECD Toolbox structural profilers for the developmental toxicity and teratogenicity, reproductive toxicity as well as for retinoic acid and estrogen receptor binding which indicate structural features with reproductive and developmental toxicity concern.

You further supported your worst-case predictions with the following experimental data:

[7] ECHA guidance R.6, Section R.6.2.2.1.f

The provided source study was conducted with mixed xylenol containing [redacted]% of the Substance. In addition to the Substance itself, the test material contains [redacted]% of the other five xylenol isomers (2,3-, 2,4-, 2,6-, 3,4- and 3,5-xylenol). In this study, reduced mating frequency was reported at the highest dose of 245 mg/kg bw/day (appr 40 mg/kg bw/d of the target). The number of female rats that mated was non-significantly reduced from 100% in the control group (10/10) to 80% at 245 mg/kg bw (8/10), and was within the historical control range that corresponds to the time when the study was conducted.

Furthermore, as explained under the 'Short-term repeated dose toxicity' above (Appendix A. Section 2.), the provided study with the mixed xylenol showed changes in the relative weights of ovaries, not observed in studies conducted with 3,5-xylenol and 2,4-xylenol (a particular constituents of the xylenol mix).

There are no substance-specific experimental data available on the Substance following repeated exposure to allow comparison. Furthermore, the complexity of the systemic interactions in reproductive and developmental toxicity and the large number of targets/mechanisms associated with those broad areas of toxicity is not covered by computational tools (profilers) to allow reliable comparisons. Therefore, it cannot be ruled out that the effects observed with the source substance mixed xylenol could be caused by exposure to the Substance.

In conclusion, you have not provided supporting data to demonstrate that the source substance mixed xylenol will present a worst case for the Substance. Most importantly, based on the provided information, it is not possible to determine if other and/or more severe effects would be observed when the Substance is tested at the higher doses than when present only at 16% in the test substance.

In the absence of such information, you have not established that the Substance and of the source substance(s) are likely to have similar properties. Therefore, you have not provided sufficient supporting information to strengthen the rationale for the read-across.

B. Conclusions on the read-across approach

As explained above, you have not established that relevant property of the Substance can be predicted from data on the analogue substance. Therefore, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. and your grouping and read-across approach is rejected.

Therefore, the information requirement is not fulfilled.

When there is no information available neither for the 28-day repeated dose toxicity endpoint (EU B.7, OECD TG 407), nor for the screening study for reproductive/developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided. Such an approach offers the possibility to avoid carrying out a 28-day study according to OECD TG 407, because the OECD TG 422 can at the same time fulfil the information requirement of REACH Annex VIII, 8.6.1 and that of REACH Annex VIII, 8.7.1.[8]

Information on the study design
A study according to the test method EU B.64/OECD TG 422 must be performed in rats with oral[9] administration of the Substance.

[8] ECHA Guidance R.7a., Section R.7.6.2.3.2.
[9] ECHA Guidance R.7a, Section R.7.6.2.3.2.

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[10].

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[11].

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

Appendix C: Procedure

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

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

The compliance check was initiated on 28 July 2020.

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

ECHA took into account your comments and did not amend the request(s) or 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[12] 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)[13]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[13]

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.

OECD Guidance documents[14]
Guidance Document on aqueous–phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

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

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.

Appendix E: Addressees of this decision and their corresponding information requirements

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.