EUROPEAN CHEMICALS AGENCY

Helsinki, 11 February 2019

Addressee: [redacted]

Decision number: CCH-D-2114457570-49-01/F
Substance name: 3-aminopropyldiethylamine
EC number: 203-236-4
CAS number: 104-78-9
Registration number: [redacted]
Submission number: [redacted]
Submission date: 16/03/2017
Registered tonnage band: 100-1000

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA requests you to submit information on:

1. **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 with the registered substance;**

   or

   Transgenic rodent somatic and germ cell gene mutation assays (Annex IX, Section 8.4., column 2; test method: EU B.58./OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach with the registered substance; germ cells and duodenum shall be harvested and stored for up to 5 years. Duodenum shall be analysed if the results of the glandular stomach and of the liver are negative or inconclusive. The test material used should be freshly prepared.

2. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2., column 2; test method: OECD TG 414) in a second species (rabbit), oral route with the registered substance;**

3. **Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method: OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **At least two 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) with extension to mate the Cohort 1B animals to produce the F2 generation;**
   - **Cohorts 2A and 2B (Developmental neurotoxicity); and**
   - **Cohort 3 (Developmental immunotoxicity).**

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI to the REACH Regulation. To ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective annex, and adequate and reliable documentation.

You have to submit the requested information in an updated registration dossier by **18 February 2022**. You also have to update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and advice and further observations are provided in Appendix 3.

Appeal

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

Authorised[1] by **Claudio Carlon**, Head of Unit, Evaluation C3

[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.
[3] ECHA Guidance on Information Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance (version 6.0, July 2017), p. 530: https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

Appendix 1: Reasons

1. **In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2) or Transgenic rodent somatic and germ cell gene mutation assays (Annex IX, Section 8.4., column 2)**

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "If there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the Registrant."

The technical dossier contains an *in vitro gene mutation study in mammalian cells* (mouse lymphoma L5178Y cells using the TK gene, 2016) performed according to *OECD TG 490* with the registered substance that show positive results in the absence of metabolic activation system. The study results with metabolic activation system were concluded as inconclusive. The dossier also contains three *in vitro* gene mutation tests in bacteria (OECD TG 471), which were performed in only four strains and which produced negative results. You also submitted an *in vitro* chromosomal aberration test (OECD TG 473) which was concluded as not showing genotoxic potential.

Thus the positive result in the *in vitro gene mutation study in mammalian cells* indicates that the substance is inducing gene mutations under the conditions of the test.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations and/or chromosomal aberrations is not available for the registered substance. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.7.6.3, the transgenic rodent somatic and germ cell gene mutation assays ("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.

Hence, ECHA considers that the TGR and the comet assay are suitable tests to follow up the concern on gene mutation for the substance subject to the decision.

In case you decide to perform the TGR assay according to the test method EU B.58/OECD TG 488, the test shall be performed in transgenic mice or rats and the substance is usually administered orally. Also the test shall be performed by analysing tissues from *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 shall be stored (at or below −70 °C) until the analysis of liver and glandular stomach is completed; the duodenum shall then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

Moreover, ECHA notes that 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. Hence, in order to limit additional animal testing male germ cells shall be collected at the same time as the other tissues (liver, glandular stomach and duodenum), and stored up to 5 years (at or below −70°C). This duration is sufficient to allow you or ECHA, in accordance to Annex X, 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.

In case you decide to perform the comet assay according to the test method OECD TG 489, the test shall be performed in rats. 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. Also, the test shall 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 sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision:

In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum

or

Transgenic rodent somatic and germ cell gene mutation assays (test method: EU B.58/OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; germ cells and duodenum shall be harvested and stored for up to 5 years. Duodenum shall be analysed if the results of the glandular stomach and of the liver are negative or inconclusive. The test material used should be freshly prepared.

In your comments on the draft decision you indicated that the *"The large increase of the proportion of small colonies* [...]*"* observed in the OECD TG 490 *"is an indication of a clastogenic origin, however, a suitable study to detect such chromosomal aberrations resulting from clastogenic effects is available showing that the registered substance is not clastogenic in vitro (OECD 473)".*

ECHA notes that paragraph 2 of OECD TG 490 states that *"The purpose of the in vitro mammalian cell gene mutation tests is to detect gene mutations induced by test chemicals".* Furthermore, while the induction of slow growing mutants (small colonies) has been

associated with substances that induce gross structural changes at the chromosomal level, they only *"... provide some insight into the type(s) of damage (mutagens vs. clastogens) induced by the test chemical"*.

You also in your comments state that the result of the OECD TG 473 is negative (not clastogenic) *in vitro* and that this would negate the increase of small colonies in the OECD TG 490 in vitro study. However, while the in vitro OECD TG 490 study shows an increase in small colonies it also shows an increase of large colonies. Such an increase in both small and large colonies is indicative of gene mutation. Therefore, it cannot be ruled out that the substance is not inducing gene mutations. ECHA therefore considers that the positive result in the OECD TG 490 study indicates a concern for gene mutation. Hence, according to Annex IX, Section 8.4., column 2, the positive *in vitro* study (OECD TG 490) should be followed up by an appropriate *in vivo* somatic cell genotoxicity.

In your comments you have also provided QSARs prediction reports for an *in vitro* mammalian chromosome aberration study and an *in vitro* gene mutation study in mammalian cells. However, ECHA notes that the provided QSARs do not cover the endpoint information requirement, that is *in vivo* mutagenicity. You also stated that *"there there are a lot of negative mutagenicity studies available for the structural similar substance Dimethylaminopropylamine (CAS 109-55-7)"*. Firstly, ECHA notes that you have failed to provide any documentation for the read-across hence ECHA cannot verify that the properties of the registered substance can be predicted from the data on the source substance. Secondly, ECHA notes that according to the ECHA dissemination website there is only one *in vivo* study, that is an OECD test Guideline 474 (Mammalian Erythrocyte Micronucleus Test) with the potential analogue substance Dimethylaminopropylamine (CAS 109-55-7). ECHA notes that an *in vivo* micronucleus study does not address the gene mutation concern. Hence, the *in vivo* micronucleus study with the analogue substance Dimethylaminopropylamine (CAS 109-55-7) would still not be sufficient to cover this information requirement for the registered substance.

Finally, in your comments you stated that you intend *"to include a detailed discussion on reasons for non-classification in IUCLID instead of conducting a further study"*. ECHA notes that for the purpose of the decision-making, this decision does not take into account any updates submitted after 23 October 2017, that is after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation. All the new information in the later update(s) of the registration dossier will however be assessed for compliance with the REACH requirements in the follow-up evaluation pursuant to Article 42 of the REACH Regulation (after ECHA has sent the final decision and the deadline to submit the information has passed).

Notes for your consideration

You are reminded that according to Annex IX, Section 8.4., column 2 of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, *"the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered"*.

In case you decide to perform the comet assay, you may consider examining gonadal cells in addition to the other aforementioned tissues, as it would optimise the use of animals. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive

result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. 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., column 2) in a second species

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.
A "pre-natal developmental toxicity study" (test method OECD TG 414) for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. Annex IX, Section 8.7.2., column 2 provides that the decision on the need to perform a pre-natal developmental toxicity study on a second species at a tonnage level of 100 to 1000 tonnes per year should be based on the outcome of the first test and all other relevant and available data. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet these information requirements.

The technical dossier contains a pre-natal developmental toxicity study with rats by the oral route. This study fulfils the standard information requirement for a pre-natal developmental toxicity study in a first species (Annex IX, Section 8.7.2.).

ECHA has reviewed the findings from the study you submitted and considers that the results of 1st PNDT in rats indicate a concern for developmental toxicity, which would trigger the need to perform a PNDT study in a second species, because ECHA considers the following:
(i) post-implantation loss values (15.5% vs 4.6%) and lower mean number of foetuses (11.4 vs 13.5) at 250 mg/kg bw/day are not the consequence of maternal toxicity at this dose, but are signs of developmental toxicity. In addition these values are outside historical control values;
(ii) skeletal malformations are real malformations and not variations, and may be all due to the same mechanisms such as disturbing the gene expression pattern during early development. This may also contribute to resorptions. From the various vertebra(e) and rib(s) findings, only the absent lumbar vertebra(e) occurred either in controls or historical controls.

More specifically, the skeletal findings comprise:
1) absent rib(s) (found only in treated animals);
2) supernumerary lumbar vertebra(e) (only in mid-dose animals);
3) absent lumbar vertebra(e) (in control, low-dose and mid-dose animals);
4) absent thoracic vertebra(e) (in mid-dose and high-dose animals);
5) absent cervical vertebra(e) (only in high-dose animals).

Finally ECHA notes that you have not classified the substance and that you have disregarded the concern.

In your comments on the draft decision you agreed to perform the second species pre-natal developmental toxicity study. Nevertheless, you also highlighted that "*the post-implantation losses were observed in a context of severe maternal toxicity*". ECHA agrees with you that there is severe maternal toxicity at the highest dose level of 750 mg/kg bw/day. However, the maternal toxicity at 250 mg/kg bw/day is considered only as slight. Severe clinical signs

and lower maternal body weight gain, which was not corrected for uterus with its content (days 6-21, -35% when compared to control), may be linked to increased incidence of postimplantation loss at 750 mg/kg bw/day. At the mid-dose level (250 mg/kg bw/day) the maternal body weight gain was only slightly lower compared to control (days 6-21, -11%), with the net weight changes of +36 g vs +42 g. At the same dose level, the mean gravid uterus weight was slightly lower compared to control (-9%). With reference to the gravid uterus weight, ECHA also notes that in the high-dose group there was only a slightly reduced foetal weight (-5% when compared to control) while in the mid-dose group there were no effects noted in the foetal body weight. Thus, the lower gravid uterus weight is due to resorptions (postimplantation loss) and not due to reduced foetal body weight at the mid-dose level of 250 mg/kg bw/day. Hence, ECHA does not agree with your conclusion that there is "maternal toxicity at both dose levels". Thus, as already indicated in the draft decision, ECHA considers that the noted post-implantation loss values and the lower mean number of foetuses at 250 mg/kg bw/day are not linked to maternal toxicity.

In your comments you also indicate that the occurrence of the various skeletal malformations "*is most probably fortuitous*" due to a number of reasons including: no dose-response relationship, occurrences in one foetus/litter or one litter/dose group, and occurrences being spontaneous findings in rats (reference literature provided). ECHA notes your considerations, however due to the various skeletal malformations observed ECHA considers that there is a high concern that cannot simply be explained by maternal toxicity.

Considering all of the above, ECHA considers that the postimplantation loss together with the skeletal findings are signs of developmental toxicity that need to be further investigated in a second species pre-natal developmental toxicity study. Hence, ECHA agrees with your comment that indeed only "*the rabbit developmental study will allow to clarify and to confirm whether the results obtained in the rats' species are of spontaneous origin (for fetal findings) and/or related to maternal toxicity (for post-implantation loss)*" and most importantly to determine whether the registered substance is a developmental toxicant.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement, because the available data contain triggers for prenatal developmental toxicity in a second species. Consequently there is an information gap and it is necessary to provide information for this endpoint

The test in the first species was carried out with rats. According to the test method OECD TG 414, the rat is the preferred rodent species and the rabbit is the preferred non-rodent species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbit as a second species

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: OECD TG 414) in a second species (rabbit) by the oral route.

Following the proposals for amendment (PfA) submitted by one of the Member States

Competent Authorities (MSCAs) you agreed to perform the pre-natal development toxicity study in rabbits. You also indicated that you would perform all studies requested in the present decision before drawing "*any conclusion on the need for classification or non-classification*" of the substance. ECHA acknowledges your comments on the PfA, however also reminds you that you should still consider the order of the studies as indicated in the "*Notes for your consideration*" (hereunder).

Notes for your consideration

You should carefully consider the order of testing of the requested pre-natal developmental toxicity study in the second species (OECD TG 414) and the extended one-generation reproductive toxicity study (EOGRTS) (section 3 of the present decision) to ensure that unnecessary animal testing is avoided.

You should consider conducting the EOGRTS first to determine whether there are sufficient grounds to classify the substance as Repr 1A or 1B: May damage the unborn child (H360D). According to Annex IX, Section 8.7., column 2, if the substance is classified as such, no further testing for developmental toxicity will be necessary. However, if the substance is not classified for developmental toxicity then you are required to conduct the study requested under this section (PNDT (second species)).

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

The basic test design of an extended one-generation reproductive toxicity study (test method OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex IX of the REACH Regulation, if the available repeated dose toxicity studies (e.g. 28-day or 90-day studies, OECD TGs 421 or 422 screening studies) indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity. If the conditions described in column 2 of Annex IX are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information requirement

ECHA considers that concerns in relation to reproductive toxicity are revealed from the studies you submitted. More specifically, you submitted results from a 90-day repeated dose toxicity study (OECD TG 408, GLP, 2016, reliability 1) and from a pre-natal developmental toxicity study (OECD TG 414, GLP, 2016, reliability 1) which showed effects raising "*other concerns in relation with reproductive toxicity*".

In the 90-day repeated dose toxicity study (with doses at 50, 250, and 750 mg/kg/day), the main findings were focused on males and females at 750 mg/kg/day: some clinical and haematological findings, changes in the blood biochemistry investigations, organ weights and histopathology. For the changes observed in oestrous cycles, you stated that *"There were no statistically significant test item-related effects on mean oestrous cycle length or mean number of cycles. However a trend towards an increase in mean oestrous cycle length was observed in females given 250 or 750 mg/kg/day at the end of the treatment period"*. Although not statistically significant, ECHA further notes the following observations related to increasing doses: a decrease of number of cycles (from 4.2 to 3.2), an increase in the cycle length (from 4.2 days to 5.9 days), and a decrease in the number of females having a mean average cycle of 4-5 days (from 7 to 4) (Table 11 in IUCLID dossier). ECHA considers that the dose-dependent increase in oestrus cycle length is a biologically relevant effect and raises a concern in relation to reproductive toxicity.

In the pre-natal developmental toxicity study (with doses at 50, 250, and 750 mg/kg/day), results showed a higher mean post-implantation loss (at 250 and 750 mg/kg bw/day: 15.5% and 20.0% vs. 4.6%, respectively, with $p<0.05$ at 750 mg/kg/day) and lower mean number of live fetuses (11.4 and 11.2 vs. 13.5, respectively). In addition all values were outside the limits of the historical control data.

Pursuant to Annex IX, Section 8.7.3. an extended one-generation reproductive toxicity study is thus an information requirement for registrations of the registered substance, due to "other concerns in relation with reproductive toxicity", namely the increased oestrus cycle length, increased mean post-implantation loss and lower mean number of live fetuses.

You did not consider the information requirement for reproductive toxicity in Annex IX, Section 8.7.3., column 1, because you stated that *"A reproductive toxicity study, one species, male and female, most appropriate route of administration, having regard to the likely route of human exposure, could be required at this tonnage level if, according to annex IX, the 28-day or 90-day study indicates adverse effects on reproductive organs or tissues."* and you added that *"according to the 90-day toxicity study (2016), no such effects on the reproductive organs were observed for the registered substance."*

However, ECHA points out that the information requirement according to Annex IX, Section 8.7.3. states that an *"Extended One-Generation Reproductive Toxicity Study (B.56 of the Commission Regulation on test methods as specified in Article 13(3) or OECD 443), basic test design (cohorts 1A and 1B without extension to include a F2 generation), one species, most appropriate route of administration, having regard to the likely route of human exposure [is required], if the available repeated dose toxicity studies (e.g. 28-day or 90-day studies, OECD 421 or 422 screening studies) indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity."* (emphasis added).

As explained above, ECHA considers that such concerns in relation with reproductive toxicity are observed from these studies.

In your comments on the draft decision you stated the following:

(i) *"the increased in mean post-implantation loss and the lower mean number of live fetuses will be addressed in OECD 414 in the second species and not in the extended one generation (OECD 443)."*

(ii) *"[redacted] confirms that the estrous cycle variations were not of biological significance"*; according to the [redacted] statement the trend towards an increase in

mean oestrous cycle length "*was considered to be of no toxicological significance in the absence of statistical significance, and absence of findings at microscopic examination of the reproductive organs [...] these findings were considered to be of no biological importance.*"

Firstly, ECHA notes that according to Annex IX, Section 8.7.3., column 1, an extended one-generation reproductive toxicity study is required at REACH Annex IX level if the available repeated dose toxicity studies indicate adverse effects on reproductive organs or tissues or reveal other concerns in relation with reproductive toxicity.

With reference to comment (i.) above, ECHA notes that these findings (*higher mean post-implantation loss and lower mean number of live foetuses*) observed in the pre-natal developmental toxicity study, indeed are concerns for developmental toxicity and will be followed-up in the second species pre-natal developmental toxicity study, however they also indicate a concern related to reproductive toxicity because in addition to developmental toxicity, increased postimplantation loss may also reflect difficulties in supporting foetal survival in utero e.g due to changes in hormonal balance. This concern is also supported by the oestrous cycle findings and should be clarified.

Regarding comment (ii.), as already indicated in this section (above), indeed the increase in mean oestrous cycle length did not reach statistical significance. However, it is a dose-dependent effect, and hence ECHA considers that it is biologically relevant, even in the absence of findings at microscopic examination. Furthermore, ECHA notes that according to ECHA's guidance document[2], the effects on oestrous cycle alone are still considered as a concern that can trigger an extended one-generation reproductive toxicity study.

Hence, ECHA considers that there are sufficient concerns to trigger an extended one-generation reproductive toxicity study since, as already indicated above, these findings "*reveal other concerns in relation to reproductive toxicity*". Hence, condition 1 of Annex IX, Section 8.7.3., column 1 is met.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint. Thus, an extended one-generation reproductive toxicity study according to Annex IX, Section 8.7.3. is required. The following refers to the specifications of this required study.

a) The specifications for the required study

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017), the starting point for deciding on the length of the premating exposure period should 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 if there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017). In this specific case, animals of Cohort 1B are mated to produce the F2 generation and, thus, the premating exposure duration will be 10 weeks for these Cohort 1B animals and the fertility parameters will be covered allowing an evaluation of the full spectrum of effects on fertility in these animals. Thus, shorter premating exposure duration for parental (P) animals may be considered. However, the premating period shall not be shorter than two weeks and must be sufficiently long to reach a steady-state in reproductive organs as advised in the ECHA Guidance. The consideration should take into account whether the findings from P animals after a longer premating exposure duration would provide important information for interpretation of the findings in F1 animals, e.g. when considering the potential developmental origin of such findings as explained in ECHA guidance.

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 with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Extension of Cohort 1B

If the column 2 conditions of 8.7.3., Annex IX are met, Cohort 1B must be extended, which means that the F2 generation is produced by mating the Cohort 1B animals. This extension provides information also on the sexual function and fertility of the F1 animals.

The use of the registered substance in the joint submission is leading to significant exposure of professionals, because the registered substance is used by professionals e.g. in coatings (use of PROCs 8a, 8b, 9, 10, 11, 13 and 15).

Furthermore, there are indications for endocrine-disrupting modes of action because the 90-day repeated dose toxicity study resulted in the following observations related to increasing doses: a decrease of number of oestrus cycles (from 4.2 to 3.2) and an increase in the oestrus cycle length (from 4.2 days to 5.9 days), and decrease number of females having a mean average oestrus cycle of 4-5 days (from 7 to 4).

Therefore, ECHA concludes that Cohort 1B must be extended to include mating of the animals and production of the F2 generation, because the uses of the registered substance is leading to significant exposure of professionals and there are indications of modes of action related to endocrine disruption from an available study (90-day repeated dose toxicity study, with doses at 50, 250, and 750 mg/kg/day) resulting in an increase of the oestrous cycle length.

The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/triggers must be documented.

Cohorts 2A and 2B

The developmental neurotoxicity Cohorts 2A and 2B need to be conducted in case of a particular concern on (developmental) neurotoxicity as described in column 2 of 8.7.3., Annex IX. When there are triggers for developmental neurotoxicity, both the Cohorts 2A and 2B are to be conducted as they provide complementary information.

ECHA notes that existing information on the registered substance itself derived from an available *in vivo* study (90-day repeated dose toxicity study, with doses at 50, 250, and 750 mg/kg/day, 2016) show evidence of lower mean landing foot splay values in females given 750 mg/kg/day (85 mm vs. 105 mm in controls), although, according to the study report, this did not correlate with any other findings. You also reported that "*Minimal to marked vacuoles were seen in the kidneys (tubules and, to a lesser severity, glomeruli), brain (choroid plexus), pars nervosa (pituitary gland), spleen, mesenteric lymph node and GALT (Gut Associated Lymphoid Tissue) in males and females treated at 750 mg/kg/day and were related with the test item administration. It is noteworthy that these vacuoles were present in the choroid plexus from only 2/10 females treated at 250 mg/kg/day (emphasis added). These vacuoles were round, of moderate to large size (15-50 µm in diameter) and devoid of any staining except those recorded in the pituitary gland which were pale and eosinophilic. The empty vacuoles in brain, spleen, lymph node and GALT were scattered, multifocal while the vacuoles in the pars nervosa were diffuse.* "

Hence at the top dose (750 mg/kg bw/day), vacuoles can be observed in many organs and sites, e.g. kidneys (tubules and, to a lesser severity, glomeruli), brain (choroid plexus), *pars nervosa* (pituitary gland), spleen, mesenteric lymph node and GALT (Gut Associated Lymphoid Tissue). However, the brain vacuolisation (choroid plexus) occurred also at the mid-dose (250 mg/kg bw/day) in 2/10 of the females. Hence, ECHA considers that the brain is more sensitive to this effect, and it should be investigated further.

In addition ECHA notes that existing information on an analogue substance (3-dimethylaminopropiononitrile, with EC number 217-090-4) derived from an available *in vivo* study (neurotoxicity study with reliability 2) show that exposure of rats to 450 mg/kg bw in drinking water caused enlarged distal motor and spindle axons with disordered neurofilaments (detected) by electron microscopy); enlarged motor nerve terminals were observed ([redacted], 1983). Finally reduced startle response was observed in males of the highest dose group.

In your comments on the draft decision you claimed that the concern on (developmental) neurotoxicity is not justified based on the following considerations:

(i) You referred to the [redacted] statement highlighting that the variations in horizontal and rearing movements were of minor importance, and that the vacuoles observed in choroid plexus observed in 2/10 females from the treated mid-dose group (250 mg/kg/day) was "*not correlated with any change in their corresponding horizontal movements, rearing or landing foot splay*". Moreover, the vacuoles noted in the high-dose and mid-dose groups were of "*small magnitude, not associated to a degenerative process and not correlated to clinical neurological signs.*"; and

(ii) With reference to the additional triggering from the analogue substance you stated that "*the comparison of the two substances and the read-across approach is not justified*". Additionally, you indicated that both the registered and the analogue substances are structurally and toxicologically different and ECHA has not supported the read-across argument.

Firstly, ECHA notes that the triggers for cohorts 2A and 2B are based on a particular concern for (developmental) neurotoxicity.

With reference to comment (i.) above ECHA agrees that the variations seen in horizontal and rearing movements were considered to be of minor importance, and hence these were not used to justify the concern for developmental neurotoxicity. ECHA also understands the reasoning and conclusions provided by the [redacted] on the choroid plexus findings noted in the sub-chronic toxicity (90-day) study. However, ECHA considers that even if the histopathological findings in the brain of adult animals were not correlated with signs of behavioural or functional adverse effects, such as observed lower mean landing foot splay in the mid dose, the findings still indicate a concern for developmental neurotoxicity. Hence the concern due to brain vacuolisation and changes in landing foot splay justify the inclusion of the developmental neurotoxicity cohorts.

As regards comment (ii.) above ECHA notes that according to the ECHA Guidance document[3] information from substances structurally analogous to the registered substance can be used for the purpose to identify triggers for the design of an extended one-generation reproductive toxicity study. The analogue substance, 3-dimethylaminopropiononitrile (EC no. 217-090-4), has been identified as a structurally analogous substances to the registered substance and the concerns are based on neurotoxicity data (enlarged distal motor and spindle axons with disordered neurofilaments; enlarged motor nerve terminals when exposed to 450 mg/kg bw), that is a relevant aspect and that is not covered by the data provided on the registered substance. Hence, the concerns observed with the analogue substance can be used as support for triggering even if there are sufficient concerns to trigger an EOGRTS at REACH Annex IX level due to the findings in the provided sub-chronic toxicity (90-day) and pre-natal developmental toxicity studies.

ECHA concludes that the developmental neurotoxicity cohorts 2A and 2B need to be conducted because there is a particular concern on (developmental) neurotoxicity based on the results from the above-identified *in vivo* studies on the registered substance itself and on one analogous substance (3-dimethylaminopropiononitrile).

The study design must be justified in the dossier and thus the existence/non-existence of the conditions/triggers must be documented.

Cohort 3

The developmental immunotoxicity Cohort 3 needs to be conducted in case of a particular concern on (developmental) immunotoxicity as described in column 2 of 8.7.3., Annex IX.

An MSCA in their PfA requested to include the Cohort 3 based on minimal to marked vacuoles seen in the spleen, mesenteric lymph nodes and Gut-Associated Lymphoid Tissue (GALT) in males and females at the highest dose level, observed in the repeated dose toxicity (90-day) study. As a response you do not agree to include the Cohort 3. You state that a) the vacuoles in the spleen, mesenteric lymph node, and GALT were reversible during recovery period; b) mortality was observed at the highest dose level; c) the substance did not induce hypersensitivity after skin contact.

ECHA notes that existing information on the registered substance itself derived from the available *in vivo* study (90-day repeated dose toxicity study, with doses at 50, 250, and 750 mg/kg/day, 2016) shows effects in immune-associated organs. Specifically, vacuoles were observed at the highest dose (750 mg/kg bw/day) in spleen, mesenteric lymph nodes and GALT, but also in some other organs. This appears to be a substance-specific effect, which manifests in multiple organs. ECHA acknowledges that there is unacceptable toxicity in the females at highest dose, as 3/14 animals showed prolonged poor clinical condition and one of these animals died. However the systemic toxicity in males is not excessive at the highest dose level; poor clinical condition was seen transiently in only one male and there were no mortalities. Since there was no excessive toxicity seen in males at the highest dose level, then the effects seen at this dose level may be used to establish a particular concern. The vacuoles seen in male were reversible in immune organs and tissues, and ECHA considers that this observation is important for establishing that the substance caused the effect. These vacuoles in spleen, mesenteric lymph nodes are considered to be an adverse effect, irrespective of their reversibility. Even though the vacuoles are seen in other organs, the vacuolisation shows a particular concern for immunotoxicity because of the moderate incidence in multiple immune organs/tissues. ECHA considers that the absence of hypersensitivity is not an adequate basis for excluding a particular concern on (developmental) immunotoxicity.

ECHA concludes that the developmental immunotoxicity Cohort 3 needs to be conducted because there is a particular concern on (developmental) immunotoxicity based on the results from the above-identified *in vivo* study on the registered substance itself.

Species and route selection

According to the test method OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

In your comments on the draft decision you proposed to perform in a stepwise approach, as follows:

(i.) pre-natal developmental toxicity study in rabbits and if study confirms classification no additional studies are performed;
(ii.) if classification is not confirmed then OECD TG 422 is to be performed to investigate any "*neurotoxicity or developmental toxicity*" concerns; and
(iii.) if triggers are observed in the OECD TG 422 then EOGRTS will be performed and "*the relevance of the suggested neurotoxicity cohort*" will be examined according to the OECD TG 422 results.

ECHA does not accept the proposed testing strategy because of the following reasons:

(i.) According to REACH Annex IX Section 8.7., column 2, "*if a substance is known to cause developmental toxicity, [...] then no further testing for developmental toxicity will be necessary. However, testing for effects on fertility must be considered.*" Hence, if following the pre-natal developmental toxicity study in rabbit the substance meets the criteria for classification as a developmental toxicant Repr 1B

(H360D) you would still need to address the observed concern for sexual function and fertility. Investigations for sexual function and fertility (i.e. extended one-generation reproductive toxicity study) are not automatically adapted due to classification following the pre-natal developmental toxicity study.

(ii.) ECHA notes that the OECD TG 422 study is not one of the requests in this decision however, you may still perform the OECD TG 422 study at your own discretion. Considering that this study is an Annex VIII requirement, you are not required to submit a testing proposal.

(iii.) As explained above there are already concerns from the sub-chronic toxicity (90-day) and pre-natal developmental toxicity studies that trigger the extended one-generation reproductive toxicity study. There is no need to conduct an OECD TG 422 in order to confirm these findings. Additionally, there are also (developmental) neurotoxicity and (developmental) immunotoxicity concerns stemming from the registered substance that trigger cohorts 2A and 2B and cohort 3 and it is not necessary to confirm the triggers.

Considering the above, ECHA concludes that there is no justified reason to accept the proposed stepwise testing approach.

b) Outcome

Based on the available information, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method OECD TG 443), in rats, oral route, according to the following study-design specifications:

- At least two 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) with extension to mate the Cohort 1B animals to produce the F2 generation;
  - Cohorts 2A and 2B (Developmental neurotoxicity); and
  - Cohort 3 (Developmental immunotoxicity).

Deadline to submit the requested information in this decision

In the draft decision communicated to you the time indicated to provide the requested information was 36 months from the date of adoption of the decision. In your comments on the draft decision, you requested an extension of the timeline to 48 months. You indicated that you intend to use a stepwise approach to perform the pre-natal developmental toxicity study and the screening study (OECD TG 422) (which has not been requested) before conducting the extended one-generation reproductive toxicity study (EOGRTS). As ECHA explained under Appendix 1, Section 3. (EOGRTS) of the decision, the proposed stepwise approach cannot be accepted. ECHA has also requested you to submit documentary evidence from the selected test laboratory indicating the scheduling timelines for the studies in question of the laboratory facility in order to justify why an extension is required. You submitted a Gantt chart from the test laboratory as documentary evidence indicating that 45 months is needed to sequentially perform the PNDT, screening and the EOGRTS studies. ECHA reminds that as the OECD TG 422 study, for which 13 weeks is reserved in the Gantt chart, is not requested in this decision. Hence, the 36 months granted in the draft decision

is sufficient to complete the requested PNDT and EOGRTS studies (sequentially or in parallel). In view of the above ECHA has not modified the deadline of the decision.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 13 September 2017.

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

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

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

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

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

In addition, you provided comments on the draft decision. These comments were not taken into account by the Member State Committee as they were considered to be outside of the scope of Article 51(5).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-62 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

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

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

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.