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Helsinki, 04 June 2024

Addressee(s)
Registrants of JS_TAPEH as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
14 June 2019

Registered substance subject to this decision ("the Substance")
Substance name: tert-pentyl 2-ethylperoxyhexanoate
EC/List number: 211-687-3

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 ***11 September 2028***.

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

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

1. Transgenic rodent somatic and germ cell gene mutation assays **OR** *in vivo* mammalian alkaline comet assay, also requested below (triggered by Annex VII, Section 8.4., Column 2).

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

2. Transgenic rodent somatic and germ cell gene mutation assays **OR** *in vivo* mammalian alkaline comet assay, also requested below (triggered by Annex VIII, Section 8.4., Column 2).

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

3. Transgenic rodent somatic and germ cell gene mutation assays (triggered by Annex IX, Section 8.4.4, Column 1; test method: OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; germ cells and duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

   OR

   In vivo mammalian alkaline comet assay (triggered by Annex IX, Section 8.4.4., Column 1 test method: OECD TG 489) in rats, or if justified, in other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

4. In case of a positive result in any of the somatic tissues in the Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488) (requested as one of the options under request 3):

- Analysis of male germ cells collected, in line with request 3, from the seminiferous tubules (Annex IX, Section 8.4.5; Column 1; test method: OECD TG 488).

5. Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: OECD TG 443) in rats, oral route, specified as follows:
   - Ten weeks premating exposure duration for the parental (P0) generation;
   - The highest dose level in P0 animals must be determined based on clear evidence of an adverse effect on sexual function and fertility without severe suffering or deaths in P0 animals as specified in request 4, or follow the limit dose concept. The reporting of the study must provide the justification for the setting of the dose levels;
   - Cohort 1A and 1B (Reproductive toxicity).

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

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

Information required depends on your tonnage band

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

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

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

How to comply with your information requirements

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

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

Appeal

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

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Failure to comply

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

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

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

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons for the request(s)

Reasons related to the information under Annex VII of REACH

1. Transgenic rodent somatic and germ cell gene mutation assays or *in vivo* mammalian alkaline comet assay

1 Under Annex VII, Section 8.4., Column 2, further mutagenicity studies must be considered in case of a positive result in an *in vitro* gene mutation study in bacteria.

2.1. Triggering of the information requirement

2 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (1999) which raises a concern for gene mutation.

3 Therefore, the information requirement is triggered.

2.2. Information requirement not fulfilled

4 The information provided, its assessment and the specifications of the study design are addressed under request 3.

Reasons related to the information under Annex VIII of REACH

2. Transgenic rodent somatic and germ cell gene mutation assays or *in vivo* mammalian alkaline comet assay

5 Appropriate *in vivo* mutagenicity studies must be considered under Annex VIII, Section 8.4., Column 2 in case of a positive result in any of the *in vitro* genotoxicity studies under Annex VII or VIII.

2.1. Triggering of the information requirement

6 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (1999) which raises a concern for gene mutation.

7 Therefore, the information requirement is triggered.

2.2. Information requirement not fulfilled

8 The information provided, its assessment and the specifications of the study design are addressed under request 3.

Reasons related to the information under Annex IX of REACH

3. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays

9 Under Annex IX, Section 8.4.4., Column 1, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and 2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

3.1. Triggering of the information requirement

10 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (1999) which raises the concern for gene mutation.

11 In your comments to the draft decision you disagree with the trigger of the information requirement. You state that "*The results obtained with the TA 102 strain in the repeat experiment are not biologically plausible, and should have raised doubts on the validity of these results*". Based on this, you indicate your intention to submit a dossier update and to "*classify the current in vitro gene mutation study in bacteria (Ames) study as invalid (Klimish 3)"* and propose to repeat it first, before taking further decisions. You have specified the reasons why you consider this study as invalid, however ECHA disagrees with your claims for the following reasons:

- You state that "*TA 102 showed negative results in the first experiment with S9-mix*". However, ECHA considers the statement as incorrect, because, in both experiments with S9, TA102 showed an increase in the number of revertant colonies above ratio 2.
- You claim that "*The results seen for TA 102 with S9 lack a clear dose response relationship, which is one evaluation criterium to conclude a positive result*". However, ECHA disagrees, because the positive results obtained for TA102 in experiments 1 and 2 increase in a demonstrated dose-response manner. Further, the preliminary test also showed a clear dose-related increase in the number of revertants for in TA102 with S9 (which reached values similar to a positive control at 2500 µg/plate).
- You state that "*Such strong positive reaction completely specific for TA 102 without any reaction in the other 4 strains, only seen with S9 while S9-mix on overall seems to lead to lower toxicity, is not biologically likely".* According to OECD TG 471 (paragraph 35) the criteria for determining positive results are (emphasis added) *"[…] a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per plate* ***in at least one strain with or without metabolic activation".*** ECHA consideres that the results from the *in vitro* gene mutation in bacteria test meet those criteria You did not explain why you consider the results "*not biologically likely*".

12 Based on the above, the *in vitro* gene mutation in bacteria study is considered valid. Therefore, the information requirement for the *in vivo* study is triggered.

3.2. Information provided

13 You have provided an *in vivo* micronucleus study (OECD TG 474, 2001) with the Substance, giving negative results (study i).

3.3. Assessment of the information provided

3.3.1. Study not adequate for the information requirement

14 Toxicological studies must comply with a recognised test method (Article 13(3) of REACH). To address the specific concern raised by the *in vitro* positive result, an *in vivo* somatic cell genotoxicity study must be conducted according to the OECD TG 488 or 489, as indicated in the Guidance on IRs and CSA, Section R.7.7.6.3. Such study must cover the key parameters of the corresponding OECD test guideline (Article 13(3) of REACH).

15 Study (i) is described as an *in vivo* micronucleus test. This study is not an *in vivo* gene mutation study addressing concerns for gene mutations.

16 The information provided does not cover the specifications required by the OECD TG 488 or 489.

17 Based on the above, study (i) is not adequate for the information requirement and the information requirement is not fulfilled.

18 In your comments to the draft decision, you state that "*For this substance, there is only industrial use under well-controlled conditions without possible consumer exposure",* therefore "[…] *even in the case of a positive result observed in a to be repeated bacterial reverse mutation study* […] *the level of concerns are limited on the basis of limited exposures*". Further, you refer to a weight of evidence approach, based on "*data available within the group of the peroxyesters"* and conclude that *the "overall weight of evidence indicating that possible genotoxicity hazard from in vitro testing is derived from the formation of hydroperoxides from the peroxyester, and for which all available results from in vivo testing do not confirm concerns for genotoxicity".* You specially refer to the negative results from a TGR (OECD TG 488) study for the structural analogue tert-amyl peroxy-2-ethylhexanoate (EC 221-110-7).

19 Although you refer to the weight of evidence, you have not provided any mulptile sources of information. Instead, you refer to a TGR (OECD TG 488) study, performed with an analogue substance tert-amyl peroxy-2-ethylhexanoate (EC 221-110-7). Since the test material is different from the Substance, we evaluated this information as a read-across adaptation under Annex XI, section 1.5 of REACH.

20 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.

21 Additional information on what is necessary when justifying a read-across approach can be found in the Guidance on IRs and CSA, Chapter R.6. and related documents (RAAF, 2017; RAAF UVCB, 2017).

22 In order to predict the properties of the Substance from information, obtained with the analogue substance tert-amyl peroxy-2-ethylhexanoate (EC 221-110-7), hereafter referred as source substance, you provide the following reasoning: "*the difference between current substance and the structural analogous substance is in one of the alkyl groups that is just one carbon shorter".* You provided a table with the structures of both substances and information on the performed genotoxicity studies.

23 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance to be quantitatively equal to those of the source substance.

24 We have analysed this information and identified the following issues:

3.3.1.1. Inadequate read-across hypothesis

25 Annex XI, Section 1.5. requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must include an explanation why the properties of the Substance may be predicted from other substances in the group, i.e. a read-across hypothesis. This hypothesis should be based on recognition of the structural similarities and differences between the substances (Guidance on IRs and CSA, Section R.6.). It should explain why the differences in the chemical structures should not influence the toxicological properties or should do so in a regular pattern, taking into account that variations in chemical structure can affect both toxicokinetics (uptake and bioavailability) and toxicodynamics (e.g. interactions with receptors and enzymes) of substances (Guidance on IRs and CSA, Section R.6.2.1.3.).

26 Your read-across hypothesis is only based on the structural similarity between the source substance, which you consider a sufficient basis for predicting the properties of the Substance. However, your hypothesis does not explain why the structural differences between the substances do not influence the toxicological properties or do so in a regular pattern.

27 While structural similarity is a prerequisite for applying the grouping and read-across approach, it does not necessarily lead to predictable or similar toxicological properties. You have not provided a well-founded hypothesis to establish a reliable prediction for a toxicological properties, explaining why the structural differences do not influence toxicokinetics and toxicodynamics of the substances, and thus why the properties of the Substance may be predicted from information on the source substance.

3.3.1.2. Missing robust study summaries

28 Annex XI, Section 1.5. requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must include robust study summary for each source study used in the adaptation.

29 Robust study summary must provide a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study (Article 3(28)).

30 In your comments you refer to the negative results from a TGR (OECD TG 488) study for the structural analogue tert-amyl peroxy-2-ethylhexanoate (EC 221-110-7). In addition, you also mention resulst (negative/positive), obtained from other genotoxicity/mutagenicity studies with the source substance that are supposed/intended to support the prediction.

31 You have not provided detailed information on the methods, results and conclusions, allowing for an independent assessment of the studies. Therefore, you have failed to provide a robust study summary for each source study used in the adaptation as required by Annex XI, Section 1.5.

3.3.1.3. Conclusion

32 For the reasons above, you have not established that relevant properties of the Substance can be predicted from data on the source substance(s). Your read-across approach under Annex XI, Section 1.5. is rejected.

33 Based on the above, the information requirement is not fulfilled.

3.4. Test selection

34 According to the Guidance on IRs & CSA, Section R.7.7.6.3., either the in vivo mammalian alkaline comet assay ("comet assay", OECD TG 489) or the transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) are suitable to follow up a positive in vitro result on gene mutation.

3.4.1. Study design

3.4.1.1. Comet assay

35 In case you decide to perform the comet assay, according to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified (OECD TG 489, paragraph 23).

36 Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

37 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.

3.4.1.1.1. Cross-linking properties

38 You are reminded that you may decide to take into account the potential cross-linking properties of the Substance in the experimental setup of the comet assay and perform a modified comet assay in order to detect cross links. Therefore, you may consider preparing and analysing two sets of slides: one set of slides submitted to the standard experimental conditions (as described in the OECD TG 489); the other set of slides submitted to modified experimental conditions that enable the detection of DNA crosslinks. The modified experimental conditions may utilise one of the following options: (1) increase of electrophoresis time, e.g. as described in reference 23 [1] in the OECD TG 489; (2) treatment of isolated cells (either in suspension or embedded in the slides) with a chemical (e.g. MMS); or (3) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options 2 and 3 are described e.g. in references 36-39 [2-5] in the OECD TG 489 or Pant et al. 2015 [6]). In order to ensure the robustness of the test result a specific positive control group of animals would be needed.

[1] Nesslany *et al.* (2007) *In vivo* comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds *Muta Res*;630(1-2):28-41.

[2] Merk and Speit (1999) Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ Mol Mutagen*;33(2):167-72.

[3] Pfuhler and Wolf (1996) Detection of DNA-crosslinking agents with the alkaline comet assay. *Environ Mol Mutagen*;27(3):196-201.

[4] Wu and Jones (2012) Assessment of DNA interstrand crosslinks using the modified alkaline comet assay. *Methods Mol Biol*;817:165-81.

[5] Spanswick *et al.* (2010) Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay. *Methods Mol Biol*;613:267-282.

[6] Pant K *et al.* (2015) Modified *in vivo* comet assay detects the genotoxic potential of 14-hydroxycodeinone, an α,β -unsaturated ketone in oxycodone. *Environ Mol Mutagen*;56(9):777-87.

3.4.1.1.2. Germ cells

39 You may consider collecting the male gonadal cells 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.

3.4.1.2. TGR assay

40 In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats.

41 Also, according to the test method OECD TG 488, the test substance is usually administered orally.

42 Based on the OECD TG 488, you are requested to follow the 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals.

43 According to the test method OECD TG 488, the test must be performed by analysing tissues from liver, as slowly proliferating tissue and primary site of xenobiotic metabolism, and from glandular stomach and duodenum, as rapidly proliferating tissue and site of direct 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 mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

3.4.1.2.1. Germ cells

44 You must collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, to limit additional animal testing. According to the OECD TG 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA to decide on the need for assessment of mutation frequency in the collected germ cells. This type of evidence is relevant under Annex IX, Section 8.4.5. in case of positive result in the *in vivo* genotoxicity test on somatic cells and for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

4. Analysis of male germ cells

45 Under Annex IX, Section 8.4.5., an appropriate *in vivo* mammalian germ cell genotoxicity study is an information requirement if there is a positive result in an available *in vivo* mammalian somatic cell genotoxicity study, which gives rise to concern. The *in vivo* mammalian germ cell genotoxicity study must address the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

4.1. Triggering of the information requirement

46 The results of request 3 will determine if an appropriate *in vivo* mammalian germ cell genotoxicity study is triggered. If triggered, this study must address the gene mutation concern.

4.2. Data in the dossier

47 Your dossier does not contain any *in vivo* mammalian germ cell genotoxicity study.

48 Therefore, this information requirement, if triggered, is not fulfilled.

4.3. Study selection and design

49 In case you will perform the TGR assay (OECD TG 488) to fulfil the information requirement for an *in vivo* mammalian somatic cell genotoxicity study and if the analysis of somatic cell is positive, you must analyse the male germ cells from the seminiferous tubules, collected and stored as specified in section 3.4.1.2.1 above. The analysis must be performed according to the OECD TG 488.

50 ECHA notes that if you decide to carry out the comet assay (OECD TG 489) and the test is positive, a subsequent germ cell genotoxicity study (TGR/OECD TG 488) may still be required under Annex IX, Section 8.4.5.

5. Extended one-generation reproductive toxicity study

51 An extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is an information requirement under Annex IX, Section 8.7.3., if the available repeated dose toxicity studies indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. Furthermore Column 2 defines the conditions under which the study design needs to be expanded.

5.1. Triggering of the information requirement

52 You claim that the "study [is] scientifically not necessary" because the "effects noted in the OECD 421 studies, occurred in the presence of maternal toxicity and effects on the pups were not duplicated in the OECD 414 study conducted on the read-across substance CAS# 3006-31-7". Further, you state that "[i]n addition, no effects on reproductive organs were reported in the 90-day study conducted on the read-across substance as well as no adverse effects reported in the OECD 414 study conducted on CAS# 3006-82-4".

53 Based on the above, you conclude that "according to Annex IX 8.7.3, this study is not required".

54 ECHA notes that in your justification as set out above, you refer to two different CAS numbers (CAS 3006-31-7 and CAS 3006-82-4) as "read-across substance" for which an OECD TG 414 study is conducted. However, in your registration dossier you have provided a PNDT study in rat, performed with the analogue substance CAS 3006-82-4, EC 221-110-7. You did not provide any other information on a PNDT study conducted with another substance.

55 In your dossier you have provided a reproduction/developmental toxicity screening study with the Substance performed according to OECD TG 421. The study shows reduced survival of offspring:

- 4 out of 9 pregnant high-dose dams had total litter loss by day 6 post partum and one female was with only still births;
- for the dams with live pups, the mean pup loss at birth was significantly higher in high dose dams (10.7 % vs 0.45 % in control) resulting in lower litter size at birth in high dose dams compared to control (13.5 vs. 15.1 in control);
- cumulative loss of pups at day 6 post-partum was significantly higher for high dose dams (35.23 % vs 2.59 % control) resulting in significantly lower live litter size in

high dose dams compared to control (9.0 vs 14.7 pups in control).

56 You state that the effects "occurred in the presence of maternal toxicity". However, while the thymus was affected in high dose females, there were no significant differences in the maternal body weights and body weight gain and no clinical signs (other than salivation) indicating that the females were not able to nurture their pups. The effects in the OECD TG 421 study with the Substance were observed in the absence of severe maternal toxicity. Therefore, the direct effects of the Substance on the survival of the offspring cannot be excluded.

57 Further, you claim that the effects on the pups were not observed in the prenatal developmental toxicity study (OECD TG 414) with the analogue substance tert-butyl peroxy-2-ethylhexanoate (EC: 221-110-7; CAS: 3006-31-7). ECHA notes that the prenatal developmental toxicity study is terminated before the dams are allowed to give birth and thus, it is not able to detect stillbirths and postnatal loss. Therefore, the lack of effects on the offspring in the PNDT study does not alleviate the concern in relation with reproductive toxicity based on the OECD 421 study with the Substance.

58 Therefore, the reported OECD TG 421 study with the Substance reveals concerns in relation with reproductive toxicity, e.g. reduced survival of offsprings which must be investigated further.

59 Therefore, the information requirement is triggered.

60 In your comments to the draft decision you reiterate your claim that the effects occurred in the presence of maternal toxicity. You argue that based on the results from the OECD TG 421 study "*it can be concluded that the dams of the high dose group showed substantial maternal toxicity during the lactation period which was reflected in statistically significantly lower body weights and food intake compared to control, resulting in limited or lack of maternal care, which finally resulted in adverse effects in pups*". Therefore, you state that the impact of the Substance on the survival of litters and pups "[…] *should not be interpreted as a direct effect of the test substance on reproduction*". In addition, you claim that "*No differences were noted among the groups between corpora lutea, implantations, pre-implantation loss, total litter size at birth, pre-birth loss and gestation length*".

61 However, your claims are not supported by the detailed results from the OECD TG 421 study. In particular, based on the results shown in Tables 3 and 9 (given both in the dossier and in the comments), the observed pups' effects seem to occur before the body weight of the dams was affected. In Table 9 there were only 4 litters left on post-natal day (PND) 1. This means that since, there were 8 live litters at birth, most of the litter loss was between birth and PND 1. However, the body weight on PND 1 of 9 females, reported in Table 3 is similar to controls (332.81 in CTR compared to 322.72 in HD). This does not indicate any significant effect on body weight at the time of litter loss. In addition, the reported clinical signs of the dams with total litter loss (all pups cold in touch and no milk in the stomach), observed already at PND 0, may be due to effects from exposure of fetus during gestation, rather then lack of maternal care.

62 Based on the above, ECHA reiterates that the observed pup effects are considered as substance-related developmental effects rather then secondary to maternal toxicity.

63 The information provided in your comments does not change the assessment.

5.2. Study design

5.2.1. Species and route selection

64 According to the test method OECD TG 443, the rat is the preferred species. Therefore, the study must be conducted in the rat.

65 As the Substance is a liquid, the study must be conducted with oral administration of the Substance (Annex X, Section 8.7.3., Column 1).

5.2.1.1. Pre-mating exposure duration

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

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

68 In your comments to the draft decision you seem to question the length of pre-mating exposure, because you consider that "based on the results of the OECD TG 421 there is no issue whatsoever regarding fertility".

69 ECHA agrees that the available data in the OECD TG 421 study does not show impairment on fertility, however notes that the OECD TG 421 study provides limited information on the mating and fertility. The study has only two-week pre-mating exposure duration, which does not cover the full spermatogenesis and folliculogenesis. Therefore, possible fertility effects, resulting from effects of the Substance on the whole cycle of gamete production, covered in the EOGRTS, cannot be excluded. In addition, the statistical power of the OECD TG 421 study is lower, compared to EOGRTS.

70 The design of the EOGRT study has to be adequate to ensure that it will fulfil regulatory requirements, including being adequate for hazard identification and risk assessment as well as classification and labelling, including categorisation (OECD TG 443, paragraph 22). For these purposes, the ten weeks premating exposure duration is one of the elements together with the appropriate dose level selection which allow production of data for an informed decision making for classification and labelling, including categorisation, for the hazard endpoint for sexual function and fertility, and for risk assessment.

71 Therefore, the requested pre-mating exposure duration is ten weeks.

5.2.1.2. Dose-level setting

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

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

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

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

(2) in case of clear evidence of an adverse effect on sexual function and fertility without severe suffering or deaths in P0 animals, the highest dose level in P0 animals must be determined based on such clear evidence, or

(3) in the absence of such clear evidence, the highest dose level in P0 animals must be set to be the highest possible dose not causing severe suffering or death, or

(4) if there is such clear evidence but the highest dose level set on that basis would cause severe suffering or death, the highest dose level in P0 animals must be set to be the highest possible dose not causing severe suffering or death, or

(5) the highest dose level in P0 animals must follow the limit dose concept.

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

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

78 In your comments to the draft decision you claim that the setting of the selection of the highest dose level cannot be followed, due to the following reasons:

i) *"In the OECD TG 421 study the highest dose tested 1000 mg/kg bw/day […] did not show any fertility findings or findings on sexual function. It is therefore highly unlikely such findings will be noted in an EOGRTS at 1000 mg/kg bw/day".*

ii) *"At this dose level reduced survival was noted in the OECD TG 421 which was considered due to maternal toxicity, resulting in lack of pup care or limited pup care and as a consequence complete or partly litter loss".*

79 Based on the above, you claim that "*an EOGRTS at the same dose levels - because a higher level than 1000 mg/kg bw/day is not warranted - will not provide any new information*". You conclude that "*Based on all this information, the registrants claim that an EOGRTS with a main focus on fertility can technically not be performed for this substance as it would be in conflict with the requirements of the developmental part of the EOGRTS*".

80 However, as noted in section 4.2.1.1. above the design of the EOGRT study has to be adequate to ensure that it will fulfil regulatory requirements. ECHA has published an *Advice on dose level selection for the conduct of reproductive toxicity studies (OECD TGs 414, 421/422 and 443) under REACH* [2]. Based on this document, "T*he top dose selection should indeed also take regulatory requirements in the EU into account (see, for example paragraph 22 of OECD TG 443), i.e. its applicability for being able to achieve conclusive decisions on classification and labelling. For the highest dose level, it should be demonstrated that the aim is that it is the highest possible dose level without severe suffering or death, or the limit dose concept shall be used*".

81 Based on the assessment of the OECD TG 421 study, at the dose of 1000 mg/kg bw/day: 1) there is no severe suffering or death in P0; 2) the effect on the litter survival is considered a toxicological property of the Substance, and 3) in case of low numbers of pups at high dose, other investigatons such as on developmental toxicity can be performed with the remaining high-dose pups as well as pups of the other dose levels. Therefore, ECHA

[2] https://echa.europa.eu/documents/10162/17220/211221_echa_advice_dose__repro_en.pdf/27159fb1-c31c-78a2-bdef-8f423f2b6568?t=1640082455275

disagrees with your claim that "*an EOGRTS at same dose levels […] would be in conflict with the requirements of the developmental part of the EOGRTS*".

5.2.1.3. Cohorts 1A and 1B

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

5.2.1.3.1. Histopathological investigations in Cohorts 1A and 1B

83 In addition to histopathological investigations of cohorts 1A, organs and tissues of Cohort 1B animals processed to block stage, including those of identified target organs, must be subjected to histopathological investigations (according to OECD TG 443, paragraph 67 and 72) if

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

5.2.1.3.2. Splenic lymphocyte subpopulation analysis

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

5.2.1.3.3. Investigations of sexual maturation

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

5.2.1.4. Further expansion of the study design

86 The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if relevant information becomes available from other studies or during conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Annex IX, Section 8.7.3., Column 2. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in Guidance on IRs & CSA, Section R.7.6.

References

The following documents may have been cited in the decision.

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

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

Guidance on data-sharing; ECHA (2017).
Guidance for monomers and polymers; ECHA (2023).
***Guidance on intermediates*;** ECHA (2010).
All guidance documents are available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

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

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

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

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

Appendix 2: Procedure

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 23 August 2022.

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

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

ECHA took into account your comments and amended the request (5. Extended one-generation reproductive toxicity study).

In your comments on the draft decision, you requested an extension of the deadline to provide information from 36 to 48 months from the date of adoption of the decision.

ECHA notes that the timeline given in the initial draft decision had not been, due to a mistake, extended by 12 months as was intended and explained above. Therefore, ECHA has corrected this mistake and extends the deadline to 48 months.

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

ECHA received proposal(s) for amendment and modified the draft decision.

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

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

The Member State Committee unanimously agreed on the draft decision in its MSC-86 written procedure. ECHA adopted the decision under Article 51(6) of REACH.

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

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

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

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

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

Appendix 4: Conducting and reporting new tests for REACH purposes

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

1.1 Test methods, GLP requirements and reporting

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

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

(3) Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries (https://echa.europa.eu/practical-guides).

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

1.2 Test material

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

(1) Selection of the Test material(s)

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

- the variation in compositions reported by all members of the joint submission,
- the boundary composition(s) of the Substance,
- the impact of each constituent/impurity on the test results for the endpoint to be assessed. For example, if a constituent/impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/impurity.

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

- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
- The reported composition must include all constituents of each Test Material and their concentration values.

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

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