Helsinki, 9 November 2017

Addressee: [redacted]

Decision number: CCH-D-2114375746-35-01/F
Substance name: BIS(2-(2-BUTOXYETHOXY)ETHYL) ADIPATE
EC number: 205-465-5
CAS number: 141-17-3
Registration number: [redacted]
Submission number: [redacted]
Submission date: 10/05/2013
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 vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;**

2. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance, provided that the study requested under 1. has negative results;**

3. **Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: OECD 421/422) in rats, oral route with the registered substance;**

4. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats with the registered substance; It is at the Registrant's discretion to perform the intended additional examinations during the testing program;**

5. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rat or rabbit), oral route with the registered substance;**

6. **Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: Alga, growth inhibition test, EU C.3./OECD TG 201) Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: Alga, growth inhibition test, EU C.3./OECD TG 201) with the registered substance.**

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 May 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 Kevin Pollard, Head of Unit, Evaluation E1.

[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

TOXICOLOGICAL INFORMATION

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.

Grouping of substances and read-across approach for toxicological information

You have sought to adapt the information requirements for an *in vitro* chromosomal aberration in mammalian cells (Annex VIII, Section 8.4.2.), an *in vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.), a screening for reproductive/ developmental toxicity (Annex VIII, Section 8.7.1.), a sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2) and pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) 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 aspects the chemical structures have in common and the differences between the structures of the source and registered substances[2]. 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 factual 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.

Thus physicochemical properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical 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.

[2] Please see for further information ECHA *Guidance on information requirements and chemical safety assessment* (version 1, May 2008), Chapter R.6: QSARs and grouping of chemicals.

The ECHA Read-across assessment framework foresees that there are two options which may form the basis of the read-across hypothesis[3] - (1) (Bio)transformation to common compound(s) and (2) Different compounds have the same type of effect(s).

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

A. Description of the grouping and read-across approach proposed by the Registrant

You seek to adapt the following information requirements by applying a read-across approach according to Annex XI, Section 1.5:

- *in vitro* chromosomal aberration in mammalian cells (Annex VIII, Section 8.4.2.);
- *in vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.);
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.);
- Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2);
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.).

You propose read-across between the structurally similar substances adipic acid (EC 204-673-3), hereafter referred to as *source (substance) AA*, and 2-(2-butoxyethoxy)ethanol (EC 203-961-6), hereafter referred to as the *source (substance) DEGBE* and the substance subject to this decision, Bis(2-(2-butoxyethoxy)-ethyl)-adipate (EC number: 205-465-5) (CAS No 141-17-3) hereafter referred to as target substance.

Your dossier contains read-across documentation as a separate attachment in the registration entitled "[redacted]".

In that document, you use the following arguments to support the prediction of properties of the registered substance from data for reference substance(s) within the group by interpolation to other substances in the group: You indicate that the target substance is rapidly hydrolysed into the source substances in gastrointestinal fluid simulants and further elaborate on the toxicokinetic properties of these substances. You also provide information establishing structural similarity, similarity in physico-chemical properties between the target and the source substances. A short narrative presenting the set of toxicological data used for the prediction of the properties under consideration is included in the read-across justification document.

You conclude on that basis that the read-across from the source substances AA and DEGBE is "*justified on basis of scope of variability and overlapping of composition, representative molecular structure, physico-chemical properties and ecotoxicological/ toxicological profiles and supported by various (Q)SAR methods*". You further consider that "*In gastrointestinal fluid simulants Bis(2-(2-butoxyethoxy)ethyl) adipate (CAS 141-17-3) is rapidly hydrolyzed into adipic acid and 2-(2-butoxyethoxy) ethanol* [redacted]*, 2013). Following hydrolysis of the ester bond, the breakdown products will be absorbed, metabolised and excreted. Given this metabolic pathway and toxicity profile of dicarboxylic acid esters in organisms, the systemic toxicity of bis(2-(2-butoxyethoxy)ethyl) adipate (CAS 141-17-3) can be characterized by the systemic toxicity of the analogue substances adipic acid and DEGBE.*"

[3] Please see ECHA's Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across).

ECHA considers that this information is your read-across hypothesis, which provides the basis whereby you intend to predict the properties of the registered substance from the source substances.

B. ECHA analysis of the grouping and read-across approach

Missing supporting information

ECHA considers that your read-across hypothesis is based upon rapid (bio)transformation of the registered substace into the source substances and subsequent systemic exposure to the source substances only. However, there is insufficient information to support this element of your read-across hypothesis in the registration dossier.

In section 2.4 of your read-across justification you indicate that after oral ingestion, the target substance "*undergoes hydrolysis of the ester bonds by gastrointestinal enzymes*" and refer to references by Lehninger, 1970 and Mattson and Volpenheim, 1972. You also mention work from Fukami and Yokoi, 2012 which, according to you, "*demonstrates that esters of fatty acids are hydrolysed to the corresponding alcohol and fatty acid by esterases*". ECHA notes that the information from Lehninger, 1970, Mattson and Volpenheim, 1972 and Fukami and Yokoi, 2012 is not included in your technical dossier and therefore the relevance, reliability and adequacy of this information in the context of this read-across approach cannot be independently evaluated by ECHA.

You further point at *in vitro* data from [redacted], 2013 establishing that "*about 97% of bis(2-(2-butoxyethoxy)ethyl) adipate are hydrolysed within one hour*". Based on the information provided in the technical dossier, ECHA understands that this study was designed to investigate the (bio)transformation of the target substance in simulated digestive fluids over 4 hours. The contents in ester and alcohol in the reaction media were measured after 0, 1, 2, and 4 hours and indicate that, under the conditions of this assay, 97% of the initial ester contents is (bio)transformed after 1 hour. You indicate that "*recovery experiments even indicate a far quicker hydrolysis*". As the 1h time point corresponds to the first time point in this assay and in the absence of further characterization of the "*far quicker hydrolysis*" from the recovery experiments, no information on the rate and extent of the (bio)transformation of the target substance within the first hour after exposure can be derived from this experiment.

ECHA further stresses that, based on the information provided in the read-across justification document, the physicochemical properties ("Lipinski rule of five", particularly the log Pow) of the target substance show a high potential for bioavailability and exposure to the target substance cannot be excluded. Therefore, ECHA considers that no evidence has been provided that a rapid or instantaneous (bio)transformation of the target substance after oral administration occurs, allowing to consider that the properties of the target substance can be predicted from the systemic toxicity of the analogue substances AA and DEGBE.

The target substance is a di-ester. In your read-across justification document you indicate on page 10 that "*in the first step of hydrolysis, the monoester is produced that is further hydrolysed to the alcohol and the dicarboxylic acid*". ECHA understands from this statement that a two-step (bio)transformation pathway is anticipated, with the formation of mono-esters as intermediate (bio)transformation products. Whilst some information on the type of metabolic reactions involved in this 2-step pathway is provided in this document, no details on the kinetics of the individual steps of this pathway are provided.

No considerations are provided addressing the potential impact of intermediate (bio)transformation products, such as monoesters, on the toxicological properties of the registered substance. In the absence of such information, ECHA considers that you have not established that the properties of the target substance can be predicted from data on the source substances as claimed in your read-across hypothesis.

ECHA understands from your read-across hypothesis that you consider that the systemic toxicological properties of the target substance can be predicted from information generated by testing its ultimate (bio)transformation products AA and DEGBE. Without prejudice to the deficiencies in this read-across approach listed above, ECHA observes that no information is provided in your read-across justification document establishing the actual prediction model that you intend to apply in order to predict the properties of the target substance. More specifically, in your read-across justification you have not accounted for the possible impact of co-exposure to the source substances, as formed from (bio)-transformation of the target substance, and did not address the potential interactions between AA and DEGBE. In the absence of such information, ECHA considers that you have not established that the properties of the target substance can be predicted from data on the source substances as claimed in your read-across hypothesis.

For the reasons presented above and on the basis of the information provided in your registration dossier, there is not sufficient support for your proposal that the target substance is immediatetly (bio)transformed to the source substances, that the potential intermediate (bio)transformation products do not affect the prediction, that no interactions occur between the source substances. Accordingly, your hypothesis based upon rapid (bio)transformation of the target substance into the source substances is not substantiated. For this reason, your hypothesis is not a reliable basis whereby the properties of the target substance may be predicted from data for reference substances within the group by interpolation to other substances in the group.

Documentation of the source studies

According to Annex XI, Section 1.5 there needs to be structural similarity among the substances within a group or category and furthermore, 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 by interpolation to other substances in the group (read-across approach). Furthermore, Annex XI, Section 1.5 lists several additional requirements, including that adequate and reliable documentation of the applied method have to be provided.

As indicated above, you intend to cover the information requirements for an *in vitro* chromosomal aberration in mammalian cells (Annex VIII, Section 8.4.2.), an *in vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.), a screening study for reproductive/ developmental toxicity (Annex VIII, Section 8.7.1.), a sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2) and pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) by applying a read-across approach in accordance with Annex XI, Section 1.5.

You have provided study records for

- *in vitro* chromosomal aberration test: (Thompson 1984, [redacted] 1974, ;
- *in vitro* mammalian gene mutation assay: Gollapudi 1993;
- oral developmental/reproductive screening study Nolen 1985;
- dietary chronic (2 year) toxicity study: Horn 1957;
- pre-natal developmental toxicity studies: [redacted] 1972, [redacted] 1974 Nolen 1985;

on the structurally-related source substances AA and DEGBE, and you propose to read-across the properties to the registered substance. However, ECHA notes that a robust study summary is required under Article 10(a)(vii), and ECHA considers that the information provided in these endpoint study records does not meet the requirements of a robust study summary, as defined in Article 3(28). Specifically, the endpoint study records do not provide details on study design and parameters investigated, which is particularly important for studies conducted before the relevant test guideline was adopted. ECHA has provided a practical guide for "How to report robust study summaries", available at: http://echa.europa.eu/documents/10162/13643/pg_report_robust_study_summaries_en.pdf. ECHA considers there is not sufficient information to make an independent assessment of the studies minimising the need to consult the full study reports, and accordingly considers that for these studies, you have failed to meet the requirement of Annex XI, 1.5 that adequate and reliable documentation of the applied method shall be provided.

C. Conclusion on the grouping and read-across approach

For the reasons as set out above, ECHA considers that this grouping and read-across approach does not provide a reliable basis whereby the human health effects of the target substance may be predicted from data for reference substance(s) 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.

As described above, further elements and supporting information would be needed to establish a reliable prediction for toxicological or ecotoxicological properties, based on recognition of the structural aspects the chemical structures have in common and the differences between the structures of the source and registered substances.

1. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)

An "*In vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study" is a standard information requirement as laid down in Annex VIII, Section 8.4.2. 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 XI, Section 1.5. of the REACH Regulation by providing the following study records;

a. *in vitro* chromosomal aberration test (OECD TG 473; Thompson 1984) in CHO cells with and without metabolic activation, with the analogous substance 2-(2-butoxyethoxy) ethanol (CAS 112-34-5, DEGBE);
b. *in vitro* chromosomal aberration test (pre-guideline; [redacted] 1974) in human embryonic lung fibroblasts, with and without metabolic activation, with the analogous substance adipic acid (CAS 124-04-9, AA).

However, as explained above in Appendix 1, "grouping of substances and read-across approach for toxicological information" of this decision, your adaptation of the information requirement is rejected. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA considers that the *in vitro* mammalian chromosome aberration test (test method OECD TG 473) and the *in vitro* mammalian cell micronucleus test (OECD TG 487) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.2. of the REACH Regulation.

In your comments according to article 50(1) of the REACH Regulation, you indicate agreement with conducting the studies. ECHA welcomes your agreement.

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 vitro* mammalian chromosome aberration test (test method: OECD TG 473) or *in vitro* mammalian cell micronucleus test (test method: OECD TG 487).

2. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

An "*In vitro* gene mutation study in mammalian cells" is an information requirement as laid down in Annex VIII, Section 8.4.3. of the REACH Regulation, "if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2." is obtained.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing study record c) in Appendix section 0, above.

c. *in vitro* mammalian gene mutation assay (OECD TG 476; Gollapudi 1993) in CHO cells with and without metabolic activation, with the analogous substance 2-(2-butoxyethoxy) ethanol (CAS 112-34-5, DEGBE).

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

Additional reasons:

ECHA stresses that if your read-across hypothesis was valid, adequate and reliable information on both (bio)transformation products (i.e. AA (CAS 124-04-9) and DEGBE (CAS 112-34-5)) would be needed for each endpoint concerned. However, ECHA observes that your technical dossier fails to address the properties of the (bio)transformation product source substance AA (AA, CAS 124-04-9) for the endpoint under consideration.

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.

ECHA considers that the *in vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes (OECD TG 476) and the *in vitro* mammalian cell gene mutation tests using the thymidine kinase gene (OECD TG 490) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.3.

In your comments according to article 50(1) of the REACH Regulation, you indicate agreement with conducting the studies. ECHA welcomes your agreement.

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 vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490) provided that the study requested under 1. has negative results.

3. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

"Screening for reproductive/developmental toxicity" (test method OECD TG 421 or 422) is a standard information requirement as laid down in Annex VIII, Section 8.7.1. of the REACH Regulation if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from *in vitro* methods that the substance may be a developmental toxicant. No such evidence is presented in the dossier. Therefore, 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 screening for reproductive/developmental toxicity in the dossier that would meet the information requirement of Annex VIII, Section 8.7.1.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing study record d) in Appendix section 0, above.

d. oral developmental/reproductive screening study (pre-guideline; Nolen 1985) in 25/m+f/dose SD rats, with the analogous substance 2-(2-n-Butoxyethoxy)ethanol (CAS 112-34-5, DEGBE).

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

Additional reasons:

> ECHA stresses that if your read-across hypothesis was valid, adequate and reliable information on both (bio)transformation products (i.e. AA (CAS 124-04-9) and DEGBE (CAS 112-34-5)) would be needed for each endpoint concerned. However, ECHA observes that your technical dossier fails to address the properties of the (bio)transformation product source substance AA (AA, CAS 124-04-9) for the endpoint under consideration.

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.

In your comments according to article 50(1) of the REACH Regulation, you state that you intend to adapt the information requirement for Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.) by providing a pre-natal developmental toxicity study in a first species and a sub-chronic toxicity study with additional reproductive parameters. ECHA acknowledges your intention to avoid unnecessary animal testing and will check your adaptation for compliance during the follow-up stage once the indicated information is available.

According to the test methods OECD TG 421/422, the test is designed for use with rats. On the basis of this default assumption ECHA considers testing should be performed with 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 5.0, December 2016) 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: Reproductive/developmental toxicity screening test (test method: OECD TG 421) *or* Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (test method: OECD TG 422) in rats by the oral route.
Notes for your considerations

For the selection of the appropriate test, please consult ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.5 and 7.6 (version 5.0, December 2016). You should also carefully consider the order of testing especially the requested screening (OECD TG 421/422) and the developmental toxicity studies (OECD TG 414) to ensure unnecessary animal testing is avoided, paying particular attention to the end point specific guidance (https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf).

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

A "sub-chronic toxicity study (90 day)" is a standard information requirement 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 sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing study records e) and f) in Appendix section 0, above.

e. oral repeated dose (90 day) toxicity study with additional fertility parameters (OECD TG 408, GLP; Johnson 2005) in F344 rats (m/f), with the analogous substance 2-(2-butoxyethoxy) ethanol (CAS 112-34-5, DEGBE), and
f. dietary chronic (2 year) toxicity study (pre-guideline, pre-GLP; Horn 1957) in Carworth Fram rats, with the analogous substance adipic acid (CAS 124-04-9, AA).

However, as explained above in Appendix 1, section 0 of this decision, 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.

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.

In your comments according to article 50(1) of the REACH regulation, you agreed to this request. In addition, you proposed to extend the sub-chronic toxicity study (90 day) by including additional examinations and parameters: *"weights of male and female gonads and accessory sex organs, stage of estrous cycle and cycle duration for females and histopathological examination of uterus and cervix, as well as the histopathological examination with special emphasis on stages of spermatogenesis in males and histopathology of interstitial testicular cell structure."*

ECHA notes that it is at your discretion to perform the intended additional examinations during the testing program provided that those additional examination do not interfere with the examinations according to test method OECD TG 408, and use the results to ensure the safe use of the substance. ECHA reminds you that the proposed extension of this study does not fulfil the standard information requirement in the registration dossier for reproductive toxicity set out in Annex X, Section 8.7.3.

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: EU B.26./OECD TG 408) in rats.

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

A "pre-natal developmental toxicity study" (test method EU B.31./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. 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 XI, Section 1.5. of the REACH Regulation by providing study records g), h) and i) in Appendix section 0, above.

g. oral pre-natal developmental toxicity study (pre-OECD TG 414; [redacted] 1972) in Wistar rats with the read-across substance adipic acid (CAS 124-04-9, AA),
h. oral pre-natal developmental toxicity study (pre-OECD TG 414; [redacted] 1974) in Dutch-belted rabbits, with the read-across substance adipic acid (CAS 124-04-9, AA),
i. dermal pre-natal developmental toxicity study (pre-OECD TG 414; Nolen 1985 in NZW rabbits, with the read-across substance 2-(2-n-Butoxyethoxy) ethanol (CAS 112-34-5, DEGBE).

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

Additional reasons specific for developmental toxicity:

In addition to the deficiencies in reporting mentioned above, ECHA observes that the one pre-natal developmental toxicity study performed with the source DEGBE (study record i), above), is a dermal study. You have not discussed how the limited dermal uptake (circa 30% in males and 50% in females, according to information from the technical dossier) would impact systemic exposure.

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.

According to the test method EU B.31./OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default assumption ECHA considers testing should be performed with rats or rabbits as a first 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 5.0, December 2016) 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 according to article 50(1) of the REACH regulation, you agreed to this request.

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: EU B.31./OECD TG 414) in a first species (rat or rabbit) by the oral route.

ECOTOXICOLOGICAL INFORMATION

6. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)

"Growth inhibition study aquatic plants" is a standard information requirement as laid down in Annex VII, Section 9.1.2. 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.

In the technical dossier you have provided a study record for a key study with the registered substance, reliability 1, [redacted], 2013, freshwater Algae Growth Inhibition Test Effect of Hexanedioic acid, bis[2-(2-butoxyethoxy)ethyl]ester (CAS 141-17-3) on the growth of *Pseudokirchneriella subcapitata* according to OECD guideline 201. However, this study does not provide the information required by Annex VII, Section 9.1.2., because the validity of the study results cannot be verified based on the information given in the study summary.

In your study summary you state that the study was conducted according to OECD Guideline 201 (Alga, Growth Inhibition Test) with no deviations, according to GLP (including certificate) and the test setting was static, with analytical monitoring.

In your summary and conclusion part of the IUCLID dossier you have not stated whether the study validity criteria were fulfilled. Furthermore, ECHA notes that the validity of this study (or its results) could not be assessed as the information related to the validity criteria of the OECD TG 201 is not available in the technical dossier. For the OECD TG 201 (adopted 23 March 2006; Annex 5 corrected 28 July 2011) test to be valid, the following validity criteria must be met:

- The biomass concentration in the control cultures should have increased by a factor of at least 16 within the test period (This criterion applies to the test algae *Pseudokirchneriella subcapitata*);
- The coefficient of variation daily growth rates in the control cultures during the course of the test (days 0-1, 1, 2 and 2-3) must not exceed 35%;
- Coefficient of variation of average growth in replicate control cultures must not exceed 15%;
- pH in the control cultures shall not increase more than 1.5 unit (For test compounds that partly ionise at a pH around the test pH, it may be necessary to limit the pH drift to obtain reproducible and well defined results. A drift of no more than 0.3 pH units is technically feasible and can be achieved by ensuring an adequate CO2 mass transfer rate from the surrounding air to the test solution, e.g. by increasing the shaking rate. Another possibility is to reduce the demand for CO2 by reducing the initial biomass density or the test duration).

In addition, in your study summary, you have not reported the following information which is important for assessing the study and must be included in the OECD TG 201 test report: initial biomass density at the beginning of the test, preparation of test solutions (including use of solvents etc.), pH, temperature, and light intensity and quality (source, homogeneity), method of determination of biomass and evidence of correlation between the measured parameter and dry weight. Furthermore, you have not reported the results and discussion according to recommendations in the OECD TG 201 (adopted 23 March 2006) and the ECHA Practical Guide 3: How to report robust study summaries (version 2.0, November 2012). Therefore, ECHA considers that the information provided in these endpoint study records does not meet the requirements of a robust study summary, as defined in Article 3(28).

In your technical dossier, you have also reported a robust study summary for a supporting study which you have given a reliability score 4 (not assignable). The study report authors are [redacted] (2011) and the study title is Adipic acid, bis[2-(2-butoxyethoxy)ethyl] ester: Algal growth inhibition test. You state that the study was performed according to OECD TG 201 and EU Method C.3 with GLP (including the certificate). The study design is static, freshwater, no limit test with 72h exposure. Test material is Adipic acid, bis[2-(2-butoxyethoxy)ethyl] ester (CAS 141-17-3), and its analytical purity is not stated. Test algae is *Desmodesmus subspicatus*. The test laboratory is [redacted] report no. [redacted], and the study owner is the [redacted]. You have accessed this study record by secondary source: US EPA High Production Volume Chemical Challenge Program.
In your robust study summary, you gave the following rationale for the reliability score 4: "*A instable test substance concentrations was observed. Further adjustment of the test condition to the substance properties was not performed. The Substance is readily biodegradable and shows relatively low water solubility. Following the standard test instable test substance concentrations are recognized only after the test has been finalized. Due to substance properties, a reasonable dose-response curve could not be established.*"

However, in the same study summary you state that the validity criteria for this study is fulfilled and you conclude that: "*The test item caused an effect on Desmodesmus subspicatus over the 72-hour study period. Reduced growth rates and biomass increases were recorded with EC50 values of 23 and 11 mg/L, respectively, based on nominal test item concentrations. When expressed as geometric mean measured concentrations, EC50 values of 0.48 and 0.35 mg/L, respectively, were reported based on growth rate and biomass increases.*"

To summarise, also the supporting study record does not provide the information required by Annex VII, Section 9.1.2., because contradictory information is given of its validity and the study results cannot be verified based on the information given in the study summary.

In addition to the two study reports indicated above, in your chemical safety report, under chapter 7 Environmental hazard assessment, in "7.6 PNEC derivation and other hazard conclusion" (on page 180) in table 42 you state that "*Studies on the toxicity to freshwater algae (OECD 201) are ongoing for Bis(2-(2-butoxyethoxy)ethyl) adipate. The dossier will be updated as soon as possible and the Chemical Safety Assessment according to Annex I of Regulation (EC) No 1907/2006 will be re-evaluated based on the outcome of the new studies.*" Also under chapter "PBT and vPvB assessment", in "8.1.2 Summary and overall conclusions" on PBT or vPvB properties on toxicity (on page 184) you state that "*A study on the toxicity to algae (OECD 201) is ongoing for Bis(2-(2-butoxyethoxy) ethyl) adipate. The dossier will be updated as soon as possible and the Chemical Safety Assessment according to Annex I of Regulation (EC) No 1907/2006 will be re-evaluated based on the outcome of the new study.*" In a same chemical safety report in references (on page 187) you refer to following study record: "[redacted] *(2013). Freshwater Algae Growth Inhibition Test Effect of Hexanedioic acid, bis[2-(2-butoxyethoxy) ethyl]ester (CAS 141-17-3) on the growth of Pseudokirchneriella subcapitata. Testing laboratory:* [redacted] *Report no.:* [redacted]*. Owner company:* [redacted]." Based on this reference, ECHA understands that these studies you are referring in your chemical safety report are not identical to those that are currently available in your technical dossier.

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.

According to ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 3.0, February 2016) Algae growth inhibition test (test method EU C.3. / OECD TG 201) is the preferred test to cover the standard information requirement of Annex VII, Section 9.1.2.

In your comments according to article 50(1) of the REACH regulation, you agreed to fulfil the information requirement for this request. Furthermore, you informed ECHA that you have aquired a letter of access to the second study (reliability score 2), and that you will consider the results of this study in your chemical safety assessment. In addition, you state that you intend to review and update your PBT/vBvP assessment, PNEC derivation and Chemical Safety Report accordingly. ECHA acknowledges your intentions, however all the new information in the later update(s) of the registration dossier will be assessed for compliance with the REACH requirements in the follow-up evaluation pursuant to Article 42 of the REACH Regulation.

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: Algae growth inhibition test, EU C.3./OECD TG 201).

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 15 March 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.

In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the request(s).

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

As no amendments were proposed, ECHA took the decision according to Article 51(3) 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.