~~Confidential~~

Helsinki, 14 January 2022

Addressees
Registrant(s) of JS_224-052-0 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
01/10/2020

Registered substance subject to this decision ("the Substance")
Substance name: (E)-anethole
EC number: 224-052-0

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***21 October 2024***.

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

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

1. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays also requested below (triggered by Annex VIII, Section 8.4., column 2)

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

1. Transgenic rodent somatic and germ cell gene mutation assay (Annex IX, Section 8.4., column 2; test method: OECD TG 488 from 2020) 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 (Annex IX, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral gavage, in one species (rat or rabbit)

3. Extended one-generation reproductive toxicity study (triggered by Annex IX, Section 8.7.3., column 1; test method: OECD TG 443) by oral gavage, in rats, specified as follows:

   - Ten weeks premating exposure duration for the parental (P0) generation;
   - Dose level setting shall aim to induce systemic toxicity at the highest dose level;
   - Cohort 1A (Reproductive toxicity);

– Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.

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

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

- Appendices entitled "Reasons to request information required under Annexes VIII to X of REACH", respectively.

Information required depends on your tonnage band

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

- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa.

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

For certain endpoints, ECHA requests the same study from registrants at different tonnages. In such cases, only the reasoning why the information is required at lower tonnages is provided in the corresponding Appendices. For the tonnage where the study is a standard information requirement, the full reasoning for the request including study design is given. Only one study is to be conducted; the registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants under Article 53 of REACH.

How to comply with your information requirements

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

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

Appeal

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

Failure to comply

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

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

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

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

1. Transgenic rodent somatic and germ cell gene mutation assay

OR

In vivo mammalian alkaline comet assay

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

The ECHA guidance R.7a states that following a positive result in an *in vitro* test, "*adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo. In cases where it can be sufficiently deduced that a positive in vitro finding is not relevant for in vivo situations (e.g. due to the effect of the test substances on pH or cell viability, in vitro-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold mechanism coming into play only at high concentrations that will not be reached in vivo has been identified (e.g. damage to non-DNA targets at high concentrations), in vivo testing will not be necessary.*"

Your dossier contains positive results for the *in vitro* gene mutation study in mammalian cells which raise the concern for gene mutation. However, no data from an appropriate *in vivo* somatic cell genotoxicity study is available in the dossier. The *in vivo* studies submitted in your dossier do not address gene mutation. In addition, they are inadequate studies for the reasons described under Section B.1.

ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern identified *in vitro*.

For the assessment of the information provided and the specifications of the study to be performed, see the request B.1.

Appendix B: Reasons to request information required under Annex IX of REACH

1. **Transgenic rodent somatic and germ cell gene mutation assay**

   OR

   ***In vivo* mammalian alkaline comet assay**

Under Annex IX, Section 8.4, column 2 of REACH, 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.

In relation to the first condition, your dossier contains positive results for the *in vitro* gene mutation study in mammalian cells which raise the concern for gene mutation.

In relation to the second condition, your dossier contains the following *in vivo* studies:

i. *in vivo* unscheduled DNA synthesis (UDS) test according to OECD TG 486 with the Substance (1996).
ii. non-guideline anti-genotoxicity study with the Substance (2002).

In the comments to the draft decision, you also refer to a publication ([redacted], 1995) to support your conclusions.

We have assessed this information and identified the following issues:

ECHA Guidance R.7a clarifies that in order to justify that an *in vivo* somatic cell genotoxicity study does not need to be performed in accordance with Annex IX, Section 8.4, column 2, the results of the available *in vivo* study(ies) must address the specific concern raised by the *in vitro* positive result.

Study (i) provides an indication of induced damage to DNA followed by DNA repair (measured as unscheduled DNA synthesis in liver cells), but does not provide direct evidence of mutation. In the comments to the draft decision, you claim that study (i) was adequately conducted and is appropriate to investigate point mutation in vivo even if it investigates DNA damage and not gene mutation specifically, like the comet assay.

As reminded in the ECHA Guidance[2], the UDS test is sensitive to some (but not all) DNA repair mechanisms and not all gene mutagens are positive in the UDS test. The sensitivity of the UDS test has been questioned (Kirkland and Speit, 2008)[3]. Therefore, a negative result in a UDS assay alone is not a proof that a substance does not induce gene mutations in the conditions of the test.

ECHA disagrees with your comment that study (i) was adequately conducted. Study (i) was not conducted under GLP and predates the OECD TG 486 published in 1997. In particular, the highest dose tested in study (i) was 1000 mg/kg bw without any dose rationale provided, whereas OECD TG 486 indicates a limit dose of 2000 mg/kg bw.

Study (ii) investigates the anti-genotoxicity potential of the Substance against known genotoxins in the *in vivo* micronucleus test and does not investigate gene mutation.

In the comments to the draft decision, you argue that, in addition to the UDS test, other *in vivo* studies available demonstrate the absence of DNA damage concern for the Substance.

[2] ECHA Guidance R.7a, R.7.7.6.3, p. 568
[3] Kirkland D and Speit G (2008) Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens III. Appropriate follow-up testing *in vivo*. Mutat Res 654:114-32.

Study (ii) does not investigate the genotoxic effects of the Substance itself and therefore does not demonstrate the absence of DNA damage concern for the Substance. In addition, you assigned study (ii) a low reliability score of 4 and ECHA agrees that it is unreliable.

Regarding the other *in vivo* studies you refer to in your comments to the draft decision ([redacted], 1995), the information in your comments is not sufficient for ECHA to make an assessment on whether they are adequate and appropriate to address the gene mutation concern, because you only provided the author's name and date of publication and nothing else. Details on the study designs, test methods followed and results obtained, as well as an explanation of how this information relates to the gene mutation endpoint are missing.

Overall, the information provided in your comments does not change the assessment.

The provided *in vivo* tests are not appropriate to address the concern identified by the *in vitro* gene mutation study in mammalian cells.

Therefore, the conditions set out in Annex IX, Section 8.4, column 2 are met and the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered.

i. Test selection

According to the ECHA Guidance Chapter R.7a, Section R.7.7.6.3, the transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive *in vitro* result on gene mutation. In your comments to the draft decision, you indicate your preference for the comet assay since the use of genetically modified animals is in conflict with your company rules.

ii. Test design

In case you decide to perform the comet assay according to the test method OECD TG 489, the test must be performed in rats.

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

In your comments to the draft decision, you agree to perform the *in vivo* comet assay if an *in vivo* mutagenicity test is requested and to use the oral route and rat as test species. However, you propose to test only one site of contact tissue since the substance induced positive results in the *in vitro* gene mutation study in mammalian cells only in the presence of S9.

In line with the test method OECD TG 489, the test must be performed by analysing tissues from the liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. The fact that the *in vitro* gene mutation study in mammalian cells was only positive in the presence of S9 indicates that the liver is a relevant target organ and that it can transform the Substance into mutagenic metabolites. However, this does not exclude a possible biotransformation of the Substance in other organs like the gastro-intestinal tract and possible mutagenic effects at the sites of contact.

Further, 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.

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 and the test substance is usually administered orally.

Based on the recent update of OECD TG 488 (2020), you are requested to follow the new 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. This updated version provides for a transitional period for the new version. However, ECHA is aware that testing according to the updated OECD TG is already available from CROs and the new study design would provide meaningful germ cell data, so this decision requires the application of the new version.

According to the test method OECD TG 488, the test must be performed by analysing tissues from the liver as slowly proliferating tissue and primary site of xenobiotic metabolism, 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 −70ºC) 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.

iii. Germ cells

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483, depending on the concern raised by the substance) may still be required under Annex IX of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

Therefore, in case you decide to perform the comet assay, you may consider to collect the male gonadal cells collected from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, in accordance to Annex IX, Section 8.4., column 2, 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.

In case you decide to perform the TGR, you must collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, in order to limit additional animal testing. According to the OECD 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, in accordance to Annex IX, Section 8.4., column 2, to decide on the need for assessment of mutation frequency in the collected germ cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

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

Pre-natal developmental toxicity study in one species is a standard information requirement under Annex IX to REACH (Section 8.7.2.).

You have provided the following study in your dossier:

i. Developmental toxicity study in rats claimed equivalent to OECD TG 414, with the Substance (1992)

We have assessed this information and identified the following issue(s):

In order to be considered compliant and enable assessing if the Substance is a developmental toxicant, the study has to meet the requirements of OECD TG 414. The criteria of this test guideline include e.g.:

- 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,
- examination of the foetuses for external, skeletal and soft tissue alterations (variations and malformations), measurement of anogenital distance in live rodent foetuses.

In the study you have provided:

- 10 pregnant females were tested for each test group. The statistical power of the information provided is not sufficient because it does not fulfil the criterion of 20 pregnant females for each test group set in OECD TG 414.
- The animals were exposed from day 7 prior to mating to postnatal day 4 and no caesarean section was performed. The study does not have the required exposure duration because the exposure duration is not from implantation until the day prior to scheduled caesarean section as required in OECD TG 414.
- The weight and histopathology of the thyroid gland has not been examined in dams, thyroid hormone measurements have not been conducted in dams, gravid uterus weight has not been measured as required in OECD TG 414.
- External, skeletal and soft tissue alterations (variations and malformations) have not been examined, anogenital distance has not been measured in live foetuses as required in OECD TG 414.

Based on the above, the information you provided do not fulfil the information requirement.

A PNDT study according to the test method OECD TG 414 must be performed in the rat or rabbit as preferred species, with oral[4] administration of the Substance.

In the comments to the draft decision, you agree with the request and acknowledge that study (i) is rather similar to an OECD TG 421 study than to an OECD TG 414 study. You agree to perform the requested study through the oral route, but you disagree with using gavage instead of feeding for administration of the Substance. In particular, you claim that:

- OECD TG 414 explicitly allows both gavage and feeding administration;
- feeding administration reflects much more the exposure scenario for humans;
- study (i) and the four-generation reproductive toxicity feeding study in rats (1971) provided in your dossier are not comparable and cannot be used to compare routes of administration;
- administration *via* feed might allow to test even higher test concentrations than gavage.

However, according to OECD TG 414 (Paragraph 18), oral gavage is the usual route of administration and if another route of administration is used, the tester should provide

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

justification and reasoning for its selection. Expected human exposure is only considered in REACH to select the most appropriate route of administration (i.e., oral, dermal or inhalation) and in OECD TG 414 (Paragraph 17) to determine whether a higher oral dose level than the limit dose is to be used in the limit test, but not to select the mode of administration (i.e. gavage or feeding).

In addition, more severe effects (increase in the average gestation length, increase in stillborn pups and decrease in pups viability index) were observed after gavage administration in study (i) than after dietary administration in the four-generation reproductive toxicity study (1971) provided in your dossier. Although study protocols and designs are different between these two studies, female rats were daily exposed from premating until after birth of the pups in both studies, therefore covering in both cases the full gestation period and (part of) the postnatal period, which are considered as critical periods for the assessment of reproductive and developmental toxicity. One major difference between the two studies lies in the mode of administration of the Substance and the results indicate that gavage administration may cause more severe reproductive and developmental toxicity than dietary administration since effects in study (i) occured at lower doses (NOAEL = 175 mg/kg bw/d) than in the four-generation reproductive toxicity study (NOAEL = 1% in diet, equivalent to approximately 600 – 1500 mg/kg bw/d).

Regarding the achievable top dose, ECHA agrees that dietary administration may allow higher doses to be reached than gavage in specific cases, if substances with irritating or corrosive properties cause local toxicity in the stomach and limit the dose that can be administered as a single dose. However, this does not apply to the Substance since no irritating or corrosive properties were reported in the studies available in your dossier. Contrary to your claim, reduced palatability of the diet caused by the Substance and difficulty in achieving and maintaining high dose levels were reported in the four-generation reproductive toxicity study (1971) and OECD TG 408 feeding study (1997) provided in your dossier. Testing must be performed at appropriately high dose levels intended to produce some toxicity to provide adequate information on developmental toxicity to ensure that the data generated are adequate for hazard identification, classification and risk assessment. Based on the above, there is risk that the doses achieved through feeding are not sufficiently high for proper hazard identification and risk assessment.

Furthermore, compared to dietary administration, oral gavage allows a better control of the doses administered and a more precise assessment of the dose-response relationship, which is also a crucial aspect of hazard identification and risk assessment. The uncertainty regarding the actual dose administered through feeding is further supported by the wide range of doses that you calculated as equivalent to a 1%-diet in the four-generation reproductive toxicity study with (1971), i.e. 600 – 1500 mg/kg bw/d, which makes it difficult to understand whether the limit dose of 1000 mg/kg bw/d was achieved or not.

Overall, the information provided in your comments does not change the assessment.

Therefore, the study must be conducted using gavage administration.

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

The basic test design of an extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is a standard information requirement under Annex IX to REACH, 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.

You have provided the following study in your dossier for a column 2 adaptation:

i. Non-guideline four-generation reproductive toxicity study in rats claimed equivalent to OECD TG 416, with the Substance (1971)

Although you indicated study (i) as equivalent to an OECD TG 416 study in your dossier, you clarify in the comments to the draft decision that study (i) was not used for column 2 adaptation.

Therefore, ECHA understands that the study is submitted to address the identified reproductive concern, i.e. whether the EOGRT study is triggered or not.

We have assessed this information and identified the following issues:

Concern triggering the EOGRT study

As already mentioned above, an EOGRT study is required if the available repeated-dose studies indicate adverse effects or concerns related to reproductive toxicity.

Adverse effects on reproductive organs or tissues or other concerns in relation with reproductive toxicity are observed in available studies with the Substance. More specifically, an increase in the average gestation length, a statistically significant increase in stillborn pups and a statistically significant decrease in pups viability index were observed in a developmental toxicity study in rats (1992) as well as various gross pathology findings in the uterus of female rats in an OECD TG 408 study (1997).

In the comments to the draft decision, you disagree with the triggering of an EOGRT study on the following basis:

- You argue that the effects observed in the developmental toxicity study in rats (1992) were obtained at massive systemic toxic doses causing lethality and that the increase in gestation lengths is likely due to delayed development of the pups following reduced feed consumption and body weight gain as well as sytemic toxicity in the dams.
- You further claim that no effect in the uterus was noted in the OECD TG 408 study (1997).
- You finally indicate that this request is not in line with the animal welfare provision of REACH considering new animal studies as a last resort only.

However, in the comments to the draft decision you also acknowledge that the developmental toxicity study (1992) is rather similar to an OECD TG 421 study than to an OECD TG 414 study.

According to OECD TGs 414 and 421, the highest dose should be chosen with the aim to induce *"some developmental and/or maternal toxicity (clinical signs or a decrease in body weight)"* (OECD TG 414) or *"toxic effects"* (OECD TG 421) but not death or severe suffering. In this study, the main signs of maternal toxicity reported at the high dose of 350 mg/kg bw/d were *"significantly reduced mean body weight and feed consumption"*, with no details provided on the percentage of decrease that would support your claim of *"massive systemic toxic doses".* One animal died in the high-dose group on gestation day 20 but, from the limited information available, it is unclear whether death is related to the treatment or not, since this was the only mortality reported and the cause of death was congested lungs. Therefore, ECHA considers the high dose used in this study as valid and its results relevant for establishing a fertility concern.

You also attribute the increased gestation length observed in the high-dose group to reduced feed consumption, delayed fetal development and systemic maternal toxicity but you do not provide more detailed information on individual/numerical data. Therefore the concern on

reproductive toxicity remains, as based on the information provided, it is not possible to exclude other possible causes, such as effects on the process of parturition.

Furthermore, *"various uterine findings in the females"* are indicated in the OECD TG 408 study in rats (1997) available in your dossier. Although no details are provided, these effects are reported as treatment-related gross pathological findings, which raise a concern for adverse effects on reproductive organs.

Regarding your comment on animal welfare considerations, minimisation of vertebrate animal testing is not on its own a legal ground for adaptation under Column 2 nor under the general rules of Annex XI. Therefore, your adaptation is rejected.

Overall, the information provided in your comments does not change the assessment outcome.

Study not addressing the reproductive and developmental concerns

In the comments to the draft decision, you argue that study (i) supports the lack of reproductive toxicity of the Substance as no effects were noted on any reproductive parameter, at doses even higher than those tested in the developmental toxicity study (1992).

However, to be considered compliant and enable concluding if the Substance is a reproductive toxicant, the study has to meet the requirements of OECD TG 443 as specified in REACH.

The key parameters of this test guideline include:

- testing of at least three dose levels and a concurrent control,
- examination of key parameters for sexual function and fertility,
- examination of key parameters for pre/peri/postnatal developmental toxicity,
- examination of key parameters for endocrine modes of action,
- examination of key parameters for systemic toxicity.

In the study you have provided:

- only one dose was tested;
- functional fertility (including gestation length), sperm parameters, oestrus cyclicity, postnatal development, lactation and nursing have not been investigated and histopathology of the gonads is missing;
- prenatal and/or peri/postnatal developmental toxicity has not been examined as required in OECD TG 443;
- investigations of endocrine modes of action, such as oestrous cycle, endocrine (including reproductive) organ weights and histopathology, anogenital distance/nipple retention, sexual maturation (vaginal opening and preputial separation, time from vaginal opening to first oestrous cycle), thyroid hormone measurements have not been performed as required in OECD TG 443;
- investigations for full clinical chemistry, full haematology, full histopathology of organs and tissues have not been performed as required in OECD TG 443.

Therefore, ECHA considers that study (i) is unreliable and that key parameters related to the effects observed in the available studies and giving raise to reproductive and developmental concerns are missing.

The reliability of study (i) is further affected by the questionable mode of administration of the Substance through the diet, and not through oral gavage, and the uncertainty regarding the dose actually administered to the test animals, as described in Section B.1.

Moreover, ECHA agrees with your comments that the OECD TG 414 requested under Section B.2 will allow to investigate embryonic or foetal developmental effects of the Substance to some extent since an OECD TG 414 provides relevant information on toxicity to the offspring before birth. However, it will not provide information on fertility, reproductive performance and developmental toxicity manifested shortly after birth, or toxicity to the offspring after birth up to adulthood (including reproductive toxicity and systemic toxicity) as foreseen to be investigated in an OECD TG 443 study.

For completeness, we note that study (i) would not meet the specifications of OECD TG 416 either since only one dose was tested, sperm parameters and oestrus cyclicity have not been investigated and gross necropsy and histopathology of organs and tissues have not been performed.

Overall, the study (i) is not reliable and therefore the information provided in your comments does not change the assessment outcome.

Based on the above, an EOGRT study according to OECD TG 443 as specified in this decision is an information requirement for your registration, because Column 1 criteria at Annex IX, section 8.7.3 are met.

Further, in the absence of any information to address this information requirement, your registration dossier does not fulfil the information requirement.

Specifications for the study design

Premating exposure duration and dose-level setting

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

Ten weeks premating 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.[1]

In order to be compliant and not to be rejected due to too low dose levels, the highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects. A descending sequence of dose levels should be selected in order to demonstrate any dose-related effect and to establish NOAELs.

If there is no relevant data to be used for dose level setting, it is recommended that range-finding results are reported with the main study.

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

Cohorts 1A and 1B

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

Species and route selection

The study must be performed in rats with oral[5] administration. More severe effects (increase in the average gestation length, increase in stillborn pups and decrease in pups viability index) were observed after gavage administration in the developmental toxicity study provided in your dossier than after dietary administration in study (i). This indicates that gavage administration may cause more severe reproductive and developmental toxicity than dietary administration. Therefore, the study must be conducted using gavage administration.

Further expansion of the study design

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and/or 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 the conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Column 2, Section 8.7.3., Annex IX. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in ECHA Guidance[6].

[5] ECHA Guidance R.7a, Section R.7.6.2.3.2.
[6] ECHA Guidance R.7a, Section R.7.6.

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

A. Test methods, GLP requirements and reporting

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

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

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[7].

B. Test material

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

1. Selection of the Test material(s)

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

2. Information on the Test Material needed in the updated dossier
   - You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[8].

[7] https://echa.europa.eu/practical-guides
[8] https://echa.europa.eu/manuals

Appendix D: 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 14 August 2020.

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

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

In addition in your comments to the initial draft decision, you raised a question concerning cost sharing, ECHA addressed this query directly with you.

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: List of references - ECHA Guidance[9] and other supporting documents

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

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

Read-across assessment framework (RAAF, March 2017)[10]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[11]

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

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

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

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

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

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

OECD Guidance documents[12]

[9] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[10] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[11] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316
[12] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

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

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

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

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

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

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

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