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Helsinki, 14 February 2024

Addressee(s)
Registrant(s) of Guanidinium_nitrate-2009-06-26 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
29 March 2022

Registered substance subject to this decision ("the Substance")
Substance name: Guanidinium nitrate
EC/List number: 208-060-1

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 ***24 May 2027***.

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

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

1. Skin sensitisation (Annex VII, Section 8.3.)

   i. *in vitro/in chemico* skin sensitisation information on molecular interactions with skin proteins (OECD TG 442C), inflammatory response in keratinocytes (OECD TG 442D) and activation of dendritic cells (OECD TG 442E) (Annex VII, Section 8.3.1.); and

   ii. only if the *in vitro/in chemico* test methods specified under point i.) above are not applicable for the Substance or the results obtained are not adequate for classification and risk assessment, *in vivo* skin sensitisation (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429);

2. 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

3. *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.

4. Adsorption/desorption screening (Annex VIII, Section 9.3.1.; test method: EU C.18/OECD TG 106)

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

5. Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.; test method: EU C.25/OECD TG 309) at a temperature of 12°C.

6. Identification of degradation products (Annex IX, Section 9.2.3.; test method: EU C.25/OECD TG 309)

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

6. 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 in request 6, 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);
    - 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.

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

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

[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)

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Reasons related to the information under Annex VII of REACH

1. Skin sensitisation

1 Skin sensitisation is an information requirement under Annex VII, Section 8.3. Under Section 8.3., Column 1, the registrants must submit information allowing (1) a conclusion whether the substance is a skin sensitiser and (2) whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

1.1. Information provided

2 You have provided:

(i) an in vivo skin sensitisation test (1988) with the Substance

(ii) an in vivo skin sensitisation test (1986) with the source substance Guanidine hydrochloride, EC 200-002-3.

1.2. Assessment of the information provided

1.2.1. Assessment whether the Substance causes skin sensitisation

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

3 To fulfil the information requirement, and to enable concluding whether the Substance causes skin sensitisation, a study must comply with the EU Method B.6/OECD TG 406 (Article 13(3) of REACH) or, in the case of a read-across adaptation, a study must have adequate and reliable coverage of the key parameters of that method (Annex XI, Section 1.5). Therefore, the following specifications must be met:

a) a dose level selection rationale is provided;
b) induction concentration is the highest causing mild irritation to the skin and the challenge dose is the highest non-irritation concentration.

4 In studies (i and ii):

a) no dose level selection rationale was provided;
b) no information was provided whether the concentration used for induction caused mild irritation and whether the challenge concentration was the highest non-irritation concentration. As 10% in isotonic saline was used in both induction and challenge, the concentration used cannot be both i.e. concentration causing mild irritation and highest non-irritating concentration.

5 The information provided does not cover the specifications required by OECD TG 406 and does not have adequate and reliable coverage of its key parameters, and does not allow to make a conclusion whether the Substance causes skin sensitisation.

1.2.2. No assessment of potency

6 To be considered compliant and enable a conclusion in cases where the substance is considered to cause skin sensitisation, the information provided must also allow a conclusion whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

7 As the currently available data does not allow to conclude whether the Substance causes skin sensitisation (see section 1.2.1 above), this condition cannot be assessed.

8 Therefore, the information requirement is not fulfilled.

1.3. Study design

9 To fulfil the information requirement for the Substance, information on molecular interaction with skin proteins and inflammatory response in keratinocytes and activation of dendritic cells (OECD TG 442C and OECD TG 442D and OECD TG 442E) must be provided. Furthermore an appropriate risk assessment is required if a classification of the Substance as a skin sensitiser (Cat 1A or 1B) is warranted.

10 In case no conclusion on the skin sensitisation potency can be made for the Substance based on the existing data or newly generated in vitro/in chemico data, in vivo skin sensitisation study must be performed and the murine local lymph node assay (EU Method B.42/OECD TG 429) is considered as the appropriate study for the potency estimation.

1.4. Your comments to the draft decision

11 In your comments on the draft decision you agreed to conduct the test(s).

2. Growth inhibition study aquatic plants

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

2.1. Information provided

13 You have provided:

(i) Growth inhibition study on aquatic plants (2010) with the Substance.

2.2. Assessment of the information provided

2.2.1. The provided study does not meet the specifications of the test guideline

14 To fulfil the information requirement, a study must comply with OECD TG 201 and the specifications of OECD GD 23 if the substance is difficult to test (Article 13(3) of REACH).

15 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 *Pseudokirchneriella subcapitata*;

Technical specifications impacting the sensitivity/reliability of the test

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

16 In study (i):

Validity criteria

Validity criteria a) – d) are not reported.

Technical specifications impacting the sensitivity/reliability of the test

e) the pH increase in the controls was 2.5 units.

17 Based on the above,

- the validity criteria of OECD TG 201 are missing.
- there are critical methodological deficiencies resulting in the rejection of the study results. More specifically, the pH increased in the controls by more than 1.5 units. High pH could have limited the algae growth in the controls by the end of the test. If the growth of the algae was reduced in the controls, then the calculation of the inhibition percentages for the different test concentrations could have been underestimated. Since no information is provided on the growth curves, we cannot rule out this phenomenon and we cannot verify that the validity criteria are met.

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

19 Therefore, the information requirement is not fulfilled.

2.3. Your comments to the draft decision

20 In the comments to the draft decision, you have attached part of a Robust Study Summary (RSS) that includes the information listed above as missing in the dossier. You have proposed to update your dossier with this information.

21 The information provided as part of your comments addresses the deficiencies identified above. However, as this information is currently not available in your registration dossier, the data gap remains. You should submit this information in an updated registration dossier by the deadline set in the decision.

Reasons related to the information under Annex VIII of REACH

3. *In vitro* micronucleus study

22 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.

3.1. Information provided

23 You have provided:

(i) an *in vitro* cytogenicity study in mammalian cells (1997) with the Substance.

3.2. Assessment of the information provided

3.2.1. The provided study does not meet the specifications of the test guideline(s)

24 To fulfil the information requirement, the study has to be an *in vitro* chromosomal aberration test or an *in vitro* micronucleus test conducted in mammalian cells. The study must comply with the OECD TG 473 or the OECD TG 487, respectively (Article 13(3) of REACH). Therefore, the following specifications must be met:

a) two separate test conditions are assessed: in absence of metabolic activation and in presence of metabolic activation;

b) the maximum concentration tested induces 55+5% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration corresponds to 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest;

c) at least 300 well-spread metaphases are scored per concentration;

d) data on the cytotoxicity and the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures is reported.

25 In study (i):

a) the test was performed only in absence of metabolic activation;

b) the maximum tested concentration was less than 10 mM, 2 mg/mL or 2 µL/mL and information on cytotoxicity of precipitation was not reported;

c) 100 metaphases (i.e., less than 300 metaphases) were scored per concentration;

d) data on the cytotoxicity for the treated and control cultures were not reported.

26 The information provided does not cover the specifications(s) required by the OECD TG 473.

27 Therefore, the information requirement is not fulfilled.

3.3. Study design

28 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).

29 In your comments on the draft decision you agree with the request. In response to your comment regarding the cell types to be used ECHA notes that the OECD TG 487 (paragraph 14.) lists examples of cells that can be used. The test laboratory should validate the use of the cells chosen and prove proficiency with the assay. The laboratory should establish positive and negative historical control distributions and ranges (paragraphs 47-52 of OECD TG 487).

3.3.1. Assessment of aneugenicity potential

30 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.

31 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)).

[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).

4. Adsorption/ desorption screening

32 Adsorption/desorption screening is an information requirement under Annex VIII to REACH (Section 9.3.1).

4.1. Information provided

33 You have adapted this information requirement by using Column 2 of Annex VIII, Section 9.2.2.1. To support the adaptation, you have provided the following information:

(i) "The study does not need to be conducted because the substance has a low octanol water partition coefficient and the adsorption potential of this substance is related to this parameter (…) and the adsorptive properties of the substance are solely driven by lipophilicity".

4.2. Assessment of the information provided

4.2.1. Low potential for adsorption based on physicochemical properties not demonstrated

34 Under Annex VIII, Section 9.3.1, Column 2, first indent, the study may be omitted if the substance can be expected to have a low potential for adsorption (e.g. the substance has a low octanol-water partition coefficient). In order to adapt this information requirement based on low octanol-water partition coefficient (log Kow), lipophilicity must be the sole characteristic driving the adsorption potential of a substance. However, for some groups of substances (e.g. ionisable substances, surfactants) other mechanisms than lipophilicity may drive adsorption.

35 You claim that the Substance has a low octanol-water partition coefficient and has therefore low potential for adsorption/desorption.

36 You have not provided any other evidence or argument that the Substance can be expected to have a low potential for adsorption.

37 In section 4.21 of your dossier you provided pKa value of 10.3 for your Substance (data from publication, 1967). In addition, the report attached in section 13.2 of your dossier explains that guanidine is a strong base with pKa 12.5 and nitrate is the corresponding base of the strong nitric acid. Considering this information, in aqueous solution the Substance is completely dissociated.

38 The information in your dossier indicates that the Substance is ionisable.

39 Therefore, other mechanisms than lipophilicity may drive absorption.

40 You have not demonstrated that lipophilicity is the sole characteristic driving adsorption potential and that log Kow is not a valid descriptor for assessing the adsorption potential of the Substance.

41 Based on the above, your adaptation is rejected.

42 Therefore, the information requirement is not fulfilled.

4.3. Your comments to the draft decision

43 Based on your comments to the draft decision we understand that you agree that the information in the registration dossier does not comply with the information requirement for Adsorption/desorption screening. You are, however, disagreeing with the study design as discussed below under section 4.4.

44 You also indicate an intention to adapt this information requirement by means of grouping and read-across according to Annex XI, Section 1.5, of the REACH Regulation. You propose to predict the adsorption/desorption properties of the Substance from new study on source substance diguanidinium carbonate, EC 209-813-7.

45 In the comments, you provide a valid read-across hypothesis, but the study on the source substance is not yet generated. Therefore, no conclusion on the compliance of the proposed adaptation can be made. You remain responsible for complying with this decision by the set deadline.

4.4. Study design

46 The OECD TG 106 Batch Equilibrium Method is the appropriate method to study the adsorption of the Substance. This method uses a range of actual soils and so represents a more realistic scenario than the HPLC (OECD 121) method. The ionisable properties of the Substance should be considered when selecting the appropriate test design. For ionisable substances, soil types should cover a wide range of pH.

47 In your comments to the draft decision you disagree to perform the study with the OECD TG 106 method. You indicate that the HPLC (OECD TG 121) method should be used instead: *"(…) when utilising a relevant range of pH values in an HPLC test according to OECD 121, obtaining a meaningful range of Koc values can be expected.(…) an OECD 106 test should only be performed if no meaningful results can be obtained with the OECD 121 test*."

48 To fulfil the information requirement, both test methods according to OECD TG 106 and OECD TG 121 are in general appropriate. However, the Substance must be within the applicability domain of the chosen test method.

49 OECD TG 121 is not applicable for moderate to strong bases. The pKa value of 10.2 provided in your registration dossier indicates that the Substance is at least a moderate base. In

addition, the pKa of guanidine (the compound to which based on your read-across hypothesis the Substance is transformed to in the aquatic solution) is ca. 13.52, which indicates that it is a strong base.

50 Having regard to the above information, ECHA considers that the Substance is outside of the applicability domain of OECD TG 121 and that OECD TG 106 is the appropriate method for the Substance.

[2] Guanidine and Derivatives, Thomas Güthner, Bernd Mertschenk, Bernd Schulz, Ullmann's Encyclopedia of Industrial Chemistry, 2006 (https://doi.org/10.1002/14356007.a12_545.pub2).

Reasons related to the information under Annex IX of REACH

5. Simulation testing on ultimate degradation in surface water

51 Simulation testing on ultimate degradation in surface water is an information requirement under Annex IX to REACH (Section 9.2.1.2.).

5.1. Information provided

52 You have provided the following information:

(i) a justification to omit the study: *"the (...) substance isn't ready biodegradable but inherent degradable, hence still the ultimate degradation is of sufficient evidence".*

53 In addition, you have adapted this information requirement by using Annex XI, Section 1.5. (grouping of substances and read-across approach) based on experimental data from the following substance:

(ii) a simulation study on ultimate degradation in surface water from the publication (1987) with the source substance guanidine hydrochloride, EC 200-002-3.

54 You provide a read-across justification document in IUCLID Section 13.

55 You provide the following reasoning for the prediction of toxicological properties: *"Guanidine hydrochloride and guanidine nitrate dissociate in aqueous media to yield the guanidine ion and the respective anion. (…). Effects of guanidine hydrochloride are expected to be based primarily on the guanidine ion. The physiological processing of the guanidine ion is expected to be independent of the individual source. Therefore read-across from guanidine hydrochloride for effects of guanidine dissociated from guanidine nitrate is considered valid. This strategy is supported by a quite similar toxicological profile of both substances (…)."*

56 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.

5.2. Assessment of the information provided

5.2.1. Your justification to omit the study has no legal basis

57 A registrant may only adapt this information requirement based on the general rules set out in Annex XI or the specific rules set out in Annex IX, Section 9.2.1.2., Column 2.

58 Therefore, you have not demonstrated that this information can be omitted.

5.2.2. Read-across adaptation rejected

59 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.

60 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).

5.2.3. Inadequate or unreliable study on the source substance

61 Under Annex XI, Section 1.5., the results to be read across must have an adequate and reliable coverage of the key parameters addressed in the test guideline for the corresponding study that shall normally be performed for a particular information requirement, in this case OECD TG 309. Therefore, the following specifications must be met:

Technical specifications impacting the sensitivity/reliability of the test

a) the purity of the test material is ≥ 95 %;

b) a reference substance known to be easily degraded under aerobic conditions (e.g. aniline or sodium benzoate) is used to verify the activity of the microbial population;

c) the repeatability of the analytical method (including the efficiency of the initial extraction) to quantify the test material and transformation/degradation products is checked by five replicate analyses of the individual extracts of the surface water;

d) the limit of detection (LOD) of the analytical method for the test material and for the transformation/degradation products is ≤ 1% of applied dose;

e) the limit of quantification (LOQ) of the analytical method for the test material and for the transformation/degradation products is ≤ 10% of applied dose;

f) the measurement of degradation and the determination of mass balances are done in at least in duplicate for each concentration and at each sampling time;

g) the surface water used to conduct the test has not been contaminated with the test material or its structural analogues within the previous 4 years;

h) to determine the transformation rates, the test material concentrations must reflect environmentally realistic concentrations and be ≤ 100 µg/L.

62 Reporting of the methodology and results

i) the mass balances during and at the end of the study are provided.

63 In study (ii):

Technical specifications impacting the sensitivity/reliability of the test

a) the purity of the test material is not provided (the report mentions only "technical grade");

b) a reference substance was not used to verify the activity of the microbial population;

c) the repeatability of the analytical method (including the efficiency of the initial extraction) to quantify the test material and transformation/degradation products was not checked by five replicate analyses of the individual extracts of the surface water;

d) the LOD of the analytical method for the test material and for the transformation/degradation products is not provided;

e) the LOQ of the analytical method for the test material and for the transformation/degradation products is not provided;

f) the measurement of degradation and the determination of mass balances was not performed in duplicate for each concentration and at each sampling time;

g) you report that surface water samples have been obtained from two streams in the vicinity of a nitroguanidine pilot production facility which does not exclude that the surface water used to conduct the test was likely contaminated with the active substance of the test material or its structural analogues within the previous 4 years;

h) to determine the transformation rates, two of the five test material concentrations were 1000 and 10000 µg/L.

64 Reporting of the methodology and results

i) the mass balances during and at the end of the study were not determined.

65 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. More, specifically you have not excluded that the surface water used to conduct the test was likely contaminated with the active substance of the test material or its structural analogues within the previous 4 years
- the reporting of the study is not sufficient to conduct an independent assessment of its reliability.

66 On this basis, the specifications of OECD TG 309 are not met.

67 Based on the above, the study submitted in your adaptation, as currently reported in your dossier, does not provide an adequate and reliable coverage of the key parameter(s) of the corresponding OECD TG.

68 As explained above, you have not established that relevant properties of the Substance can be predicted from data on the source substance. On this basis, your read-across approach under Annex XI, Section 1.5. is rejected.

69 Therefore, the information requirement is not fulfilled.

5.3. Your comments to the draft decision

70 In your comments to the draft decision you agree that the information in the registration dossier does not comply with the information requirements for simulation on ultimate degradation in surface water. You indicate an intention to adapt this information requirement by means of grouping and read-across according to Annex XI, Section 1.5, of the REACH Regulation. You propose to fulfil this information requirement for the Substance from new study on source substance diguanidinium carbonate, EC 209-813-7.

71 In the comments, you provide a valid read-across hypothesis, but the study on the source substance is not yet generated. Therefore, no conclusion on the compliance of the proposed adaptation can be made. You remain responsible for complying with this decision by the set deadline.

5.4. Study design

72 Simulation degradation studies must include two types of investigations (Guidance on IRs and CSA, Section R.7.9.4.1):

(2) a degradation pathway study where transformation/degradation products are quantified and, if relevant, are identified, and

(3) a kinetic study where the degradation rate constants (and degradation half-lives) of the parent substance and of relevant transformation/degradation products are experimentally determined.

73 You must perform the test, by following the pelagic test option with natural surface water containing approximately 15 mg dw/L of suspended solids (acceptable concentration between 10 and 20 mg dw/L) (Guidance on IRs and CSA, Section R.11.4.1.1.3.).

74 The required test temperature is 12°C, which corresponds to the average environmental temperature for the EU (Guidance on IRs and CSA, Table R.16-8) and is in line with the applicable test conditions of the OECD TG 309.

75 As specified in Guidance on IRs and CSA, Section R.7.9.4.1., the organic carbon (OC) concentration in surface water simulation tests is typically 2 to 3 orders of magnitude higher than the test material concentration and the formation of non-extractable residues (NERs) may be significant in surface water tests. Paragraph 52 of the OECD TG 309 provides that the "total recovery (mass balance) at the end of the experiment should be between 90% and 110% for radiolabelled substances, whereas the initial recovery at the beginning of the experiment should be between 70% and 110% for non-labelled substances". NERs contribute towards the total recovery. Therefore, the quantity of the (total) NERs must be accounted for the total recovery (mass balance), when relevant, to achieve the objectives of the OECD TG 309 to derive degradation rate and half-life. The reporting of results must include a scientific justification of the used extraction procedures and solvents.

76 For the persistence assessment by default, total NERs is regarded as non-degraded Substance. However, if reasonably justified and analytically demonstrated a certain part of NERs may be differentiated and quantified as irreversibly bound or as degraded to biogenic NERs, such fractions could be regarded as removed when calculating the degradation half-life(s) (Guidance on IRs and CSA, Section R.11.4.1.1.3.). Further recommendations may be found in the background note on options to address non-extractable residues in regulatory persistence assessment available on the ECHA website (NER - summary 2019 (europa.eu)).

77 Relevant transformation/degradation products are at least those detected at ≥ 10% of the applied dose at any sampling time or those that are continuously increasing during the study even if their concentrations do not exceed 10% of the applied dose, as this may indicate persistence (OECD TG 309; Guidance on IRs and CSA, Section R.11.4.1.).

6. Identification of degradation products

78 Identification of abiotic and biotic degradation products is an information requirement under Annex IX to REACH (Section 9.2.3.).

79 You have not submitted any information for this requirement.

80 Therefore, the information requirement is not fulfilled.

6.1. Your comments to the draft decision

81 In your comments to the draft decision you agree with the request. You indicate an intention to adapt this information requirement by means of grouping and read-across according to Annex XI, Section 1.5, of the REACH Regulation. You propose to fulfil this information requirement for the Substance from new study on source substance diguanidinium carbonate, EC 209-813-7.

82 In the comments, you provide a valid read-across hypothesis, but the study on the source substance is not yet generated. Therefore, no conclusion on the compliance of the proposed adaptation can be made. You remain responsible for complying with this decision by the set deadline.

6.2. Study design

83 Simulation degradation studies must include two types of investigations (Guidance on IRs and CSA, Section R.7.9.4.1.):

(1) a degradation pathway study where transformation/degradation products are quantified and, if relevant, are identified, and

(2) a kinetic study where the degradation rate constants (and degradation half-lives) of the parent substance and of relevant transformation/degradation products are experimentally determined.

84 Identity, stability, behaviour, and molar quantity of the degradation/transformation products relative to the Substance must be evaluated and reported. In addition, identified transformation/degradation products must be considered in the CSA including PBT assessment.

85 You must obtain this information from the degradation study requested in request 5.

86 To determine the degradation rate of the Substance, the requested study according to OECD TG 309 (request 5) must be conducted at 12°C and at a test concentration < 100 µg/L. However, to overcome potential analytical limitations with the identification and quantification of major transformation/degradation products, you may consider running a parallel test at higher temperature (but within the frame provided by the test guideline, e.g. 20°C) and at higher application rate (i.e. > 100 µg/L).

Reasons related to the information under Annex X of REACH

7. Extended one-generation reproductive toxicity study

87 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.

7.1. Information provided

88 You have adapted this information requirement by using Annex XI, Section 3. (substance-tailored exposure-driven testing). To support the adaptation, you have provided the following justification:

(i) *"the study does not need to be conducted because relevant human exposure can be excluded as demonstrated in the relevant exposure assessment".*

7.2. Assessment of the information provided

7.2.1. Substance-tailored exposure-driven testing adaptation rejected

89 A substance-tailored exposure-driven testing adaptation must fulfil the cumulative conditions set out under Annex XI, Sections 3(1) as well as 3(2)(a), (b) or (c).

7.2.1.1. Lack of appropriate DNEL

90 Under Annex XI, Section 3.2(a)(ii), a relevant and appropriate derived no effect level (DNEL) must be derived. Further, a DNEL derived from a 90-day repeated dose toxicity study or from the 1st species pre-natal developmental toxicity (PNDT) study must not be considered appropriate to omit an EOGRT study since they address different information requirements.

91 You have used a 90-day repeated dose toxicity study and 1st species PNDT study study to derive the worker long-term systemic DNEL for inhalation effects and worker long-term, systemic DNEL for dermal effects.

92 Therefore, you have not provided a relevant and appropriate DNEL.

93 Based on the above, your substance-tailored exposure driven testing adaptation under Annex XI, Section 3. is rejected.

94 Therefore, the information requirement is not fulfilled.

7.3. Study design

7.3.1. Species and route selection

95 According to the test method OECD TG 443, the rat is the preferred species. Therefore, the study must be conducted in the rat.

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

7.3.2. Pre-mating exposure duration

97 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.

98 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.).

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

7.3.3. Dose-level setting

100 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; introductory part of Annex IX/X to REACH; Annex I, Section 1.0.1. to 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.

101 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.

102 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.

103 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.

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

105 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.

7.3.4. Cohorts 1A and 1B

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

7.3.4.1. Histopathological investigations in Cohorts 1A and 1B

107 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.

7.3.4.2. Splenic lymphocyte subpopulation analysis

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

7.3.4.3. Investigations of sexual maturation

109 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.

7.3.5. Cohorts 2A and 2B

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

111 Guanidium chloride, EC No. 200-002-3, is structurally analogous to the Substance, since both have the same cation. Existing information on a substance structurally analogous to the Substance (guanidium chloride, EC No. 200-002-3) derived from available OECD TG 408 study (2015) shows evidence of functional adverse effects on the nervous system which suggests that the Substance has (developmental) neurotoxicity effects. Specifically, the grip strength of the hind legs was dose-dependently decreased in the dosed groups compared to the control group in both males and females. Furthermore, the hind limb reflex was impaired in 2/15 high dose males and 15/15 high dose females as well as in 3/10 females of both the low and mid dose group. These effects are likely functional adverse effects on the nervous system, and they are not likely to be secondary to general toxicity (nephropathy observed at high dose level only; local irritant effects in the glandular stomach at a 'very minor incidence' in all dose groups).

112 In your comments on the Proposal for Amendment which requested the inclusion of cohorts 2A and 2B, you acknowledged that the effects on grip strength and on hind limb reflex might be regarded as a trigger for further investigation of developmental neurotoxicity (DNT) and you agreed that the generation of additional data may be required. However you argue that the DNT cohorts should not be included for the following reasons:

> (1) guanidine causes physiologically-relevant effects on the nervous system in humans, and Kalia & Swartz (inter alia) characterises one mode of action as presynaptic inhibition of voltage-gated potassium channels, indirectly leading to enhanced cholinergic activity at the neuromuscular junction. You then argue that based on a mechanistic understanding of the mode of action and the difference in effects seen in rat and human, that the rat is not a suitable model system for the assessment of (developmental) neurotoxicity.

(2) You argue that the Cohorts 2A and 2B have general shortcomings.

(3) *in vitro* approaches provide a more mechanistic approach to assess developmental neurotoxicity.

(4) You argue that *in vitro* approaches (e.g. Zebrafish and or test battery) should be preferred over vertebrate testing.

113 ECHA notes your agreement that generation of additional data may be required. In respect of the arguments you raise, these are not related to the legal condition for triggering the DNT cohort and you did not identify any legal ground for adaptation and therefore your comments must be rejected. For completeness:

(1) according to OECD TG 443, para. 10: "*The choice of species for the reproductive toxicity test should be carefully considered in light of all available information. However, because of the extent of background data and the comparability to general toxicity tests, the rat is normally the preferred species, and criteria and recommendations given in this TG refer to this species. If another species is used, justification should be given and appropriate modifications to the protocol will be necessary*."

In this case, evidence of nervous system activity in humans is an additional basis that the Substance is of particular concern for neurotoxicity.

Further, your conclusions on the rat being inappropriate are not supported by the information you provide, particularly noting (a) the information you have provided, both in human and in rat, does not provide a comprehensive comparison of the dose-dependency and nature of neurotoxic effects between rat and human, in particular you have not addressed the equivalence of doses between species nor the comparability of outcomes between species and so it is not possible to conclude that they have different effects. (b) even if some apical responses are different between human and rat, this difference does not in itself demonstrate that the rat is an inappropriate model species for (developmental) neurotoxicity; key mechanisms of action and neurotoxicity outcomes may still be conserved. (c) you have not demonstrated that inhibition of voltage-gated potassium channels is the sole mode of action of guanidine in the human, not least because Kalia and Swartz studied a *Drosophila* potassium channel, i.e. in a different species, and you have provided only assumptions but no mechanistic information and not demonstrated mode of neurotoxic action in rat.

(2) the DNT cohort is an information requirement. In any case, you have provided only generic considerations and not excluded that there are test laboratories offering the DNT cohorts and able to demonstrate proficiency in the conduct of such studies.

(3) The *in vitro* approaches you identify do not provide the information that would be obtained from the conduct of cohorts 2A and 2B, and you have not demonstrated that these *in vitro* methods would address all possible key events /adverse outcome pathways, nor even explained how these methods would address the key events/ adverse outcome pathways that have already been identified, i.e. inhibition of voltage-gated potassium channels. As there is a data gap for a standard information requirement, ECHA has no discretion but to request the test specified in the legal text (i.e. cohorts 2A and 2B).

(4) Minimisation of vertebrate animal testing is not on its own a legal ground for adaptation under the general rules of Annex XI.

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

7.3.6. Further expansion of the study design

115 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 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.

7.3.7. Your comments on the draft decision

116 In your comments you agree with the request except, in your comments on the Proposal for Amendment, with the DNT cohort as discussed above.

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 (2023).
***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 22 November 2022.

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

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

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. In your comments you request a prolongation of the deadline to allow time for a dose-range finding study and update of the CSR. ECHA notes that time for such steps is already included in the standard deadline. Furthermore, you consider that "*The laboratory capacities to perform an EOGRTs may also be a time-critical factor*". You did however not provide any documentary evidence for this claim. Therefore, the deadline has not been amended.

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-85 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]

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 (https://echa.europa.eu/practical-guides).

(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.
- The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

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).