ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 23 November 2018

Addressee: [redacted]

Decision number: CCH-D-2114447743-44-01/F
Substance name: p-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline
EC number: 225-716-2
CAS number: 5026-74-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 31/10/2017
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. **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;**

   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 with the registered substance;

2. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: OECD TG 408) in rats with the registered substance modified to include analysis of extra parameters (sperm measurements and optional measurements of female reproductive function, e.g. oestrus cycle);**

3. **Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.; test method: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25./OECD TG 309) at a temperature of 12 °C with the registered substance;**

4. **Identification of degradation products (Annex IX, 9.2.3.) using an appropriate test method with the registered substance;**

5. **Classification and labelling (Annex VI, Section 4.): Apply classification and**

labelling on the registered substance for reproductive toxicity *or* provide a justification for not classifying.

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 ***1 June 2020***. 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

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

Appendix 1: Reasons

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.

Your registration dossier contains adaptation arguments in the form of a grouping and read-across approach under Annex XI, Section 1.5. of the REACH Regulation for the following information requirements:

- Appropriate in vivo somatic cell genotoxicity study (Annex IX, 8.4 column 2)
- Sub-chronic toxicity (90-day) study (Annex IX, 8.6.2.)

ECHA has considered first the scientific and regulatory validity of your read-across approach in general before assessing the individual information requirement in sections 1 and 2.

Grouping of substances and read-across approach

You have sought to adapt the information requirements listed above by applying a read-across approach in accordance with Annex XI, Section 1.5. According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach). ECHA considers that the generation of information by such alternative means should offer equivalence to prescribed tests or test methods.

Based on the above, a read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological or ecotoxicological property is reliable and should be based on recognition of the structural similarities and differences between the source and registered substances. This hypothesis explains why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.

Due to the different nature of each endpoint and consequent difference in scientific considerations (e.g. key parameters, biological targets), a read-across must be specific to the endpoint or property under consideration. Key physicochemical properties may determine the fate of a compound, its partitioning into a specific phase or compartment and largely influence the availability of compounds to organisms, e.g. in bioaccumulation and toxicity tests. Similarly, biotic and abiotic degradation may alter the fate and bioavailability of compounds as well as be themselves hazardous, bioaccumulative and/or persistent. Thus, physicochemical and degradation properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical and degradation properties is only a part of the read-

across hypothesis, and it is necessary to provide additional justification which is specific to the endpoint or property under consideration.

The ECHA Read-across assessment framework foresees that there are two options which may form the basis of the read-across hypothesis- (1) (Bio)transformation to common compound(s)- the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed and (2) Different compounds have the same type of effect(s)- the read-across hypothesis is that the organism is exposed to different compounds which have similar (eco)toxicological and fate properties as a result of structural similarity (and not as a result of exposure to common compounds).

Finally, Annex XI, Section 1.5. lists several additional requirements, which deal with the quality of the studies which are to be read-across.

You consider to achieve compliance with the REACH information requirements for the registered substance *p-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline* using data of structurally similar substance: *m-(2,3-epoxypropoxy)-N,N-bis(2,3-epoxypropyl)aniline* (EC No 275-662-9) (hereafter the 'source substance').

You have provided a read-across documentation as a separate attachment in the Section 13 of IUCLID dossier.

In the read-across justification you provided the identifiers and the structures for the source and target substances and explains the impurity profiles. You also provided:

- a summary of the PC data of the p- and m- isomers showing that the two isomers " *have comparable physical-chemical properties and are therefore expected to behave similarly in biological systems*"
- a summary of environmental fate and pathway showing that both isomers are not inherently biodegradable
- a summary of ecotoxicological data showing similar results in short term toxicity from three trophic levels (Daphnia, Fish and Algae) for the m-isomer and for two trophic levels (Fish and Algae) for the p-isomer substance.
- a summary of mammalian toxicological data:
    - The m-isomer and p-isomer substances are of moderate acute oral toxicity (LD50 between 300 and 2000 mg/kg) with common systemic signs of toxicity were noted (Sedation, ruffled fur and hunched posture).
    - Both substances are of low acute dermal toxicity, LD50 > 2000 mg/kg for the m-isomer and greater 4000 mg/kg for the p-isomer.
    - The m-isomer and p-isomer are slightly irritant to rabbit eyes
    - p-isomer showed moderate persisting irritating effects to rabbit skin (applied under occlusive dressing and during 24) whereas the m-isomer showed also an irritating effect but reversible (semi-occlusive dressing during 4 hours).
    - Both substances were found to be sensitizers.
    - The p- and m-isomer showed positive responses when tested for mutagenicity in all studies in vitro (bacterial reverse mutation assay, mammalian chromosome aberration test and mammalian gene mutation test).
    - While the p-isomer substance showed a positive response in a pre-GLP and pre-OECD guideline test (the test having some similarity with the current OECD 474 guideline), the m-isomer substance did not show a mutagenic potential in a recent study performed up to 875 mg/kg the maximum

tolerated dose. (The above mentioned in vivo study performed on the p-isomer as well the in vivo sister chromatide exchange study are not considered to be reliable studies due to deficiencies on the data reported compared to current guideline and the lack of cytotoxicity measurement.) Furthermore, you argued with regard to mutagenicity that "*based on metabolic studies in vivo performed on other glycidyl compounds, it is expected that glycidyl ethers would be rapidly and effectively detoxified by epoxide hydrolases. These enzyme systems are largely absent in in vitro bacterial and mammalian cell systems providing a plausible explanation for the positive genotoxicity results in vitro but negative genotoxicity results in vivo in the recent study performed on the m-isomer*" and that "*Considering that both substances are structural isomers, that the critical functional group for mutagenicity being the epoxide groups is present equally presents in both substances, we assume that the detoxification process occurring with the epoxide hydrolase will occur for both substances and in the same manner in an in vivo micronucleus study.*"

- A 28-day oral repeated dose toxicity was performed on the m-isomer. A systemic NOAEL of 50 mg/kg was based on effects noted on female reproductive organs. Local effects were also noted in the gastro intestinal tract at all dose levels. You concluded that "*Considering that both substances are structural isomers, that the critical functional group for toxicity being the epoxide groups is present equally presents in both substances, we assume that the detoxification process occurring with the epoxide hydrolase will occur for both substances and in the same manner in repeated toxicity studies, as well as in other mammalian toxicity endpoints like reproductive toxicity.*"

The read-across justification also contains a data matrix with the available information for p- and m-isomers. You concluded that "*Based on the similarity of structure and chemical functionality, as well as on the above mentioned experimental results confirming that both the m- and p-isomer substances have the same toxicological profile, the read-across approach proposed is justified.*" Based on the information provided, ECHA understands that the read-across hypothesis proposed by you is based on the substances being structural isomers with the same functional groups, molecular weight, and similar lipophilicity and molecular size. In addition, they share the same mechanism of action, i.e. toxicity is caused by three reactive glycidyl ethers responsible for DNA- and/or protein binding, which effect is not depending on the isomer position of the groups.

As an integral part of this prediction, you propose that the source and registered substance have similar properties for the above-mentioned information requirements. ECHA considers that this information is your read-across hypothesis.

ECHA's evaluation and conclusion

Your proposed adaptation argument is that the similarity in chemical structure and in some of the physico-chemical/ ecotoxicological/ toxicological properties between the source and registered substance is a sufficient basis for predicting the properties of the registered substance for other endpoints.

You provided sufficient information on the composition and impurities for both registered and analogue substance. However, you did not address the differences in the impurity profiles of the registered and analogue substances as explained below. The main impurities

(typical concentration [redacted]%) are the p- and m-isomers of the same compound (1-{4-[bis(oxiran-2-ylmethyl)amino]phenoxy}-3-{[4-(oxiran-2-ylmethoxy)phenyl](oxiran-2-ylmethyl)amino}propan-2-ol and 1-{3-[bis(oxiran-2-ylmethyl)amino]phenoxy}-3-{[3-(oxiran-2-ylmethoxy)phenyl](oxiran-2-ylmethyl)amino}propan-2-ol). The impact of the different isomers of these impurities on the toxicological profile of the substances has not been addressed. Moreover, you did not address the impact of the remaining impurities ([redacted]% and [redacted]% in the analogue and registered substance, respectively), which are different chemical entities with varying number of glycidyl ether moieties and are present at different concentrations.

You stated "*Considering that both substances are structural isomers, that the critical functional group for toxicity being the epoxide groups is present equally presents in both substances, we assume that the detoxification process occurring with the epoxide hydrolase will occur for both substances and in the same manner in repeated toxicity studies, as well as in other mammalian toxicity endpoints like reproductive toxicity.*" ECHA notes that indeed since the substances are isomers, they do have same functional groups, same molecular weight, similar lipophilicity and molecular weight, and glycidyl ether groups. However, you did not provide any mechanistic/kinetic/other data or explanation to support why the m- and p- positional isomerism has no influence on the toxicity profile of the substances. ECHA notes that different isomers do not necessarily have similar reactivity, toxicokinetic properties and toxicological profiles, and a similar molecular weight, composition and functional groups does not provide a sufficient evidence to support similar behaviour of the two isomers. Even if there is similar reactivity due to the epoxide groups, it does not follow that the repair of the reacted epoxide, or the damage caused by the reactive epoxide, is similar between the m- and p- isomers. Your read-across hypothesis has not addressed this issue, and is accordingly an inadequate basis for predicting the properties of the registered substance. ECHA further notes that you referred to the metabolism of glycidyl ethers in general but has not provided data on the metabolism of the registered and analogue substances. In addition, no data has been provided to explain the expected similar behaviour of the isomers.

You concluded that "*Based on the similarity of structure and chemical functionality, as well as on the above mentioned experimental results confirming that both the m- and p-isomer substances have the same toxicological profile, the read-across approach proposed is justified* " ECHA notes that based on the data provided it can be concluded that the substances have similar physico-chemical properties (e.g. melting/freezing point, Log Pow and water solubility). ECHA also notes that for the target substance the dossier contains two high tier toxicity data, a two generation study and a pre-natal developmental toxicity study and for the source substance only a 28d study is available. While the two generation study also identifies pathological changes in the gastrointestinal tract as does the 28-d study performed with the m-isomer, these changes are more severe and at lower doses. Since the exposure times for all these studies are different, in the absence of similar studies with the target and source substance, a clear conclusion cannot be drawn on similarity of toxicological profiles of the two isomers. Furthermore, while the p-isomer substance showed a positive response in a pre-GLP and pre-OECD guideline test (the test having some similarity with the current OECD 474 guideline), the m-isomer substance did not show a mutagenic potential in a recent study performed up to 875 mg/kg the maximum tolerated dose. ECHA notes that the read-across in its current form cannot be accepted due to uncertainties explained above, i.e. the impact of different impurity profiles, the explanation as to why the different isomers would have similar toxicokinetic and toxicological behaviour, and the lack of comparable toxicity data for the target and source substance. Therefore, the

prediction of properties from the results conducted with the analogue substance to the registered substance may lead to underestimation of the hazard.

Structural similarity is a prerequisite for applying the grouping and read-across approach. However similarity in chemical structure and similarity of some of the physico-chemical/ ecotoxicological/ toxicological properties does not necessarily lead to predictable or similar human health properties in other endpoints. Your justification based on structural similarity, similar physico-chemical, ecotoxicological and toxicological properties has not established why the prediction is reliable for the human health end-points for which the read across is claimed.

Additionally, ECHA has taken into account all of your arguments together. ECHA firstly notes that you have not provided a reasoning as to why these arguments add to one another to provide sufficient basis for read-across. Secondly, the defects of each individual argument are not mitigated by the other arguments you have provided, and so ECHA considers that the arguments when taken all together do not provide a reliable basis for predicting the properties of the registered substance.

Therefore, ECHA considers that this grouping and read-across approach does not provide a reliable basis whereby the human health effects of the registered substance may be predicted from data for reference substances within the group. Hence, this approach does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. of the REACH Regulation. ECHA notes that there are specific considerations for the individual endpoints which also result in a failure to meet the requirement of Annex XI, Section 1.5., and these are set out under the endpoint concerned.

As described above, further elements are needed to establish a reliable prediction for a toxicological or ecotoxicological property, based on recognition of the structural similarities and differences between the source and registered substances. This could be achieved (if it is possible) by a well-founded hypothesis of (bio)transformation to a common compound(s), or that the registered and source substance(s) have the same type of effect(s), together with sufficient supporting information to allow a prediction of human health properties.

1. ***Transgenic rodent somatic and germ cell gene mutation assays (Annex IX, Section 8.4., column 2) or In vivo mammalian alkaline comet assay (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 several *in vitro* studies that show positive results: *in vitro* gene mutation in bacterial cells, *in vitro* chromosomal aberration and *in vitro* gene mutation

in mammalian cells performed similar to pre- and non-GLP guideline methods (OECD TG 471, 472). In addition, the technical dossier also contains two more recent studies performed according to OECD TG 473 (2011) and OECD TG 476 (2012) and GLP with the registered substance that show positive results. The positive results indicate that the substance is inducing gene mutations and chromosomal aberrations under the conditions of the tests.

The technical dossier contains several pre-guideline and non-GLP *in vivo* studies; a sister chromatid exchange test and an *in vivo* chromosome aberration test with the registered substance that show positive results. These studies are not following a test guideline and you consider that these are of Klimisch score 3 (i.e. not reliable). In view of your assessment that these studies are not reliable, ECHA cannot consider these as reliable information. Additionally, ECHA notes that a sister chromatid exchange test is not listed as an accepted somatic cell mutagenicity test in ECHA's guidance (Table R.7.7-3; the ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017, Chapter R.7a). Data on human health and environmental properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3) must meet the requirements of Annex XI, 1.1.2. The *in vivo* chromosome aberration test with the registered substance is not performed according to GLP or a Test Guideline, and fails to have adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3). Specifically, and by comparison to OECD TG 474, (i) there is not counting of 4000 immature erythrocytes per animal for the incidence of micronucleated immature erythrocytes [1000 per slide cited]; (ii) there is no statement that the manual scorer is unaware of the treatment condition; (iii) the number of animals per group is less than 5 analysable animals per sex [3 per sex per group]; (iv) there is no established historical positive and negative control range and distribution; and (v) sufficient experience with the conduct of the assay is required to demonstrate the ability to reproduce expected results. In this experiment five animals (out of 30) died from gavage misdosing, which demonstrates insufficient experience. Finally, the dossier contains an *in vivo* micronucleus test performed according to OECD TG 474 and GLP with an analogue substance (3-(oxiran-2-ylmethoxy)-N,N-bis(oxiran-2-ylmethyl)aniline / 71604-74-5 / 275-662-9). However, and as explained above in the Grouping of substances and read-across approach section of this decision, your adaptation of the information requirement is rejected. Hence, ECHA concludes that the *in vivo* tests already provided with the registered and the analogue substances are not appropriate to follow-up the concern for gene mutations and chromosomal aberrations.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations and 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 assay 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. According to the test method EU B.58/OECD TG 488, 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 ºC) 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 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.

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.

According to the test method OECD TG 489, 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:

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.
or

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

In your comments on the Member State Competent Authorities' proposal for amendment (which proposes to request the collection of somatic cells from three tissues -liver, glandular stomach and duodenum- and the analysis of two tissues -liver and glandular stomach- in the TGR assay), you have mentioned your agreement to conduct the TGR/OECD 488 assay. You have also highlighted the scientific debate concerning sampling times and source of germ cells and requested further guidance in that regard from ECHA.

ECHA acknowledges that some scientific work is ongoing on this topic and is aware of the recent publication by Marchetti et al. 2018[2] that you cited. You referred to some conclusions of the article of Marchetti et al. and proposed using it as a benchmark for the test design: "*i) sperm from the cauda epididymis at 28+3d does not provide meaningful mutagenicity data; ii) tubule germ cells at 28 + 3d provides reliable mutagenicity data only if the results are positive; iii) tubule germ cells at 28+28d produces reliable positive and negative results in rats. Thus, the 28 + 28d regimen provides an approach for simultaneously assessing mutagenicity in somatic tissues and germ cells from the same animals*". You proposed that a "*testing regiment [sic] fulfilling points (ii) and (iii) and conducted in two-steps would be the most scientifically valid and within the interests of animal welfare*".

However, ECHA does not consider the approach proposed by you as a sufficient and adequate basis to deviate from the recommendations of the current OECD TG 488, with respect to the sampling schedule, for the reasons explained below:
Firstly, the sampling schedule you propose does not seem feasible, since you propose to take germ cells at two different sampling times (28+3d, and 28+28d), and this cannot be performed on the same group of animals, since they are killed during sample collection.
Secondly, ECHA notes that some somatic tissues you are requested to collect in the TGR are fast proliferating tissues, and in that respect the 28+28d schedule has not yet demonstrated its suitability, according to Marchetti et al. ("*further work is required to support the 28+28d protocol for tissues other than slowly proliferating tissues as per current TG 488*"). Thirdly, at present there is not even a draft updated OECD TG 488 that recommends for 28+28d schedule instead of 28+3d. Finally, the primary aim of the current request for an in vivo follow up study is to investigate the mutagenic effects in somatic tissues. The 28+3d protocol is appropriate for the investigation of somatic cells and is recommended by the current OECD TG 488, therefore the germ cells should be collected, at the same time as the somatic tissues, 3 days after the end of the 28-day treatment (28+3d).

Concerning the compartment where the germ cells should be sampled, ECHA acknowledges the wording of the OECD TG 488 from 2013 that indicates sampling from two sources: "*sampling cells from seminiferous tubules in addition to spermatozoa from the vas deferens/cauda epididymis following only a 28 + 3 day sampling regimen would provide some coverage of cells exposed across the majority of phases of germ cell development, and may be useful for detecting some germ cell mutagens*". The conclusion of Marchetti et al (2018) however indicates that "*sperm from the cauda epididymis at 28+3d does not provide meaningful mutagenicity data*". Taking into account these elements, ECHA would advise you to collect the cells from the seminiferous tubules only and store them for a

[2] Marchetti F, Aardema MJ, Beevers C, van Benthem J, Godschalk R, Williams A, Yauk CL, Young R, Douglas GR. Identifying germ cell mutagens using OECD test guideline 488 (transgenic rodent somatic and germ cell gene mutation assays) and integration with somatic cell testing (2018). Mut. Res. 832-833, 7-18.

possible analysis of mutant frequencies.

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

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.

A "sub-chronic toxicity study (90 day)" is a standard information requirement as laid down in Annex IX, Section 8.6.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have not provided any study record of a sub-chronic toxicity study (90 day) in the dossier that would meet the information requirement of Annex IX, Section 8.6.2.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for a "repeated dose 28-day oral toxicity study" (OECD TG 407) with the analogue substance(s) 3-(oxiran-2-ylmethoxy)-N,N-bis(oxiran-2-ylmethyl)aniline / 71604-74-5 / (EC no 275-662-9). However, this study does not provide the information required by Annex IX, Section 8.6.2., because exposure duration is less than 90 days and the number of animals per dose group is significantly lower. Therefore, the sensitivity of a 28-day study is much lower than that of a 90-day study. Furthermore, and as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

Therefore, your adaptation of the information requirement is rejected.

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. Since adverse effects were observed in the reproductive organs in the 28-d study with the

analogue substance, the requested 90-d study should be modified to include sperm measurements and any optional measurements of female reproductive function.

. ECHA has evaluated the most appropriate route of administration for the study. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers that the oral route - which is the preferred one as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 5.0, December 2016) Chapter R.7a, Section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the substance is a liquid of very low vapour pressure and no uses with spray application are reported that could potentially lead to aerosols of inhalable size.
Hence, the test shall be performed by the oral route using the test method EU B.26./OECD TG 408.According to the test method EU B.26./OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

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: Repeated dose 90-day oral toxicity study (test method: OECD TG 408) in rats modified to include sperm measurements, and any optional measurements of female reproductive function.

Notes for your consideration

ECHA notes that a revised version of OECD TG 408 was adopted this year by the OECD. This revised version contains enhancements of certain endocrine disrupting relevant parameters. You should test in accordance with the revised version of the guideline as published on the OECD website for adopted test guidelines (https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788).

3. Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.)

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.

"Simulation testing on ultimate degradation in water" is a standard information requirement as laid down in Annex IX, section 9.2.1.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex IX, Section 9.2., column 2. You provided the following justification for the adaptation:*"In accordance with column 2 of REACh (Regulation (EC) No 1907/2006) Annex IX, the simulation testing on ultimate degradation in surface water, and sediment simulation testing (required in section n 9.2.1.2, and 9.2.1.4) do not need to be conducted based on the findings of the Chemical Safety Assessment; the substance does not fulfil classification criteria according to the applicable regulations and does not fulfil the criteria for vPvB or PBT."*

However, ECHA considers that your adaptation does not meet the specific rules for adaptation of Column 2 of Annex IX, Section 9.2 for the following reasons. Firstly the substance was shown to hydrolyse in an OECD 111 study: at 20°C half lives are estimated as 3.9 days at pH 4, 4.1 days at pH 7 and 4.6 days at pH 9. You do not suggest the identity of hydrolysis products. Nevertheless, as the substance contains epoxide groups it is reasonable to expect these may undergo hydrolysis. Therefore ECHA considers that you have not provided adequate justification in your chemical safety assessment (CSA) or in the technical dossier for why there is no need to investigate further the degradation of the substance and its degradation products. As explained further below, ECHA considers that the information is needed for the PBT/vPvB assessment and for the identification of the degradation products in relation to the PBT/vPvB assessment.

In addition, according to Annex IX, Section 9.2.1.2, column 2 of the REACH Regulation, simulation testing on ultimate degradation in surface water does not need to be conducted if the substance is highly insoluble in water or is readily biodegradable. ECHA notes that based on the information in the technical dossier, the registered substance is not readily biodegradable in a ready biodegradability CO2 evolution test according to OECD 301B (<10% degradation after 29 days) and has a water solubility of 3.34 g/l at 20°C.

Therefore, your adaptation of the information requirement cannot be accepted.

In your comments on the draft decision you provided a QSAR prediction that shows formation of five hydrolysis products from the registered substance. Four of those are epoxides and you predicted the quantity at day 28.

ECHA considers that the five hydrolysis products forecast in the QSAR prediction are those expected based on the known propensity for epoxide groups to hydrolyse. However, you have not provided a validation of the QSAR prediction of hydrolysis products. In addition the quantity of the fifth degradant (the ultimate hydrolysis product with no epoxide group remaining) has not been estimated. Therefore, ECHA is not able to assess the reliability of the prediction of the rate of hydrolysis of the registered substance.

You are reminded that a simulation test of ultimate degradation in surface water would give information on degradants formed by both hydrolysis and biodegradation. As discussed in section 4, you provided QSAR estimates for ultimate biodegradation of the registered substance and of the five hydrolysis products. However, you have not provided a validation of the QSAR prediction. In addition, there is no QSAR forecast of likely metabolites from biodegradation. Therefore, ECHA is not able to assess the reliability of the prediction of biodegradation of the parent substance and of the five hydrolysis products.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) Aerobic mineralisation in surface water – simulation biodegradation (test method EU C.25. / OECD TG 309) is the preferred test to cover the standard information requirement of Annex IX, Section 9.2.1.2.

One of the purposes of the simulation test is to provide the information that must be considered for assessing the P/vP properties of the registered substance in accordance with Annex XIII of the REACH Regulation to decide whether it is persistent in the environment. Annex XIII also indicates that "*the information used for the purposes of assessment of the PBT/vPvB properties shall be based on data obtained under relevant conditions*". The Guidance on information requirements and chemical safety assessment R.7b (version 3.0, February 2016) specifies that simulation tests "attempt to simulate degradation in a specific environment by use of indigenous biomass, media, relevant solids [...], and a typical temperature that represents the particular environment". The Guidance on information requirements and chemical safety assessment Chapter R.16 on Environmental Exposure Estimation, Table R.16-8 (version 3.0 February 2016) indicates 12°C (285K) as the average environmental temperature for the EU to be used in the chemical safety assessment. Performing the test at the temperature of 12°C is within the applicable test conditions of the Test Guideline OECD TG 309. Therefore, the test should be performed at the temperature of 12°C.

In the OECD TG 309 Guideline two test options, the "pelagic test" and the "suspended sediment test", are described. ECHA considers that the pelagic test option should be followed as that is the recommended option for P assessment. The amount of suspended solids in the pelagic test should be representative of the level of suspended solids in EU surface water. The concentration of suspended solids in the surface water sample used should therefore be approximately 15 mg dw/L. Testing natural surface water containing between 10 and 20 mg SPM dw/L is considered acceptable. Furthermore, when reporting the non-extractable residues (NER) in your test results you should explain and scientifically justify the extraction procedure and solvent used obtaining a quantitative measure of NER.

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: Aerobic mineralisation in surface water – simulation biodegradation test (test method: EU C.25./OECD TG 309).

Notes for your consideration

Before conducting the requested test you are advised to consult the ECHA Guidance on information requirements and chemical safety assessment, Chapter R7b, Sections R.7.9.4 and R.7.9.6 (version 4.0, June 2017) and Chapter R.11, Section R.11.4.1.1 (version 3.0, June 2017) on PBT assessment.

In accordance with Annex I, Section 4, of the REACH Regulation you should revise the PBT assessment when results of the test detailed above are available. You are also advised to consult the ECHA Guidance on information requirements and chemical safety assessment (version 3.0, June 2017), Chapter R.11, Section R.11.4.1.1. and Figure R. 11-3 on PBT assessment for the integrated testing strategy for persistency assessment in particular taking into account the degradation products of the registered substance.

4. Identification of degradation products (Annex IX, 9.2.3.)

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 identification of the degradation products is a standard information requirement according to column 1, Section 9.2.3. of Annex IX of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

The biodegradation section in the technical dossier does not contain any information in relation to the identification of degradation products, nor an adaptation in accordance with column 2 of Annex IX, Sections 9.2 or 9.2.3. or with the general rules of Annex XI for this standard information requirement. "

According to Annex IX, Section 9.2.3., column 2 of the REACH Regulation, identification of degradation products is not needed if the substance is readily biodegradable. ECHA notes that based on the information in the technical dossier, the registered substance is not readily biodegradable, as also discussed in section 3 above.

Furthermore, ECHA notes that you have not provided any justification in your chemical safety assessment (CSA) or in the technical dossier for why there is no need to provide information on the degradation products. ECHA considers that this information is needed in relation to the PBT/vPvB assessment and risk assessment.

In your comments on the draft decision you provided QSAR estimates for biodegradation of the five hydrolysis products and an estimation that ultimate biodegradation would take place over weeks or months.

You are reminded that a simulation test of ultimate degradation in surface water would give information on degradants formed by both hydrolysis and biodegradation. As discussed in section 3, ECHA considers that the five hydrolysis products forecast in the QSAR prediction are those expected, but due to missing documentation of the prediction (QPRF and QMRF) ECHA cannot assess the reliability of the prediction of the rate of hydrolysis of the registered substance. Although you have provided QSAR estimates for ultimate biodegradation of the registered substance and the five hydrolysis products, you have not provided a validation of the QSAR prediction. In addition, there is no QSAR forecast of likely metabolites from biodegradation. Therefore, ECHA is not able to assess the reliability of the prediction of biodegradation of the parent substance and the five hydrolysis product.

Furthermore, ECHA reminds you that information on the degradation products is needed in relation to the vPvB/PBT assessment. Therefore, ECHA notes that validated QSAR predictions of log Kow of the five hydrolysis products could provide relevant information regarding bioaccumulation potential.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

Regarding appropriate and suitable test method, the methods will have to be substance-specific. When analytically possible, identification, stability, behaviour and molar quantity of metabolites relative to the parent compound should be evaluated. In addition, degradation

half-life, log Kow and potential toxicity of the metabolite may be investigated. You may obtain this information from the Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25./OECD TG 309 also requested in this decision, or by some other measure. You will need to provide a scientifically valid justification for the chosen method.

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:

Identification of the degradation products (Annex IX, Section 9.2.3.) by using an appropriate and suitable test method, as explained above in this section.

Notes for your consideration

Before providing the above information you are advised to consult the ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), Chapter R.7b., Sections R.7.9.2.3 and R.7.9.4. These guidance documents explain that the data on degradation products is only required if information on the degradation products following primary degradation is required in order to complete the chemical safety assessment. Section R.7.9.4. further states that when substance is not fully degraded or mineralised, degradation products may be determined by chemical analysis.

5. Classification and labelling (Annex VI, Section 4.): Apply classification and labelling on the registered substance for reproductive toxicity *or* provide a justification for not classifying

Pursuant to Article 10(a)(iv) of the REACH Regulation your technical dossier shall contain information on classification and labelling of the substance as specified in Annex VI, Section 4 of the REACH Regulation in conjunction with Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation).

Annex VI, section 4.1. of the REACH Regulation clarifies that the hazard classification of the substance shall result from the application of Title I and II of the CLP Regulation. In addition, for each entry, the scientifically justified reasons why no classification is given for a hazard class or differentiation of a hazard class should be provided. According to Article 5(1) of Title I of the CLP Regulation, a substance shall be classified on the basis of available information.

Furthermore, the technical dossier must include the resulting hazard label for the substance in line with Title III of the CLP Regulation (Annex VI, section 4.2 of the REACH Regulation).

You have provided for the reproductive toxicity a two-generation reproductive toxicity study (OECD 416, GLP, reliability 1, from 2017) in rats by gavage with the registered substance, at 0, 5, 15 and 25 mg/kg/bw/d, showing similar adverse effects in reproductive parameters in the mid-dose and high-dose groups.

At 25 mg/kg bw/day the combination of implantation count and post-implantation loss, resulted in statistically significant lower litter size at birth/Day 1 compared to control, which persisted to weaning (Day 21 of age). A total of five F0 females failed to maintain their litter

to weaning. In F1 females only one female failed to rear offspring to weaning. In the original report it is stated that "*The significance of these litter losses at this dosage is uncertain as only one F1 female at the same dose level failed to rear offspring to weaning. It would normally be expected for similar litter losses to be observed in the next generation although it could be argued that the most susceptible animals had not been able to contribute to the F1 generation, so effectively had been filtered out.* " Furthermore, the original report concluded that "*Although only one F1 female at 25 mg/kg bw/day showed similar total litter loss, an association with the high incidence of total litter loss at 15 mg/kg bw/day and maternal treatment cannot be discounted.* "

At 15 mg/kg bw/day, the same effects are reported with the combination of implantation count and post-implantation loss, resulting in statistically significant lower litter size at birth/Day 1 compared to control, which persisted to weaning (Day 21 of age). At this dosage a total of six F1 females failed to maintain their litter to weaning but there were no instances of total litter loss for F0 females.

In addition, for males at 15 and 25 mg/kg bw/day, the age of attainment of balano preputial separation was statistically significantly later than control. You argued that the mean body weight at the age of attainment was similar to control and thus, the apparent delay in sexual maturation reflects lower growth/maturity for males at these dosages rather than any underlying disturbance of sexual development. ECHA notes that this conclusion is speculative and considers that a disturbance of sexual development cannot be excluded.

According to Annex I, Section 3.7. of the CLP Regulation, classification of the substance as reproductive toxicant, Category 1B is indicated when there is clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate. The above effects demonstrate adverse effects sexual function and fertility. You have not self-classified the substance for reproductive toxicity despite the adverse effects observed in the two-generation study.

You considered that "*the substance should not be classified for reprotoxicity as effects only occurred at doses where animals were compromised by the test substances primary damaging effects on gut epithelia and subsequent stress /secondary responses on the physiology rather than intrinsic toxicity. This interpretation of the results is further supported by the STOT RE Cat 2. Classification that is applied based on the findings of the repeated dose study with the m-isomer.*"

However, it is noted that at 15 mg/kg bw/d only the following effects were reported:

- Microscopic changes in stomach restricted to degeneration/atrophy of the glandular mucosa for only one F0 male.
- Minimal or mild regenerative hyperplasia of the mucosa of dueodenum in 13/27 males and 11/28 females.
- Minimal regenerative hyperplasia of the mucosa of jejunum in 3/28 females.
- Minimal or mild sinusoidal ectasia was present 9/28 animals
- Body weight relative thymus weight for F1-F2 offspring was lower than control.

- No treatment related effects on females mean body weight during the ten week pre-pairing period (at all doses).
- No effects on body weight gain in both generations throughout the ten week pre-pairing period and the first two weeks of gestation.
- Body weight gain was lower than control during the last week of gestation across both generations. This was considered to be influenced by a lower contribution by the developing litter (based on litter weight at birth).
- No effects on food consumption during the pre-pairing phase and gestation in both generations.
- Food intake during lactation was lower than control from Day 4 of lactation, with statistical significance being reached during the second week (Days 7-14) of lactation. You explained in the dossier that "*Food consumption during lactation at this dosage may have been influenced by the lower demand on the lactating female from the smaller litter size, compared to control.*"

ECHA observes that the effects noted above are not severe and serious adverse effects in the maternal animals, and there is no explanation for why these comparatively mild effects cause fertility effects. Therefore there is no plausible explanation to relate the less marked systemic maternal toxicity to the combination of implantation count and post-implantation loss, resulting in statistically significant lower litter size at birth/Day 1 compared to control, which persisted to weaning (Day 21 of age). Since similar effects are seen at 25 mg/kg bw/d where there is more maternal toxicity (in terms of gut lesions) as well at 15 mg/kg bw/d where maternal toxicity is too low to justify the reproductive effects, it cannot be simply assumed that the reprotoxicity effects are just "*subsequent stress /secondary responses on the physiology rather than intrinsic toxicity".*

The Guidance on the Application of the CLP criteria (Version 5.0, July 2017), specifies in Section 3.7.2.2.1.1. that "*There is no established relationship between fertility effects and less marked systemic toxicity. Therefore, it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity.*"

Based on the adverse effects observed at the 15 and 25 mg/kg bw/ day in the two generations study, it appears you have provided clear evidence of an adverse effect on sexual function and fertility or on development. Although these reprotoxic effects are present when there are also other toxic effects on the maternal animals, ECHA considers that you have not demonstrated that the adverse effect on reproduction is a secondary non-specific consequence of other toxic effects.

In your comments on the draft decision, you have provided a justification for not classifying the substance for reproductive toxicity. ECHA notes that the current decision will not take into account any dossier updates submitted after the date when the draft decision was notified to you. However, any new information in the updated registration dossier will be assessed for compliance with the REACH requirements in the follow-up evaluation pursuant to Article 42 of the REACH Regulation (i.e., after the deadline set out in the final decision has passed).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to review the classification and labelling of the substance, taking into account the information above, and to provide scientifically justified reasons why no classification for reproductive

toxicity is given. In the alternative, you may change the classification and labelling and provide the information on the hazard classification as set out in Annex VI, Section 4 of the REACH Regulation. You are also reminded that for a differentiation of a hazard class, scientifically justified reasons need to be provided.

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 16 August 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.

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.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-61 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. The substance subject to the present decision is provisionally listed in the Community rolling action plan (CoRAP) for the start of substance evaluation in 2019.

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

3. 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.

4. 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.