Helsinki, 03 April 2020

Addressees
Registrants of 905-588-0 (LOA Reaction mass) listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of this decision
18/09/2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: reaction mass of ethylbenzene and xylene
List number: 905-588-0
CAS number: NS

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

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***11 July 2022***.

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

1. Justification for an adaptation of a Short-term repeated dose toxicity (28-day), (Annex VIII, Section 8.6.1.) based on the study requested under Section B.1

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

1. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method OECD TG 408) in rats with the Substance

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

C. Requirements applicable to all the Registrants subject to Annex X of REACH

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

Conditions to comply with the requests

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.

To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annexes VII and VIII of REACH, if you have registered a substance at 10-100 tpa;
- you have to comply with the requirements of Annexes VII, VIII and IX of REACH, if

you have registered a substance at 100-1000 tpa;

- you have to comply with the requirements of Annexes VII to X of REACH, if you have registered a substance at above 1000 tpa.

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

The Appendix on general considerations addresses issues relevant for several requests while the other Appendices state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH.

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

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

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

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 on general considerations

(i) Assessment of the Grouping of substances and read-across approach, in light of the requirements of Annex XI, Section 1.5.

You seek to adapt the following standard information requirements by applying (a) read-across approach(es) in accordance with Annex XI, Section 1.5:

- Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
- Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study in a first species (Annex IX, Section 8.7.2.)

ECHA has considered the scientific and regulatory validity of your read-across approach(es) in general before assessing the specific standard information requirements in the following appendices.

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.

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

You read-across between ethylbenzene with EC No. 202-849-4 (CAS No. 100-41-4) as source substance and the Substance as target substance. You have provided a read-across justification document in IUCLID Section 13 entitled "[redacted]".

In addition, in your technical dossier you also report the result of studies conducted with other substances identified as "*mixed xylenes / 924-522-1; xylene / 1330-20-7 / 215-535-7*" hereafter called "mixed xylenes". You indicate that the corresponding test materials are "technical grade" mixed xylenes and you report various composition as summarized in the following table 1. The substance identity profile (SIP) of the Substance is broadly defined as follows: Mixed xylene (EC 215-535-7): 30-90%; Ethylbenzene (EC 202-849-4): 10-70%; Benzene (EC 200-753-7): < 0.1%; Isopropylbenzene (EC 202-704-5): < 3%; Toluene (EC 203-625-9): < 2%. For some of these test materials it is not possible to verify if their composition falls within the SIP of the Substance either because insufficient information is provided or because the SIP of the Substance does not include a clear description of the isomeric composition. Other test substances can be concluded to be outside the SIP of the Substance. Therefore, these test substances are regarded as analogue substances.

In your comments on the draft decision, you have provided a read-across justification document entitled "[redacted]". The new information provided in this document is discussed below under section A. 'Prediction of toxicological properties'.

Table 1. Composition of test materials referred to as "*mixed xylenes / 924-522-1; xylene / 1330-20-7 / 215-535-7*"

Test material	Reference	Composition				
		m-xylene	p-xylene	o-xylene	ethylbenzene	other constituents
MX no. 1						
MX no. 2						
MX no. 3						
MX no. 4						
MX no. 5						
MX no. 6						
MX no. 7						
MX no. 8						
MX no. 9						
MX no. 10						
MX no. 11						

n/a: no information available. * m-xylene and p-xylene were co-eluted.

Based on the above we understand that you seek to adapt the standard information requirements listed above using data on various analogue substances including ethylbenzene and various mixtures of ethylbenzene and xylene isomers (described in Table 1 above).

A. Predictions for toxicological properties

1. Description of your read-across adaptation

a) In the technical dossier

You provided the following justification for the read-across from ethylbenzene in your techical dossier:

In the read-across justification document for the read-across from ethylbenzene to xylenes and in your CSR, you provide the following reasoning for the prediction of toxicological properties:

- You define the structural similarity between the source and target substance as "*alkyl substituted aromatic ring (one ethyl group in the case of ethylbenzene, two methyl groups in the case of xylene isomers)*".
- Xylene isomers and ethylbenzene have similar physico-chemical properties (i.e. density, vapour pressure, Log K_{ow}, water solubility).
- Regarding metabolism, you state that:
  - "*Metabolism of xylene isomers* [...] *primarily involves oxidation of the alkyl group* [...] *to form a methylbenzoic acid metabolite via a methylbenzyl alcohol intermediate* [...]. *Methylbenzoic acid is subsequently excreted* [...] *in the form of the methylhippuric acid metabolite, with glucuronidation being a minor pathway; a small amount (3-5%) is lost as unmetabolized xylene in expired air.* [...]. *This pattern of metabolism is generally applicable to other xylene isomers*";
  - "*Ethylbenzene is primarily metabolized through the oxidation of the alkyl side chain to 1-phenylethanol and ultimately to mandelic acid, which is the major urinary metabolite in man* [...]. *Similar to xylene metabolism, the side chain oxidation products are excreted in urine as glucuronide conjugates*";
  - You state that, for xylene isomers and ethylbenzene, ring oxidation is of negligible importance;
  - You state that "*the metabolism of xylene and ethylbenzene are similar and the presence of each substance in a mixture has no apparent effect on their individual metabolic profiles*".
- You consider that "*a comparison of toxicological data available for xylenes (including mixed xylenes and the individual isomers) demonstrates that the effects seen are generally similar and that the effect levels are of the same order of magnitude*".
- However, you acknowledge that "*ototoxicity* [...] *seems to be specific to mixed xylenes (where ototoxic potency appears related to the level of ethyl benzene present) and p-xylene*". You further state that "*for other endpoints, slight differences in effect levels are apparently related more closely to dose selection (and potentially other factors such as vehicle, gavage procedures) rather than to intrinsic hazard*" and that "*the presence of up to 10% ethyl benzene is not expected to significantly alter this hazard profile, with overall effects on human health influenced primarily by xylenes*".

b) In your comments on the draft decision

You provide additionnal justification for the read-across from individual constituents of the Substance and from Reaction mass of ethylbenzene and xylene in your comments on the draft decision.

More specifically, you provide a new read-across justification document to support the prediction of the properties of the Substance from the properties of the following source substances: the Substance, *i.e.* Reaction mass of ethylbenzene and xylene (EC No. 905-588-0), Ethylbenzene (EC No. 202-849-4), o-xylene (EC No. 202-422-2), p- xylene (EC No. 203-396-5) and m-xylene (EC No. 203-576-3).

You provide the following additional reasoning for the prediction of toxicological properties:
- "[The Substance] *and the Reaction masses of Ethylbenzene and Xylenes* [i.e. EC No. 905-588-0, EC No. 905-562-9 and EC No. 905-570-2] *will exhibit similar toxicological effects as their composition consists predominantly of the xylene isomers (ortho-, para- and meta-)*". Therefore you imply that compositional differences between these mixtures will not impact their toxicological properties;
- "*the reaction masses of Ethylbenzene and Xylenes are expected to contain a higher percentage of ethylbenzene (>10%)* [compared to the Substance], *the toxicological*

effects for ethylbenzene would be a worst-case scenario for Xylene". Therefore you imply that ethylbenzene may be considered as a worst-case to predict the toxicological properties of xylene isomers or any mixture of these;

- To define and support your read-across hypothesis, you refer to the Read Across Assessment Framework (RAAF) and the associated document in multi-constituents. You have listed the assessment elements (AEs) for an analogue approach where it is assumed that different compounds have the same type of effect(s) (i.e. Scenario 2). You have not provided a specific justification for each of the identified AEs but instead you refer to specific sections of the justification document;
- Ethylbenzene and xylene isomers have similar environmental fate and ecotoxicity properties;
- In addition to the information toxicokinetic properties and metabolism described above, you state:
    - "[The] *pattern of metabolism is the same for all three xylene isomers*". You refer to a study from your dossier by [redacted] (1976) for an inhalation study in humans showing similar pulmonary retention and excretion patterns using xylene isomers as well as a 1:1:1 mixture of the three isomers. In all cases, more than 95% of the absorbed xylenes were excreted in the urine in the form of hippuric acid derivatives;
    - Referring to a published study by [redacted] (1978) for an inhalation study in human using industrial xylene (60% xylene isomers and 40% ethylbenzene), you state that "*the metabolism of xylene isomers was similar and the presence* [of] *ethylbenzene in a mixture has no effect on* [xylene isomers] *metabolic profiles*";
    - Referring to a report on ethylbenzene by [redacted] (2010), you state that "*similar to xylene metabolism, the side chain oxidation products are excreted in urine as conjugates*".
- You consider that xylene and each of the xylene isomers (ortho-, meta- and para-) have similar toxicological properties. You have provided a data matrix summarizing available information on acute toxicity, skin/eye irritancy, mutagenicity, repeated-dose toxicity and developmental and reproductive toxicity. More specifically:
    - with regard to sub-chronic repeated dose toxicity, you refer to the studies by [redacted] (1986) and Condie *et al.* (1988) already in your dossier. You also refer to two studies by [redacted] (1988) on meta-xylene and para-xylene and to an ongoing study by [redacted] (2019) on ortho-xylene. You state that "*from each of these studies it is apparent that very similar, minor effects were observed, limited mainly to body weight depression in the absence of any relevant compound-related gross or microscopic pathologic lesions*";
    - with regard to developmental toxicity, you specify that "*the effects of the individual xylene isomers reported in the* [redacted] *(2003) study were essentially the same as those seen with the mixed or technical xylene*". You consider that "*the presence of 15.3% ethylbenzene in the xylene sample tested by* [redacted] *(2003) had no significant impact on the study results, which is further supported by the fact that ethylbenzene itself is not a reproductive toxicant in OECD 414 studies with rats and rabbits (* [redacted] *1981;* [redacted] *1981)*". You also note that in the study by [redacted] (2003), the authors state that "*the adverse effects of the technical mixture* [...] *were roughly similar to those caused by the single substances. No clear evidence of synergism or amelioration of effects was found*".
    - you consider that there is sufficient data available to conclude that "*for all toxicological effects (except ototoxicity) and all toxicology REACH endpoints, xylenes, o-xylene, m-xylene and p-xylene behave very similarly*". On repeat dose toxicity and rodent developmental studies, you consider that any

"*potential synergy or additivity between the 4 components of the streams*" can be ruled out based on available evidence. However, you acknowledge that there is no rabbit developmental toxicity data on any xylene and ethylbenzene mixture. You propose to conduct a prenatal developmental toxicity study (OECD TG 414) in rabbit on the Substance.

- To further support similarity in toxicological properties, you have conducted a ToxCast (for EPA's Toxicity Forecaster) analysis of the xylene isomers. You specify that m-xylene was mainly found to be active in some oestrogen receptor alpha assays, some androgenic receptor assays and the pregnane X receptor assay. The o-xylene isomer mainly showed some activity on the pregnane X receptor and p-xylene and mixed xylenes are reported to be negative in all assays. You conclude that "*from the more than one hundred assays in which the xylene isomers have been tested, there appear very few (if any) differences*".

2. Assessment of your read-across adaptations

ECHA understands that you consider that quantitative differences in the composition in xylene isomers will not impact the prediction as they share a common metabolic pathway and will form common (bio)transformation products. For the read-across from ethylbenzene, 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.

You have provided short-term repeated dose toxicity studies, screening for reproductive/ developmental toxicity studies and pre-natal developmental toxicity studies in rat on ethylbenzene and 'mixed xylenes' as listed in Table 1 above (further details on these studies are discussed under the relevant Appendices). You have not provided any such studies for the Substance. With this data you intend to predict the relevant properties of the Substance.

ECHA notes the following shortcomings with regards to predictions of toxicological properties:

a) Supporting information

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)*". The ECHA Guidance R.6., Section R.6.2.2.1.f indicates that "*it is important to provide supporting information to strengthen the rationale for the read-across*". 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 selected source substances.

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar substances cause the same type of effect(s) and you have proposed similar toxicokinetics as a basis for predicting the properties of the Substance.

In this context, supporting information must include, among others, relevant, reliable and adequate toxicokinetic and toxicodynamic information and bridging studies to enable a comparison of the properties of the Substance and the selected source substances.

In your technical dossier, you provide some toxicokinetic data on individual constituents o-xylene, m-xylene and p-xylene. You have not provided supporting information on any of the source substances (i.e. mixture of xylene isomers and ethylbenzene) or on the Substance to compare their properties to those of the individual constituents o-xylene, m-xylene and p-xylene. In your CSR, you acknowledge that "*In complex mixtures* [...] *the toxicokinetics of*

even well-studied pure substances may vary depending upon interaction with other chemical species available within the mixture. For example, the substances present may compete for the uptake, metabolism, and/or elimination of the complex mixture. This situation, already complicated, is further exacerbated when the composition of the mixture is uncertain and variable".

In your comments on the draft decision, you clarify that, based on the study by [redacted] (1976), similar metabolism and excretion is expected for individual isomers and for a 1:1:1 mixture of these. In addition, you have provided a reference to a study by [redacted] (1978) to support that the metabolism of xylene isomers is not impacted by the presence of c.a. 40% ethylbenzene.

However, in the study by [redacted] (1978), the composition of the test material was "*ethylbenzene 40.4 %,p-xylene 1.4 %, m-xylene 49.4 %, and o-xylene 8.8 %*", hence essentially a mixture of m-xylene and ethylbenzene. In addition, the authors specify that "*measurements were made of two of the components of xylene, i.e., ethylbenzene and m-xylene*" and therefore this study does not provide any information on p-xylene and o-xylene. Finally, this study only provides information on uptake and blood concentrations of ethylbenzene and m-xylene and therefore does not cover metabolism or excretion. Accordingly, this study cannot be considered as a relevant source of information to demonstrate that the toxicokinetics of the consituents of the Substances and any mixtures of these are similar.

Based on the above, the data set provided in the technical dossier and in your comments on the draft decision do not include such relevant, reliable and adequate information for the Substance and source substances to support your read-across hypothesis of similar toxicokinetics.

In addition, knowledge of both the toxicokinetic and toxicodynamic properties of a substance are necessary to predict its toxicological properties (with the exception of those cases where a substance is not systemically available) and you have not provided any information on toxicodynamic properties in either your technical dossier or your comments on the draft decision.

Finally, your dossier does not contain any bridging study to support similar toxicological properties between the Substance and the source substances.

Therefore you have not provided sufficient supporting information to strengthen the rationale for the read-across.

b) Read-across hypothesis contradicted by existing data

In the context of the supporting information provided to strengthen the rationale for the read-across (ECHA Guidance R.6., Section R.6.2.2.1.f), the observation of differences in the toxicological properties between the source substance(s) and the Substance or their constituents must be regarded as a warning sign. An explanation for such a difference resulting in a contradiction between the similarities in properties claimed in the read-across hypothesis and the observation of different properties needs to be provided and supported by scientific evidence.

As indicated above, your read-across hypothesis is based on the assumption that the Substance and source substances cause the same type of effect(s) as the toxicological properties of their constituents are expected to be similar.

The available information contradicts your hypothesis for the following reasons:

1. In your dossier, you state that "*the metabolism of xylene and ethylbenzene are similar*".

   In your comments on the draft decision, you explain that in the study [redacted] (1976) more than 95% of the absorbed xylenes were excreted in the urine in the form of hippuric acid derivatives for individual isomers and for a 1:1:1 mixture of these. For ethylbenzene, you state that "*similar to xylene metabolism, the side chain oxidation products are excreted in urine as conjugates*". Based on the information you have provided, it appears that the main metabolites of xylenes isomers are formed through glycine conjugation to form different hippuric acid isomers and that glucuronide conjugates are only found in trace amounts (unless high levels of xylenes are administered). Similarly, for ethylbenzene, glucorinide conjuguates are expected to be only minor metabolites (based on excretion data). The main metabolites of ethylbenzene are formed through oxidation (hydroxylation) of the side chain (i.e. to form mandelic acid and phenylglyoxylic acid) and not through glycine conugation (as for xylenes).

   Therefore, the metabolic pathways of xylene isomers and ethylbenzene are significantly different (formation of glycine conjugates *versus* side-chain hydroxylation) and they do not have a single metabolite in common. In your dossier or in your comments on the draft decision, you have not addressed why these differences are not relevant for the prediction of the toxicological properties of the Substance.
2. As explained above, you claim that xylenes (including mixed xylenes and the individual xylene isomers) have similar toxicological properties. However, available toxicological data does not support your claim. In fact, the data demonstrates dissimilar toxicological properties among xylene isomers and with ethylbenzene. In particular, an increased auditory threshold and a loss of outer hair cells have been observed for p-xylene or mixed xylenes containing 10 - 20% ethyl benzene (Gagnaire *et al.* 2001, 2007; [redacted] 2006). In this context we note that ethylbenzene has a harmonised classification for STOT RE 2 / H373: hearing organs. Furthermore, in a sub-chronic toxicity study (OECD TG 408) with ethylbenzene (Mellert *et al.* 2006), increased mean corpuscular volume, reduced platelet counts, reduced prothrombin times and reduced thymus weights in females were observed. In a sub-chronic toxicity study (OECD TG 408) with mixed xylenes (composition MX no. 2 in Table 1), mild polycythaemia and leukocytosis in some of the dose groups, and increased spleen and heart weights were noted. Available data on ethylbenzene indicates some carcinogenic potential. Finally, the information from the registration dossiers of o-xylene (EC No. 202-422-2), m-xylene (EC No. 203-576-3) and p-xylene (EC No. 203-396-5) support positive results in the *in vitro* comet assay whereas *in vitro* genetic toxicity is negative for mixed xylenes and ethylbenzene. The above information suggests that a small change in structure can result in significantly different toxicological properties.

   In your comments on the draft decision, you state that the available repeated-dose toxicity studies show "*very similar, minor effects* [...], *limited mainly to body weight depression in the absence of any relevant compound-related gross or microscopic pathologic lesions*". You consider that "*the mild polycythaemia and leukocytosis* [...] *in reference to the Condie (1988) study, is misleading* [...] *since they do not show a dose-response, are not seen in any of the other RDT studies, and the clinical health of the rats did not appear to be affected*". You acknowledge there are variations in the NOAELs among studies but you consider that these are mainly due to differences in the rat strain used and in dosing frequency. With regard to difference in ototoxicity between xylene isomers and ethylbenzene, you state that "*in the absence of further studies with*

sufficient data on the exposure of workers to mixed xylene and xylene isomers it is not possible to make a definitive conclusion regarding the ototoxicity of para-xylene to humans". Finally, to support similar toxicological properties, you have conducted a ToxCas analysis on xylene isomers. You conclude that "*based on over hundred assays on which the isomers have been tested, very few differences between the isomers are observed*".

Considering the information in your technical dossier and your comments on the draft decision, we note that you have not provided a justification on why all the toxicological differences listed above should not be regarded as indicative of dissimilar toxicological properties. Furthermore, the results of the ToxCast analysis appear to confirm to some extent that small differences in the structure of xylene isomers may lead to different toxicological properties. We note however that there are major uncertainties with this analysis as the tests were not conducted on all substances, the composition of the test materials used in these tests is unclear and no information on pure ethylbenzene is provided.

Given the above contradictions to your read-across hypothesis and the lack of explanation for them in either your technical dossier or your comments on the draft decision, ECHA concludes that you have not demonstrated and justified that the properties of the target substance are likely to be similar despite the observation of these differences.

B. Conclusions on the grouping of substances and read-across approach

As explained above, you have not established that relevant properties of the Substance can be predicted from data on the source substances. 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.

Appendix A: Reasons for the requests to comply with Annex VIII of REACH

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

1. Justification for an adaptation of the Short-term repeated dose toxicity (28-day), (Annex VIII, Section 8.6.1.)

A Short-term repeated dose toxicity study (28 days) is a standard information requirement in Annex VIII to REACH. This information may take the form of a study record or a valid adaptation in accordance with either a specific adaptation rule under Column 2 of Annex VIII or a general adaptation rule under Annex XI.

You have not provided a short-term repeated dose toxicity study (28 days) with the Substance. Instead, you have adapted the information requirement according to Annex XI, Section 1.5. and you have provided the following studies:

i. Condie *et al.* 1988, key study (similar to OECD TG 408 and EPA OPP 82-1 in rats; test material: mixed xylenes with composition "MX no. 2" in Table 1);
ii. [redacted] 1986, key study (similar to EU Method B.32, oral route, in rats; test material: mixed xylenes with composition "MX no. 1" in Table 1);
iii. [redacted] 1986, two supporting studies (non guideline 13-week studies, oral route in rats and mice; test material: mixed xylenes with composition "MX no. 1" in Table 1);
iv. Mellert *et al.* 2006, supporting study (similar to OECD TG 408 in rats; test material: ethylbenzene, purity: 99.7%);
v. Carpenter *et al.* 1975, two supporting studies (non guideline 13-week studies, inhalation route in rats and dogs; test material: mixed xylenes with composition "MX no. 3" in Table 1).

In addition, in Section 7.9.1. of your technical dossier you report the following repeated dose toxicity studies:

vi. [redacted] 1994, key study (non guideline repeated dose toxicity study, inhalation route, in rats; exposure duration: 3 months; test material: m-xylene);
vii. [redacted] 2010, supporting study (13-weeks study according to EPA OPPTS 870.6200, oral route, in rats; test material: ethylbenzene);
viii. [redacted] 1992, supporting study (non guideline repeated dose toxicity study, inhalation route, in rats; exposure duration: 3 months at 1000 ppm and 6 months at 100 ppm; test material: m-xylene);
ix. [redacted] 2001, supporting study (non guideline 28-day study, inhalation route, in rats; test material: ethylbenzene).

In Section 7.9.2. of your technical dossier you report the following repeated dose toxicity study:

x. [redacted] 2010, supporting study (28-day study according to EPA OPPTS 870.7800, inhalation route, in rats; test material: ethylbenzene, purity: 99.96%).

Finally in Section 7.9.4. of your technical dossier you report the following repeated dose toxicity studies:

xi. Gagnaire *et al.* 2001, three key studies (non guideline 13-weeks studies, inhalation route, in rats; test material: o-xylene, m-xylene and p-xylene);
xii. [redacted], two key studies (non guideline 13-weeks studies, inhalation route, in rats; test material: ethylbenzene and mixed xylenes with composition "MX

no. 9" and "MX no. 10" in Table 1);

xiii. [redacted] 2006, three supporting studies (non guideline 3-weeks studies, inhalation route, in rats; test material: o-xylene, m-xylene and p-xylene);

xiv. [redacted] 1987, supporting study (non guideline 6-weeks studies, inhalation route, in rats; test material: mixed xylenes with composition "MX no. 11").

We have assessed this information and identified the following issues:

A. All the studies above were conducted with analogue "Mixed xylene" substances or ethylbenzene. However, as explained in the Appendix on general considerations your adaptation according to Annex XI, Section 1.5 is rejected.

B. Tests on substances must be conducted in accordance with the OECD test guidelines or another recognised international test method (Article 13(3) of REACH). To fulfil the information requirement, the study has to meet the requirements of OECD TG 407. The key parameters of this test guideline include:
   - testing of at least three dose levels and a concurrent control;
   - highest dose level should aim to induce some systemic toxicity, but not death or severe suffering;
   - 5 female and 5 male animals should be used at each dose level (including control group);
   - Recording of body weight, histopathology (including thyroid gland, and if there is an indication for an effect on the pituitary-thyroid axis thyroid hormone measurements), organ weights, haematological and clinical biochemistry observations.

   The studies listed ii., vi., viii., ix. and xiii. above were conducted with only two dose levels. For the studies listed in v. to xiv. above no NOAEL/C in reference to systemic toxicity is provided and therefore the information reported does not demonstrate that the highest dose level in the study induced any systemic toxicity. The studies listed in v., vi. and viii. to xiv. were conducted in males or females only. In the study listed in i., histopathology was conducted only on liver and kidneys. For studies listed in ii. and iii., no haematological or clinical biochemistry observations were conducted and the organ weights were not determined. For the studies listed in v., one of the study was not conducted on a rodent species and in both studies the weights of all relevant organs was not determined. Finally, the studies listed in vi. to ix. investigate functional and behavioural response, the study listed in x. investigates immunotoxicity and the studies xi. to xiv. investigate ototoxicity.

   With the exception of study iv., none of the studies you have provided were performed according to the criteria of the OECD TG 407, since some of the following key parameters are missing: organ weights and/or histopathology of all tissues specified in the OECD TG 407 and/or haematological observations and/or clinical biochemistry observations. Therefore these studies do not provide equivalent information to a short-term repeated dose toxicity study (28 day). Study iv. provides adequate coverage of the key parameters for this endpoint but, as explained under issue A. above, this study is not relevant to cover the information requirement of the Substance.

Therefore, the information requirement is not fulfilled.

Column 2 of Annex VIII, Section 8.6.1. provides that an experimental study for this endpoint is not needed if a reliable sub-chronic (90 days) or chronic toxicity study is available.

The present decision requests the registrants concerned to generate and submit a reliable sub-chronic toxicity study (90 days) (see Section B.1). According to Column 2 of Annex VIII, Section 8.6.1., and to prevent unnecessary animal testing, a short term toxicity study (28 days) does not therefore need to be conducted.

Because you still must comply with the information requirement in Annex VIII, Section 8.6.1., you are requested to submit a justification for the adaptation provided in Column 2 of that provision.

In your comments on the draft decision, you have provided additional justification on why you consider that generating further information on repeated-dose toxicity is unnecessary. We have addressed your comments under Section B.1 below.

Appendix B: Reasons for the requests to comply with Annex IX of REACH

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

1. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.)

A Sub-chronic toxicity study (90 day) is a standard information requirement in Annex IX to REACH.

In your dossier you have not provided a study with the Substance. Instead, you have adapted the information requirement according to Annex XI, Section 1.5. and you have provided the following studies:

i. Condie *et al.* 1988, key study (similar to OECD TG 408 and EPA OPP 82-1 in rats; test material: mixed xylenes with composition "MX no. 2" in Table 1);
ii. [redacted] 1986, key study (similar to EU Method B.32, oral route, in rats; test material: mixed xylenes with composition "MX no. 1" in Table 1);
iii. [redacted] 1986, two supporting studies (non guideline 13-week studies, oral route in rats and mice; test material: mixed xylenes with composition "MX no. 1" in Table 1);
iv. Mellert *et al.* 2006, supporting study (similar to OECD TG 408 in rats; test material: ethylbenzene, purity: 99.7%);
v. Carpenter *et al.* 1975, two supporting studies (non guideline 13-week studies, inhalation route in rats and dogs; test material: mixed xylenes with composition "MX no. 3" in Table 1).

In addition, in Section 7.9.1. of your technical dossier you report the following repeated dose toxicity studies:

vi. [redacted] 1994, key study (non guideline repeated dose toxicity study, inhalation route, in rats; exposure duration: 3 months; test material: m-xylene);
vii. [redacted] 2010, supporting study (13-weeks study according to EPA OPPTS 870.6200, oral route, in rats; test material: ethylbenzene);
viii. [redacted] 1992, supporting study (non guideline repeated dose toxicity study, inhalation route, in rats; exposure duration: 3 months at 1000 ppm and 6 months at 100 ppm; test material: m-xylene);
ix. [redacted] 2001, supporting study (non guideline 28-day study, inhalation route, in rats; test material: ethylbenzene).

In Section 7.9.2. of your technical dossier you report the following repeated dose toxicity study:

x. Li *et al.* 2010, supporting study (28-day study according to EPA OPPTS 870.7800, inhalation route, in rats; test material: ethylbenzene, purity: 99.96%).

Finally in Section 7.9.4. of your technical dossier you report the following repeated dose toxicity studies:

xi. Gagnaire *et al.* 2001, three key studies (non guideline 13-weeks studies, inhalation route, in rats; test material: o-xylene, m-xylene and p-xylene);
xii. [redacted], two key studies (non guideline 13-weeks studies, inhalation route, in rats; test material: ethylbenzene and mixed xylenes with composition "MX no. 9" and "MX no. 10" in Table 1);
xiii. [redacted] 2006, three supporting studies (non guideline 3-weeks studies, inhalation route, in rats; test material: o-xylene, m-xylene and p-xylene);

xiv. [redacted] 1987, supporting study (non guideline 6-weeks studies, inhalation route, in rats; test material: mixed xylenes with composition "MX no. 11" in Table 1).

We have assessed this information and identified the following issues:

A. All the studies above were conducted with analogue "Mixed xylene" substances or ethylbenzene. However, as explained in the Appendix on general considerations your adaptation according to Annex XI, Section 1.5 is rejected.

B. Tests on substances must be conducted in accordance with the OECD test guidelines or another recognised international test method (Article 13(3) of REACH). To fulfil the information requirement, the study has to meet the requirements of OECD TG 408. The key parameters of this test guideline include:
   - testing of at least three dose levels and a concurrent control;
   - At least 10 female and 10 male animals should be used at each dose level (including control group);
   - ophthalmological examination, sensory reactivity to various stimuli and functional observations of the animals, pathology of sexual (male and female) organs, recording of body weight, histopathology (including thyroid gland), organ weights, haematological and clinical biochemistry observations (including thyroid hormone measurements).

   The studies listed ii., vi., viii., ix. and xiii. were conducted with only two dose levels. For the studies listed in v. to xiv. no NOAEL/C in reference to systemic toxicity is provided and therefore the information reported does not demonstrate that the highest dose level in the study induced any systemic toxicity. The studies listed in v., vi. and viii. to xiv. were conducted in males or females only. In the study listed in i., histopathology was conducted only on liver and kidneys. For studies listed in ii. and iii., no haematological or clinical biochemistry observations were conducted and the organ weights were not determined. For the studies listed in v., one of the study was not conducted on a rodent species and in both studies the weights of all relevant organs was not determined. Finally, the studies listed in vi. to ix. investigate functional and behavioural response, the study listed in x. investigates immunotoxicity and the studies xi. to xiv. investigate ototoxicity .

   With the exception of study iv., none of the studies you have provided were performed according to the criteria of the OECD TG 408, since some of the following key parameters are missing: organ weights and/or histopathology of all tissues specified in the OECD TG 408 and/or haematological observations and/or clinical biochemistry observations and/or pathology of sexual (male and female) organs and/or sensory reactivity and/or ophthalmological examination. Therefore these studies do not provide equivalent information to a sub-chronic toxicity study (90 day). Study iv. provides adequate coverage of the key parameters for this endpoint but, as explained under issue A. above, this study is not relevant to cover the information requirement of the Substance.

In your comments on the draft decision, you acknowledge that the "*toxicology database consists of a large number of toxicity studies which are historical and, therefore* [not] *conducted to the contemporaneous OECD test guidelines or were non-OECD test guideline studies*". However, you consider that "*these studies are still valid for both hazard characterisation and for derivation of risk assessment endpoints such as DNEL's, etc*". You explain that "*this is further supported by* [the publication from] *Bitsch et al., 2006* [*Regul.*

Toxicol. Pharm., 2006, 46:202–210] *that identified that the frequently affected targets are: liver, kidney, body weight, and clinical effects, while other effects are infrequent*". You further state that based according to Batke *et al.* (*Toxicol. Lett.*, 2013, 218:293–298) "*the majority of the key targets are usually covered in non-guideline studies, and therefore the NOELs are likely to be well derived*".

In your comments you also state that you have identified studies on isomers. You consider that the "*properties of all the reaction masses can be inferred from the properties of the individual isomers and ethylbenzene*" and that "*there is adequate data already, or data is in preparation to make a robust read across*".

We have evaluated this information and have the following comments:

A. Although you do not explicitly claim an adaptation, ECHA understands that you consider the information provided was submitted in order to meet the required information by way of adaptation under Annex XI, Section 1.1.2. This adaptation rule enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods where a number of cumulative conditions are met, in particular it must provide an adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case OECD TG 408.

   However, as already explained under B. above, with the exception of the study iv. by Mellert *et al.* (2006) on ethylbenzene, none of the reported studies fulfils this requirement.

   On the publication by Bitsch *et al.* (2006), the authors merely describe how the REPDOSE database was developed and we note that your interpretation of the study results may be biased by the fact that the identified target organs are also the once that are most often investigated (especially in older study). The study by Batke *et al.* (2013) investigates the relevance of individual targets for determining the LOEL from guideline and non-guideline studies. However, it does not provide any critical analysis on the sensitivity of guideline *versus* non-guideline studies. In this regard, the authors acknowledge that "*the observation of frequent targets is not conclusive as such but has to be extended to the effects occurring at these targets*" and that for this study they have combined "*all effects types referring to organs, i.e. gross pathology, organ weights, histopathology*". Therefore, these studies do not provide a definitive proof that the NOEL of any chemical can be reliably identified based on restricted set of key parameters compared to the requirements of OECD TG 408.

   Therefore the information provided in your comments and in your dossier do not fulfil the requirement of an adaptation according to Annex XI, Section 1.1.2.

B. From your comments, we also understand that you intend to cover this information requirement based on a read-across using information on the constituents of the Substance. As this information is not yet available or included in your dossier, we will assess this information at the follow-up of the decision making process.

Therefore, the information requirement is not fulfilled.

Information on 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 although the information indicate that human exposure to the Substance by the inhalation route is likely, there is no concern for severe local effects following inhalation exposure

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

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH.

In your dossier you have not provided a study with the Substance. Instead, you have adapted the information requirement according to Annex XI, Section 1.5. and you have provided the following studies:

i. [redacted] 2003, two key studies (developmental toxicity study according to OECD TG 414, inhalation route, in rats; test material: ethylbenzene and mixed xylenes with composition "MX no. 6" in Table 1);
ii. [redacted] 2007, key study (2-generation reproduction study according to EPA.OPPTS 870.3800 with evaluation of F2 offspring for nervous system functional and morphological endpoints, inhalation route, in rats; test material: ethylbenzene);
iii. [redacted] 1978, supporting study (developmental toxicity study according to EPA OPP 83-3, inhalation route, in rats; test material: mixed xylenes with composition "MX no. 8" in Table 1);
iv. [redacted] 1983, supporting study (non guideline developmental toxicity study, inhalation route, in rats; test material: mixed xylenes with composition "MX no. 7" in Table 1);
v. [redacted] 1993, two supporting studies (pre-natal and post-natal toxicity studies, inhalation route, in rats; test material: mixed xylenes with composition "MX no. 5" in Table 1);
vi. [redacted] 1995; 1997, two supporting studies (post-natal toxicity studies, inhalation route, in rats; test material: mixed xylenes with composition "MX no. 4" in Table 1).

We have assessed this information and identified the following issues:

A. All the studies above were conducted with analogue "Mixed xylene" substances or ethylbenzene. However, as explained in the Appendix on general considerations your adaptation according to Annex XI, Section 1.5 is rejected.

B. Tests on substances must be conducted in accordance with the OECD test guidelines or another recognised international test method (Article 13(3) of REACH). To fulfil the information requirement, the study has to meet the requirements of OECD TG 414. The key parameters of this test guideline include:
   - testing of at least three dose levels and a concurrent control,
   - 20 female animals with implantation sites for each test and control group,
   - dosing of the Substance from implantation until the day prior to scheduled caesarean section,
   - examination of the dams for weight and histopathology of the thyroid gland/thyroid hormone measurements/gravid uterus weight/uterine content/body weight of the dams/clinical signs of the dams,
   - examination of the foetuses for sex and body weight/external, skeletal and soft

tissue alterations (variations and malformations)/number of resorptions and or live foetuses/ measurement of anogenital distance in live rodent foetuses.

The studies listed in iii. to vi. you have provided were conducted with less than three dose levels. The studies listed in iv. and vi. were conducted with less than 20 pregnant females for each test group and therefore the statistical power of the information provided is not sufficient. Studies iii. and vi. do not have a required exposure duration because the exposure duration is not from implantation. Study ii. and vi. do not cover the endpoints foreseen in a pre-natal developmental toxicity study because it is limited to evaluate neurodevelopmental toxicity and postnatal neurodevelopment, respectively. Hence, studies ii. to vi. do not provide equivalent information to a pre-natal developmental toxicity study. Study i. provides an adequate coverage of the key parameters for this endpoint but, as explained under issue A. above, this study is not relevant to cover the information requirement of the Substance.

In your comments on the draft decision, you state that "*with regards to developmental toxicity, the effects of the individual xylene isomers reported in the* [redacted] *(2003) study were essentially the same as those seen with the mixed or technical xylene*" and therefore "*xylene isomers were not selectively toxic to the foetus under the conditions of this study*". You conclude that "*it seems quite reasonable and logical to read-across for xylenes for reproductive toxicology*".

From your comments, we understand that you intend to cover this information requirement based on a read-across using information on the constituents of the Substance. Your technical dossier currently does not include information on prenatal developmental toxicity in a first species (rat). Furthermore, for the reasons explained under point B. above, you have not provided reliable information on ethylbenzene for this endpoint. Therefore, we will assess the proposed adaptation at the follow-up of the decision making process.

Therefore, the information requirement is not fulfilled.

A PNDT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral[2] administration of the Substance.

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

Appendix C: Reasons for the requests to comply with Annex X of REACH

Under Articles 10(a) and 12(1) of REACH, a technical dossier at a tonnage abve 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to REACH.

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

Pre-natal developmental toxicity (PNDT) studies (OECD TG 414) in two species is a standard information requirement under Annex X to REACH.

In your dossier you have not provided a study with the Substance. Instead, you have adapted the information requirement according to Annex XI, Section 1.5. and you have provided the studies already listed under request B.2. with regard to prenatal developmental toxicity in a first species.

We have assessed this information and identified the following issue:

A. All the studies above were conducted with analogue "Mixed xylene" substances or ethylbenzene. However, as explained in the Appendix on general considerations your adaptation according to Annex XI, Section 1.5 is rejected.

B. In order to be compliant and enable concluding if the Substance is a developmental toxicant, information provided has to meet the requirements of OECD TG 414 in two species.

   All the studies you have provided were conducted on rats.

   You have not provided pre-natal developmental toxicity (PNDT) on a second species.

Therefore, the information requirement is not fulfilled.

In your comments on the draft decision, you specify that you intend to conduct a rabbit developmental toxicity study on the Substance.

Information on study design

A PNDT study according to the OECD TG 414 study should be performed in rabbit or rat as the preferred second species, depending on the species tested in the first PNDT study (request B2 in this decision). The study shall be performed with oral[3] administration of the Substance.

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

Appendix D: Procedural history

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

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

The compliance check was initiated on 29 August 2018.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

ECHA took into account your comments and did not amend the requests.

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 E: Observations and technical guidance

1. The substance subject to the present decision is provisionally listed in the Community rolling action plan (CoRAP) for the start of substance evaluation in 2020.

2. The information requirements under Section 8.7.1 of Annex VIII (Screening for reproductive/developmental toxicity) and Section 8.7.3. of Annex IX/X to REACH (Extended one-generation reproductive toxicity study, EOGRTS) are not addressed in this decision, because the information from the Sub-chronic toxicity study (90-day), requested in the present this decision, is relevant for the triggering and the design of the EOGRTS, and the information requirement under Section 8.7.3 covers the information requirement under Section 8.7.1.

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

4. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of the Member States.

5. Test guidelines, GLP requirements and reporting

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

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

   Under Article 10 (a) (vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide: 'How to report robust study summaries'[4].

6. Test material

   Selection of the test material(s)

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

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

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

Technical reporting of the test material

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

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[5].

7. List of references of the ECHA Guidance and other guidance/ reference documents[6]

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

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

ECHA Read-across assessment framework (RAAF, March 2017)[7]

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.

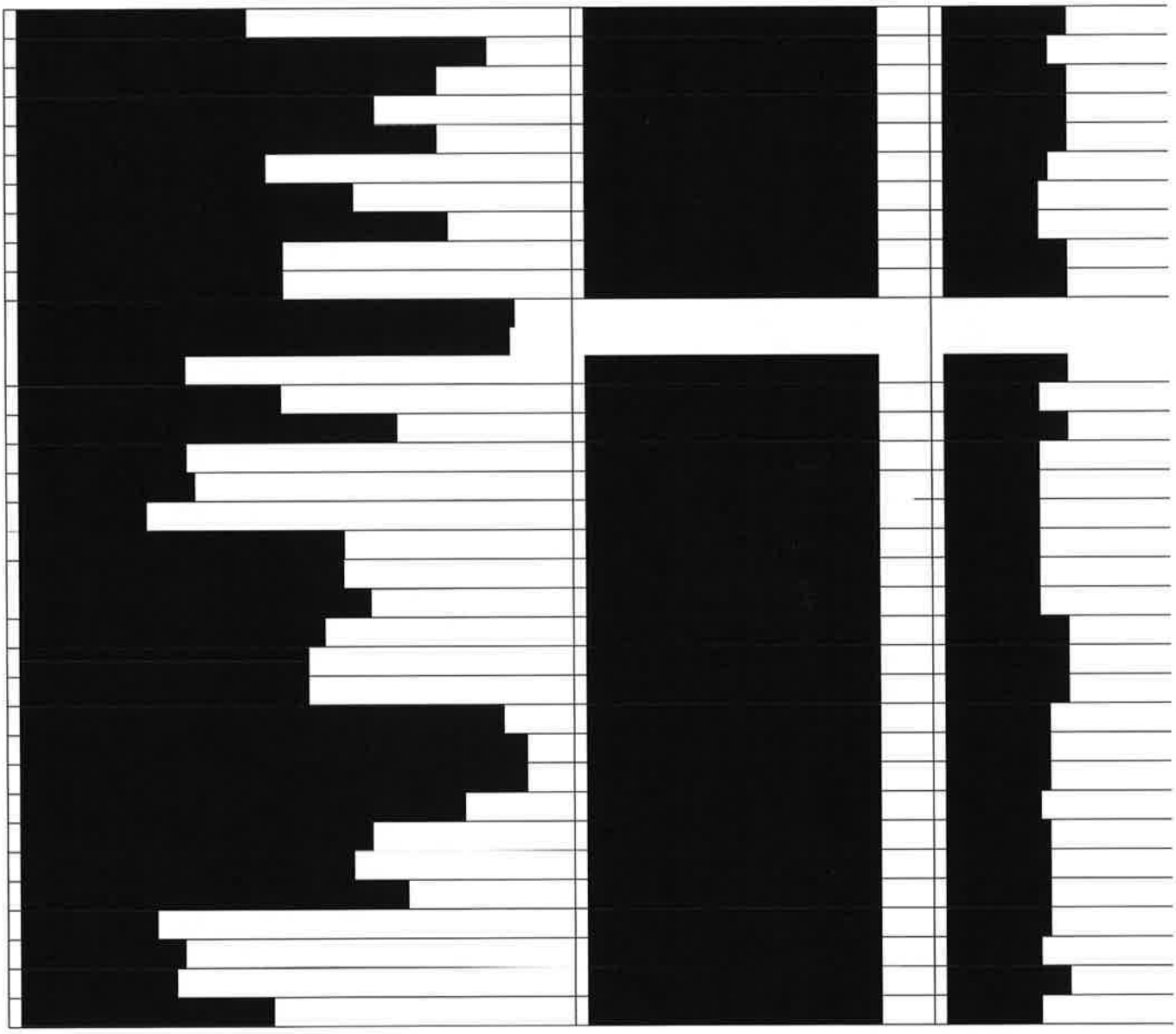
Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

OECD Guidance documents[8]
Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment – No 43, referred to as OECD GD43.

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

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

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



Note: where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas the decision is sent to the actual registrant.