~~Confidential~~

Helsinki, 26 May 2023

Addressee(s)
Registrant(s) of gamma-Butyrolactone (GBL) as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
26/07/2018

Registered substance subject to this decision ("the Substance")
Substance name: gamma-butyrolactone
EC/List number: 202-509-5

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***31 August 2026***.

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

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

1. Growth inhibition study on aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3/OECD TG 201)

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

2. In vitro micronucleus study (Annex VIII, Section 8.4.2.; test method: OECD TG 487) The aneugenic potential of the Substance must be assessed with an additional control group for aneugenicity on top of the control group for clastogenicity, if the Substance induces an increase in the frequency of micronuclei.

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

3. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211)
4. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: EU C.47./OECD TG 210)

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

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

- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation; and
- Cohorts 2A and 2B (Developmental neurotoxicity).

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

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

Information required depends on your tonnage band

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

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

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

How to comply with your information requirements

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

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

Appeal

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

Failure to comply

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

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

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

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

Appendix 1: Reasons for the request(s)

Contents

Reasons related to the information under Annex VII of REACH

1. Growth inhibition study aquatic plants

1 Growth inhibition study on aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).

1.1. Information provided

2 You have provided:

(i) A growth inhibition study on algae (1988-06-03) with the Substance;

(ii) A growth inhibition study on algae (1988-05-24) with the Substance.

1.2. Assessment of the information provided

1.2.1. The provided studies do not meet the specifications of the test guideline(s)

3 To fulfil the information requirement, a study must comply with OECD TG 201 (Article 13(3) of REACH). Therefore, the following specifications must be met:

Validity criteria

b) exponential growth in the control cultures is observed over the entire duration of the test;

c) at least 16-fold increase in biomass is observed in the control cultures by the end of the test;

d) the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is ≤ 35%;

e) the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is ≤ 7% in tests with *Desmodesmus subspicatus.*

Technical specifications impacting the sensitivity/reliability of the test

f) three replicates at each test concentration and at least three replicates for controls (including solvent controls, if applicable) are included;

g) for *Desmodesmus subspicatus* the initial cell density is 2-5 x10^3 cells/mL;

h) the pH of the control medium does not increase by > 1.5 units.

Characterisation of exposure

i) analytical monitoring must be conducted. Alternatively, a justification why the analytical monitoring of exposure concentrations is not technically feasible must be provided.

Reporting of the methodology and results

j) the method for determination of biomass and evidence of correlation between the measured parameter and dry weight are reported. Algal biomass is normally determined based on dry weight per volume, or alternatively as cell counts or biovolume using microscopy or an electric particle counter. If an alternative method is used (e.g. flow cytometry, in vitro or in vivo fluorescence, or optical

density), a satisfactory correlation with biomass must be demonstrated over the range of biomass occurring in the test;

k) the results of algal biomass determined in each flask at least daily during the test period are reported in a tabular form;

l) microscopic observation performed to verify a normal and healthy appearance of the inoculum culture are reported. Any abnormal appearance of the algae at the end of the test is reported.

4 For both studies (i) and (ii), the following issues have been identified:

Validity criteria

a) no information is provided to verify whether exponential growth occurred in the control cultures over the entire duration of the test;

b) no information is provided on the increase in biomass in the control cultures by the end of the test;

c) no information is provided on the mean coefficient of variation for section-by-section specific growth rates in the control cultures;

d) no information is provided on the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures.

Technical specifications impacting the sensitivity/reliability of the test

e) no information is provided on the number of replicates;

f) the test was conducted on *Desmodesmus subspicatus* and the initial cell density was 10^4 cells/mL;

g) no information is provided on the pH in the controls.

Characterisation of exposure

h) no analytical monitoring of exposure was conducted;

Reporting of the methodology and results

i) you report that algal biomass was determined using fluorescence. You indicate that since no calibration curve data were available, fluorescence data were equated with cell numbers. However, you have not reported evidence of correlation between the fluorescence and dry weight or cell numbers over the range of biomass occurring in the test;

j) tabulated data on the algal biomass determined daily for each treatment group and control are not reported;

k) microscopic observations to verify a normal and healthy appearance of the inoculum culture are not reported.

5 Based on the above,

- the validity criteria of OECD TG 201 cannot be verified,
- there are critical methodological deficiencies resulting in the rejection of the study results:
  - A sufficient number of replicates is necessary to ensure that a statistically robust result can be derived.
  - A too high initial cell density may result in the deprivation of nutrients and of dissolved CO_2 from the test medium before the end of the test. Those

may in turn limit the algal growth and the requirement for an exponential growth throughout the entire duration of the test would be violated.

- It is important to measure the evolution of pH in the control as it may indicates a deprivation of CO_2 from the test medium.

- the reporting of the study is not sufficient to conduct an independent assessment of its reliability.

6 On this basis, the specifications of OECD TG 201 are not met.

7 Therefore, the information requirement is not fulfilled.

1.3. Comments on the draft decision

8 In your comments to the draft decision you agree to perform the requested study.

Reasons related to the information under Annex VIII of REACH

2. In vitro micronucleus study

9 An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is an information requirement under Annex VIII, Section 8.4.2.

2.1. Information provided

10 You have provided the following in vitro study:

(i) chromosome aberration test (1989)

2.2. Assessment of the information provided

11 Study (i) cannot be evaluated as the results are equivocal.

12 Based on the above, the study is not adequate for the information requirement.

2.3. Comments on the draft decision

13 In your comments to the draft decision you agree to perform an OECD TG 487 study.

2.4. Specification of the study design

14 According to the Guidance on IR & CSA, Section R.7.7.6.3., either the *in vitro* mammalian chromosomal aberration ("CA") test (test method OECD TG 473) or the *in vitro* mammalian cell micronucleus ("MN") test (test method OECD TG 487) can be used to investigate chromosomal aberrations *in vitro*. However, while the MN test detects both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the CA test detects only clastogenicity, as OECD TG 473 is not designed to measure aneuploidy (see OECD TG 473, paragraph 2). Therefore, you must perform the MN test (test method OECD TG 487), as it enables a more comprehensive investigation of the chromosome damaging potential *in vitro.* Moreover, in order to demonstrate the ability of the study to identify clastogens and aneugens, you must include two concurrent positive controls, one known clastogen and one known aneugen [1] (OECD TG 487, paragraphs 33 to 35).

2.4.1. Assessment of aneugenicity potential

15 If the result of the MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance.

16 In line with the OECD TG 487 (paragraph 4), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragment(s)) and/or aneugenic events (i.e. micronuclei contain whole chromosome(s)).

17 [1] According to the TG 487 (2016) 'At the present time, no aneugens are known that require metabolic activation for their genotoxic activity' (paragraph 34).

Reasons related to the information under Annex IX of REACH

3. Long-term toxicity testing on aquatic invertebrates

18 Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

3.1. Information provided

19 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1. To support the adaptation, you have provided following information:

(i) "*According to Annex IX Column 2 of Regulation (EC) No 1907/2006, long-term toxicity testing is to be proposed if the chemical safety assessment indicates the need to investigate further the effects on aquatic organisms. The hazard assessment of γ-butyrolactone reveals neither a need to classify the substance as dangerous to the environment, nor is it a PBT or vPvB substance, nor are there any further indications that the substance may be hazardous to the environment. Therefore, a chronic test in aquatic invertebrates is not provided or proposed*".

3.2. Assessment of the information provided

3.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

20 Under Annex IX, Section 9.1., Column 2 is not a basis for omitting information on long-term toxicity to aquatic invertebrates referred to under Column 1, Section 9.1.5.

21 Your adaptation is therefore rejected.

22 Therefore, the information requirement is not fulfilled.

3.3. Comments on the draft decision

23 In your comments to the draft decision you agree to perform the requested study.

4. Long-term toxicity testing on fish

24 Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

4.1. Information provided

25 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1. To support the adaptation, you have provided following information:

(i) "*According to Annex IX Column 2 of Regulation (EC) No 1907/2006, it is laid down that chronic tests on fish shall be proposed by the registrant if the chemical safety assessment indicates the need to investigate further the effects on fish. According to Annex I of this regulation, the chemical safety assessment triggers further action when the substance or the preparation meets the criteria for classification as dangerous according to Directive 67/548/EEC or Directive 1999/45/EC or is assessed to be a PBT or vPvB. The hazard assessment of γ-butyrolactone reveals*

neither a need to classify the substance as dangerous to the environment, nor is it a PBT or vPvB substance, nor are there any further indications that the substance may be hazardous to the environment. Therefore, and for reasons of animal welfare, a chronic test in fish is not provided".

4.2. Assessment of the information provided

4.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

26 Under Annex IX, Section 9.1., Column 2 is not a basis for omitting information on long-term toxicity to fish referred to under Column 1, Section 9.1.6.

27 Your adaptation is therefore rejected.

28 Therefore, the information requirement is not fulfilled.

4.3. Comments on the draft decision

29 In your comments to the draft decision you agree to perform the requested study.

4.4. Study design and test specifications

30 To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (Guidance on IRs and CSA, Section R.7.8.2.).

Reasons related to the information under Annex X of REACH

5. Extended one-generation reproductive toxicity study

31 An extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is an information requirement under Annex X, Section 8.7.3. Furthermore Column 2 defines the conditions under which the study design needs to be expanded.

5.1. Information provided

32 ECHA understands you have adapted this information requirement by using Annex XI, Section 1.2. (weight of evidence) based on the following:

33 You have provided:

(i) Screening for reproductive / developmental toxicity (1999) with the analogue substance 1,4-Butanediol, EC 203-786-5

(ii) Toxicity to reproduction (ovulation, 1976) with the Substance

(iii) Sub-chronic toxicity: oral, mice (1992) with the Substance

(iv) Sub-chronic toxicity: oral, rat (1992) with the Substance

5.2. Assessment of the information provided

34 Annex XI, Section 1.2. states that there may be sufficient weight of evidence from several independent sources of information enabling, through a reasoned justification, a conclusion on the information requirement, while the information from each single source alone is insufficient to fulfil the information requirement.

35 The justification must have regard to the information that would otherwise be obtained from the study that must normally be performed for this information requirement.

36 According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude on the corresponding information requirement.

37 Relevant information that can be used to support weight of evidence adaptation for the information requirement of Annex X, Section 8.7.3 includes similar information that is produced by the OECD TG 443. OECD TG 443 requires the study to investigate the following key elements:

(1) sexual function and fertility, and
(2) toxicity to offspring
(3) systemic toxicity

5.2.1. Sexual function and fertility

38 Sexual function and fertility on both sexes must include information on mating, fertility, gestation (length), maintenance of pregnancy (abortions, total resorptions), parturition,

lactation, organ weights and histopathology of reproductive organs and tissues, oestrous cyclicity, sperm count, sperm analysis, hormone levels, litter sizes, nursing performance and other potential aspects of sexual function and fertility.

39 The source of information (i) may provide relevant information on sexual function and fertility. Source (ii) contains very limited information on this key element, namely on ovulation. Studies (iii) and (iv) do not contain any relevant information on sexual function and fertility as the animals were not mated.

40 However, the reliability of these sources of information is significantly affected by the following deficiency:

41 Information on sexual function and fertility (functional fertility and histopathology of reproductive organs and tissues) must be investigated in parental P0 animals as indicated in OECD TG 443 after at least ten weeks premating exposure duration if extension of Cohort 1B is not included[2] to ensure the exposure of full spermatogenesis and folliculogenesis before mating.

42 In the case of your Substance, the conditions to include the extension of Cohort 1B are currently not met. The source of information (i) investigates sexual function and fertility with the premating exposure duration of two weeks for the parental P0 animals. Source (ii to iv) did not involve mating of animals.

43 Neither sources of information investigate the sexual function and fertility in the P0 generation with sufficient premating exposure duration to ensure the coverage of full spermatogenesis and folliculogenesis before mating.

44 In the absence of information on the sexual function and fertility after exposure to the Substance over a pre-mating period of 10 weeks, no conclusion can be drawn on sexual function and fertility as required by the information requirement.

45 Therefore the provided study cannot be considered a reliable source of information that could contribute to the conclusion on this key parameter investigated by the required study.

5.2.2. Toxicity to the offspring

46 Toxicity to offspring must cover information on deaths before, during or after birth, growth, external malformations, clinical signs, sexual maturity, oestrous cyclicity, organ weights and histopathology of reproductive organs and tissues in adulthood and other potential aspects of toxicity to offspring.

47 The source of information (i) provides some information on toxicity to the offpsring up to post-natal day 3. Sources (ii to iv) do not inform on toxicity to offspring.

48 Information provided on toxicity to offspring is limited and does not cover all relevant and essential aspects as defined above. Source (i) does not inform on toxicity to the offspring up to adulthood. Therefore, no conclusion can be drawn on toxicity to the offspring as required by the information requirement.

5.2.3. Systemic toxicity

49 Systemic toxicity must include information on clinical signs, survival, body weights, food consumption, haematology (full-scale), clinical chemistry (full-scale), organ weights and histopathology of non-reproductive organs and tissues (full-scale) and other potential aspects of systemic toxicity in the parental P and F1 generation up to adulthood.

50 Sources of information (i, iii and iv) provide relevant information on systemic toxicity in animals exposed as adults. Information provided on systemic toxicity does not cover all

[2] ECHA Guidance R.7a, Section R.7.6

relevant and essential aspects as defined above. In particular, there is no information on systemic toxicity from F1 generation. Therefore, the information on systemic toxicity does not cover the required aspect on systemic toxicity.

5.3. Conclusion

51 In summary, the sources of information (i) to (iv) provide limited relevant information on sexual function and fertility, toxicity to the offspring, and systemic toxicity. However, in particular information on toxicity to the offspring up to adulthood is missing, and information on sexual function and fertility is not reliable.

52 It is not possible to conclude, based on any source of information alone or considered together, on the information requirement for extended one-generation reproductive toxicity study.

53 Based on the above, your adaptation is rejected.

54 Therefore, the information requirement is not fulfilled.

5.4. Comments on the draft decision (read across)

55 In your comments to the draft decision you agree with ECHA's rejection of your adaptation according to Annex XI, Section 1.2. (weight of evidence). Instead you propose an adaptation according to Annex XI, Section 1.5 (grouping of substances and read-across) to fulfil this information requirement.

56 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

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

57 You provide a read-across justification document in IUCLID Section 13 and a revised version of this document together with your comments (attachment 5).

58 You predict the properties of the Substance from information obtained from the following source substance(s):

- Butane-1,4-diol (BDO), EC 203-786-5

59 You provide the following reasoning for the prediction of toxicological properties: "*After uptake, the source substance BDO is rapidly metabolised by alcohol/aldehyde dehydrogenases to form gamma-hydroxybutyric acid (GHB) which is the main metabolite also of the target substance gamma-butyrolactone (GBL)."*

60 ECHA understands that your read-across hypothesis is based on the formation of common (bio)transformation products. You predict the properties of your Substance to be quantitatively equal to those of the source substance.

61 We have identified the following issue(s) with the prediction(s) of toxicological properties:

5.4.2. Missing source study

62 According to Annex XI, Section 1.5., if the grouping concept is applied then in all cases the results to be read across must have adequate and reliable coverage of the key parameters addressed in the corresponding study that shall normally be performed for a particular information requirement.

63 In your comments to the draft decision you present a testing proposal for an OECD TG 443 study with the source substance, but the study is currently not available.

5.4.3. Missing supporting information

64 Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide supporting information to scientifically justify the read-across explanation for prediction of properties. The set of supporting information should strengthen the rationale for the read-across in allowing to verify the crucial aspects of the read-across hypothesis and establishing that the properties of the Substance can be predicted from the data on the source substance(s) (Guidance on IRs and CSA R.6, Section R.6.2.2.1.f.).

65 Your testing strategy submitted with the comments includes the intention to conduct an OECD TG 422 study with the Substance as supporting information. This study is currently not available.

5.4.4. Conclusion on the read-across approach

66 As the outlined strategy relies on an approach that has not yet been fully described and justified, as well as on data which are yet to be generated, no assessment or conclusion on the compliance of the proposed adaptation can presently be made. For the reasons explained above your dossier is currently not compliant with the information requirement and therefore, you remain responsible for complying with this decision by the set deadline.

5.5. Specification of the study design

5.5.1. Species and route selection

67 A study according to the test method OECD TG 443 must be performed in rats with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

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

5.5.2. Pre-mating exposure duration

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

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

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

5.5.3. Dose-level setting

72 The aim of the requested test must be to demonstrate whether the classification criteria of the most severe hazard category for sexual function and fertility (Repr. 1B; H360F) and developmental toxicity (Repr. 1B; H360D) under the CLP Regulation apply for the Substance

(OECD TG 443, paragraph 22; OECD GD 151, paragraph 28; Annex I Section 1.0.1. of REACH and Recital 7, Regulation 2015/282), and whether the Substance meets the criteria for a Substance of very high concern regarding endocrine disruption according to Art.57(f) of REACH as well as supporting the identification of appropriate risk management measures in the chemical safety assessment.

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

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

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

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

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

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

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

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

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

5.5.4. Cohorts 1A and 1B

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

5.5.4.1. Histopathological investigations in Cohorts 1A and 1B

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

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

5.5.4.2. Splenic lymphocyte subpopulation analysis

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

5.5.4.3. Investigations of sexual maturation

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

5.5.5. Cohorts 2A and 2B

82 The developmental neurotoxicity Cohorts 2A and 2B must be conducted in case of a particular concern on (developmental) neurotoxicity.

83 Existing information on the Substance itself derived from available *in vivo* studies (90 day rat and mouse studies) show evidence of sedation after dosing, with mice becoming recumbent at high dose. Further, you have self-classified the substance as STOT Single Exp. category 3, stating that the affected organ is the Central nervous system, based on the results of an acute inhalation study. The above-mentioned effects are adverse effects on the nervous system.

84 In your comments, you agree that the Substance causes narcotic effects in adult animals, and you have self-classified the Substance accordingly. You note that the narcotic effects are observed following high acute and lower repeated exposure. You consider that this is not automatically linked to (developmental) neurotoxicity. Therefore, you consider that inclusion of Cohorts 2A and 2B is not necessarily justified.

85 In accordance with ECHA's Guidance (R.7a page 529), narcosis is a functional adverse effect on the nervous system and thus is a substance specific finding which may indicate a particular concern justifying inclusion of the developmental neurotoxicity cohorts.

86 For the reasons stated above, the developmental neurotoxicity Cohorts 2A and 2B must be conducted.

87 In your comments, you propose to limit the exposure of F1 animals '*to the sensitive window of CNS development in rats, i.e., in utero and during early life (i.e. lactation)*'. You consider that extending exposure beyond weaning could lead to narcotic effects being falsely identified as developmental neurotoxicity. You also raise considerations which must be taken into account in top-dose setting.

88 You have not provided a valid reason for deviating from the Test Guideline. ECHA notes that Cohort 2B of an EOGRT study is terminated on PND 21 or 22 and therefore the offspring are exposed only *in utero* via their mother and during the lactation period. In Cohort 2A of a EOGRTS, the offspring are exposed via the mother *in utero*, through lactation and directly at least after weaning until termination on ~PND 66-77. ECHA acknowledges that it is generally not possible to distinguish the precise origin or timing of the toxicological insult if adverse neuropathological, functional, or behavioural outcome is observed after sexual maturation in cohort 2A. However, any effects investigated or detected in Cohorts 2A and 2B are relevant for developmental toxicity and the respective hazard classification[3]. ECHA

[3] RAC Guidance Note '*Addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes*': https://www.echa.europa.eu/documents/10162/17090/rac_clh_guidance_note_neurotoxicity_en.pdf/96717ed9-55d3-10e0-785b-093d07e267f3?t=1665034511575

agrees that you must take relevant considerations into account for dose-setting, as set out in section 5.5.3 above.

89 Finally, you note that you have initiated an OECD TG 422 study which will investigate e.g. brain weight and histopathology, functional observation battery, and motor activity. You consider that the need for investigating the potential of the Substance to induce developmental neurotoxicity should depend on the outcome of that OECD TG 422 study. ECHA acknowledges that further information on neurological parameters will be available, however notes that, as explained above, a concern for developmental neurotoxicity has already been identified. Therefore, Cohorts 2A and 2B must be conducted.

5.5.6. Additional considerations

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

5.5.7. Comments on the draft decision (study design)

91 In your comments to the draft decision you disagree with ECHA on the numbers of F1 animals needed for the evaluation of sexual maturation. You base your argumentation on a recent publication by Oldenburger et al., 2021 who could demonstrate that two instead of three F1-animals/sex/litter/dose would suffice for this evaluation. Therefore you disagree with ECHA's specification of maintaining all F1 animals until sexual maturation to ensure that sufficient animals are available for the required examinations.

92 To fulfil the information requirement, a study must comply with the relevant OECD TG (Article 13(3) of REACH). In this case the specifications of OECD TG 443 must be met:

93 ECHA's specifications of the study design are in accordance with OECD TG 443 and its supporting guidance document, OECD GD 151.

94 According to OECD GD 151, paragraph 12: "*The design of the EOGRTS is provided in TG 443. A general summary is outlined in Figure 1 and Table 1, to give some context to the following sections of this guidance. A detailed list of endpoints is given in Annex 1. In case the DNT (cohort 2) and/or DIT (cohort 3) cohorts are omitted or the F1 generation bred to produce an F2 generation (see paragraph 1), resulting changes should however maintain the required number of pups for reproductive assessment as detailed in this GD. Thus, whether the DNT and/or DIT assessments are performed or not, all animals, including those in cohorts 2 and 3 should be maintained until sexual maturation to ensure that sufficient animals (3/sex/dose) are available for evaluation of critical endpoints*."

95 Further, in paragraph 60 of the same document: "*…In addition, it is important that, unless earlier testing is required (i.e. cohort 2B), all the animals included in each cohort are monitored to sexual maturation (vaginal patency or preputial separation). In cases where the DNT or DIT elements are omitted, then cohorts 2A and 3 should be maintained and evaluated for sexual maturation. In this way, the probability to detect rare or low incidence malformations such as hypospadias which would appear postnatally, or other effects on the reproductive axis will be increased. The following discussion provides the rationale for using these numbers of animals."*

96 Therefore, ECHA's specification follows the current OECD TG 443 guideline and must be met for a study to be compliant with Article 13(3) of REACH.

References

The following documents may have been cited in the decision.

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

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

Guidance on data-sharing; ECHA (2017).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).
All guidance documents are available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

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

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

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

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

Appendix 2: Procedure

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

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 17 November 2021.

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

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

In your comments you requested an additional extension of the deadline due to lacking laboratory capacity with an additional 6 to 12 months.

In the instructions for the webform for Registrant's comments on a draft decision it is indicated that if you request extension based on lab availability, documentation of the correspondence with laboratory/ies including the scheduling timelines for the studies in question of the lab facility/ies should be included as an attachment to justify why an extension to the stated deadline is required. You have not provided documentary evidence for your request. ECHA has therefore not amended the deadline.

Based on the information provided with your comments on the initial draft decision ECHA agrees that the results of the cytogenicity study provided in your dossier need to be interpreted as equivocal. Therefore the request for an In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4.4., Column 1; test method: OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) was removed from the decision.

ECHA took into account your comments and amended the request(s) but did not amend the deadline.

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

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

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

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

The Member State Committee unanimously agreed on the draft decision in its MSC-82 written procedure. ECHA adopted the decision under Article 51(6) of REACH.

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

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

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

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

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

Appendix 4: Conducting and reporting new tests for REACH purposes

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

1.1. Test methods, GLP requirements and reporting

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

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

(3) Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[4].

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

1.2. Test material

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

(1) Selection of the Test material(s)

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

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

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

- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.

[4] https://echa.europa.eu/practical-guides

- The reported composition must include all constituents of each Test Material and their concentration values.

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

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