EUROPEAN CHEMICALS AGENCY

Helsinki, 22 March 2016

Addressee: [redacted]

Decision number: CCH-D-2114321118-60-01/F
Substance name: trisodium 2-(carboxylatomethyl(2-hydroxyethyl)amino)ethyliminodi(acetate)
EC number: 205-381-9
CAS number: 139-89-9
Registration number: [redacted]
Submission number: [redacted]
Submission date: 22 May 2013
Registered tonnage band: 100 to 1000 tonnes

DECISION ON A COMPLIANCE CHECK

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

1. **High-pressure liquid chromatogram, gas chromatogram (Annex VI, Section 2.3.6) of the registered substance;**
   - **Complete chromatogram**
2. **Description of the analytical methods (Annex VI, Section 2.3.7)**
   - **Identification and quantification of the counter-ion**
   - **Description of the analytical protocol and identification and quantification of the main constituent(s) and impurities**
3. **Composition of the substance (Annex VI, Section 2.3.)**
   - **Concentration values**
4. **In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, EU B.13/14 /OECD TG 471) with the registered substance using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102;**
5. **In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2, test method: EU B.10/OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;**
6. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3; test method: EU B.17/OECD TG 476) with the registered substance provided that both studies requested under 4. and 5. have negative results;**
7. **Screening study for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: OECD TG 421 or 422) in rats, oral route with the registered substance;**

8. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2; test method: EU B.26/OECD TG 408) in rats with the registered substance;**

9. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2; test method: EU B.31/OECD TG 414) in rats or rabbits, oral route with the registered substance;**

10. **Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5; test method: Daphnia magna reproduction test, EU C.20/OECD TG 211) with the registered substance;**

11. **Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) with the registered substance;**

12. **Exposure assessment and risk characterisation (Annex I, Sections 5. and 6.) for environment: generate an exposure assessment for all relevant exposure scenarios and revise the risk characterisation accordingly;**

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

You are required to submit the requested information in an updated registration dossier by **29 March 2018**. **You shall also update the chemical safety report, where relevant.** The timeline has been set to allow for sequential testing.

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

Appeal

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

Authorised[1] by Guilhem de Seze, Head of Unit, Evaluation E1

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

Appendix 1: Reasons

IDENTIFICATION OF THE SUBSTANCE

In order to ensure that potential hazardous properties of substance are not underestimated, the information that is necessary to resolve the substance identification deficiencies, below, must be available to you before identifying the test sample to be used for the testing requested in the present decision.

1. **High-pressure liquid chromatogram, gas chromatogram (Annex VI, Section 2.3.6.);**

Pursuant to Article 10(a)(ii) of the REACH Regulation, the technical dossier shall contain information on the identity of the substance as specified in Annex VI, Section 2 of the REACH Regulation. In accordance with Annex VI, Section 2, the information provided shall be sufficient to enable the identification of the registered substance.

"High-pressure liquid chromatogram or gas chromatogram" is a formal information requirement of Annex VI Section 2.3.6. Adequate information needs to be present in the technical dossier for the registered substance to meet this information requirement.

ECHA observes that in your IUCLID dossier, you did not provide any chromatogram or comprehensive report for the chromatographic analysis which is required to establish the identity of the registered substance. As such, there is no chromatographic data on the constituent(s) and impurities that are present in the registered substance. Therefore, it is not possible to verify the reported composition.

ECHA regards this required information scientifically relevant for the registered substance and points out that the identity of the substance cannot be confirmed without providing appropriate chromatographic data in the dossier.

Therefore, you are requested to submit an appropriate chromatographic report including the chromatogram and a peak table containing the retention times, identification of the peaks, peak areas and peak area % of the constituent(s) and impurities. In order to verify the composition of the substance the analytical data must be consistent with the composition reported in IUCLID section 1.2.

As for the reporting chromatographic data in the registration dossier, they should be included in IUCLID section 1.4.

2. **Description of the analytical methods (Annex VI, Section 2.3.7.);**

Pursuant to Article 10(a)(ii) of the REACH Regulation, the technical dossier shall contain information on the identity of the substance as specified in Annex VI, Section 2 of the REACH Regulation. In accordance with Annex VI, Section 2 the information provided shall be sufficient to enable the identification of the registered substance.

"Description of the analytical methods" is an information requirement as laid down in Annex VI, Section 2.3.7. of the REACH Regulation. Adequate information needs to be present in the technical dossier for the registered substance to meet this information requirement. ECHA observes that you did not provide sufficient description of the analytical methods used for the identification and quantification of the constituents and impurities required to be reported in the composition of the registered substance, as requested according to Annex VI section 2.3.7.

More specifically, the following information is not provided in the dossier:

i) Information on the identification and quantification of the counter-ion (sodium-ion);

ii) The description of the analytical methods used for the identification and quantification of the constituent(s) and impurities.

Accordingly, you are required to provide proper information on the identification and quantification of the counter-ion and on the method(s) used to determine the composition of the registered substance, as reported in section 1.2 of the IUCLID dossier.

The description shall be sufficient for the methods to be reproduced and shall therefore as a minimum include details of the followings:

- experimental protocol followed for carrying out the analysis;
- information as to how the analytical data provided translate into the concentration levels of the constituents required to be reported in the composition information, including the calculations;

As for the reporting of the data in the registration dossier, the information should be attached in IUCLID section 1.4.

You shall ensure that the analytical data are consistent with the composition reported in section 1.2 of the IUCLID dossier.

3. Composition of the substance (Annex VI, Section 2.3.);

Pursuant to Article 10(a)(ii) of the REACH Regulation, the technical dossier shall contain information on the identity of the substance as specified in Annex VI, Section 2 of the REACH Regulation. In accordance with Annex VI, Section 2 the information provided shall be sufficient to enable the identification of the registered substance.

Annex VI, section 2.3. of the REACH Regulation requires that each registration dossier contains sufficient information for establishing the composition of the registered substance and therefore its identity.

In that respect, according to chapter 4.3 of the Guidance for identification and naming of substances under REACH and CLP (Version: 1.3, February 2014) – referred to as "the Guidance" thereinafter, you shall note that, for well-defined substances, the following applies:

- Each main constituent (i.e. the constituent present at ≥80% for mono-constituent substance or each constituent present at ≥10% and 80% for multi-constituent substance) shall be identified and reported individually;

- Each impurity present at ≥1% or relevant for the classification and/or PBT assessment of the registered substance shall be identified and reported individually;

- For each constituent, the typical, minimum and maximum concentration levels shall be specified regardless of the substance type;

- The compositional information should be completed up to 100%.

In the present dossier, you identified the registered substance as the well-defined mono-constituent substance "*trisodium 2-(carboxylatomethyl(2-hydroxyethyl)amino)ethyliminodi(acetate)*" and specified for the main constituent a typical concentration of [redacted]% (w/w). Three impurities were reported with typical concentration levels which sum up to [redacted]% (w/w). Consequently, about [redacted]% (w/w) of the composition of the registered substance has not been accounted for. In addition the three impurities were reported without concentration ranges.

ECHA, therefore, concludes that the compositional information has not been provided to the required level of detail for establishing the composition of the registered substance. In addition, due to the missing analytical information for the identification and quantification of the composition, as described in the section above, it is not possible to verify that the composition reported in section 1.2 of the IUCLID dossier is representative of the registered substance.

Accordingly, you are requested to revise the composition of the registered substance providing the missing compositional information. You shall specify the identity and typical, upper and lower concentration level of the constituents, including impurities, required to be reported in the IUCLID dossier.

Regarding how to report the composition of the registered substance in IUCLID, the following applies: you shall report individually any impurity required to be identified and specify at least one of the following identifiers: chemical name, CAS number, EC number and/or molecular formula, as well as the minimum, maximum and typical concentration, in the appropriate fields in section 1.2 of the IUCLID dossier.

Further technical details on how to report the composition of well-defined substances in IUCLID are available in the Data Submission Manual – Part 18: How to report the substance identity in IUCLID 5 for registration under REACH (version: 2.0, July 2012) on the ECHA website.

You shall ensure that the composition is verifiable and therefore supported by analytical methods to be provided in IUCLID section 1.4 as required under Annex VI.2.3.7. of the REACH Regulation.

PROPERTIES OF THE SUBSTANCE

Grouping of substances and read-across approach

In the registration, you have adapted the standard information requirements for:

- *In vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1.);
- *In vitro* cytogenicity study in mammalian cells or *In vitro* micronucleus study (Annex VIII, Section 8.4.2.);
- *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.);
- Screening study for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.);
- Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2);
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2);
- Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5); and
- Long-term toxicity testing on fish (Annex IX, Section 9.1.6)

by applying a read-across adaptation according to Annex XI, Section 1.5 of the REACH Regulation.

Article 13(1) of the REACH Regulation provides that information on intrinsic properties of substances may be generated by means other than tests. Such other means include the use of information from structurally related substances (grouping of substances and read-across), "*provided that the conditions set out in Annex XI are met*".

Annex XI, 1.5. requires a structural similarity among the substances within a group or category such that relevant properties of a substance within the group can be predicted from the data on reference substance(s) within the group by interpolation. Such prediction for properties need to be based on a similar or regular pattern of these properties as a result of the structural similarity. The following analysis presents your justification for the proposed grouping approach and read-across hypothesis, together with ECHA's analysis concerning the justification in both a generic and in property-specific context.

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

You have summarised the read-across approach in your technical dossier as follows: "*The substance considered for use as surrogates in read-across for the registration of HEDTA are the sodium salts of EDTA. These substances are considered to be sufficiently similar to HEDTA to supplement the registration of HEDTA under REACH where environmental fate and (eco)toxicological data are unavailable for the registered compound. Both compounds are chelating agents and are expected to display similar effects on biological systems based upon their structural similarities and their comparable physical chemistry. As a result, the toxicological data on the surrogate are considered appropriate for use in a read-across manner when registering HEDTA under REACH. Based on the chemical structure and consideration of the ionisation and binding potential of the compounds it is likely that HEDTA will behave similarly to EDTA with respect to toxicokinetics, environmental fate and (eco)toxicology.*"

B. Information submitted by the Registrant to support the grouping approach and read-across hypothesis

ECHA understands that the read-across hypothesis is based on the assumption that source and target substances are expected to have similar effects and that the (eco)toxicological properties (for the endpoints listed above) can be predicted from the proposed source substances on this basis.

You have provided a read-across justification document in IUCLID section 13. The document provides a comparision of the chemical stuctures of the proposed analogues with the registered substance and outlines the reasoning behind selecting the analogues. It also provides a side-by-side comparison of the physical and chemical properties of the substances. This includes ionisation and binding potential for all substances up to pH 12. There is no information available for the registered substance for absorption, metabolism, distribution or elimination. The proposed source substances are different EDTAs ($CaNa_2$ and Na_2 salts) that are absorbed across the gastrointestinal tract (1-18% in rats; <5% in humans). EDTA salts are claimed not to undergo biotransformation and are, thus, excreted unchanged in urine. Using the OECD QSAR toolbox both EDTA and the registered substance are predicted not to be metabolised in the liver.

A summary of the available toxicological studies in support of human health effects is provided. For the registered substance there are several toxicological studies provided in the technical dossier. You also state that "*However, the majority of these studies have a Klimish rating of 3 or 4, largely due to the small number of test animals, the unknown purity of the compound under study, and the age of the studies. Many of the studies are pre-GLP and the adherence to a standard protocol is considered to be unreliable*". You claim that these studies can serve to demonstrate similarities with the proposed source studies (on EDTA salt) despite the fact that the studies on the registered substance were not considered "*to be sufficiently rigorous to indicate the toxicity of HEDTA*".

C. ECHA analysis of the grouping approach and read-across hypothesis in light of the requirements of Annex XI, 1.5.

ECHA notes that you have sufficiently characterised the proposed source substances. You have clearly indicated the structural similarities beween the proposed source substances and the registered substance. Furthermore, ECHA notes that the registered substance differs from the source substance due to the presence of a hydroxyethyl group rather than an acetate group on one of the nitrogen atoms. Moreover, ECHA understands that you base your read-across hypothesis on the fact that both sources and target substances are, despite the identified structural differences, likely to cause the same effects.

You present the following arguments to support the read-across approach:

i. Both the registered substance and the proposed source substances are ionised at physiological pHs, thus, all substances are likely to be poorly absorbed. There is no information available on the absorption of the registered substance. However, the EDTA salts show low absorption.
ii. The QSAR Toolbox predicts that none of the substances are metabolised in the liver. This is supported by limited toxicokinetics information on the proposed analogue substances. No toxicokinetics information is available for the registered substance.

iii. You claim that the available (eco)toxicological data on the registered substance (despite the deficiencies listed above) demonstrate similarities with the available information on the source substances and therefore the *"data for EDTA may justifiably be used"* when there is no reliable information on the registered substance.

ECHA makes the following observations:

i. ECHA notes that both source and target substances may have low absorption. However, the proposed source substances are absorbed and do exhibit toxicity. In these circumstances, low absorption cannot be used to predict toxicity.

ii. The available information indicates that the proposed source substances are not metabolised. The results from the OECD QSAR Toolbox claim to support (for liver metabolism) this notion also for the registered substance. However, ECHA notes that you have not provided the detailed QSAR predictions to support this claim. Thus, ECHA is unable to assess the validity of this claim. In addition, ECHA notes that metabolism occurs also in other tissues than liver.

iii. ECHA notes that there are four reliable studies conducted using the registered substance available in the technical dossier: Acute toxicity by the inhalation route, skin irritation in rabbits, eye irritation in rabbits, *In vitro* gene mutation study in bacteria using four bacterial strains. The results available for the proposed source substances are similar for these endpoints. However, ECHA notes that none of the available studies address repeated dose toxicity or toxicity to reproduction. Hence, there is no information available from repeated dose toxicity studies which allow comparison of systemic toxicity. You have not explained as to how and why these studies lead to the conclusion that the toxicological properties of the registered substance (for each concerned endpoint), in qualitative and quantitative terms, can be accurately predicted from the information available on the proposed source substances. Therefore, ECHA concludes that the argument of similar toxicity is not supported and as a consequence cannot be considered as a reliable basis for the prediction.

iv. In the technical dossier, you have indicated that all data on the registered substance (with the exception of what is listed in the point above) is either '*not reliable*' or that reliability is '*not assignable*'. Despite this, you argue that this information is sufficient to support the read-across approach. ECHA notes that you have not explained as to how and why these studies (despite the identified deficiencies and questionable reliability of the studies) can lead to the conclusion that the toxicological properties of the registered substance (for each concerned endpoint), in qualitative and quantitative terms, can be accurately predicted from the information available on the proposed source substances. Therefore, ECHA concludes that the argument of similar toxicity is not supported and as a consequence cannot be considered as a reliable basis for the prediction.

v. Concerning aquatic toxicity, you use data from analogue substances Na2-EDTA and CaNa2-EDTA to predict the long-term effects for *Daphnia* and fish, respectively. From the short-term data for Na2-EDTA and the registered substance, there seems to be some evidence that the proposed read-across for aquatic invertebrates may be plausible. However, you do not explain why the short-term fish study for Na2-EDTA indicates that it is less toxic than the target (registered) substance, nor do you explain why the provided data indicates that the toxicity is not logKow-driven.

Additionally, there is some evidence from the provided bioaccumulation data that the proposed read-across approach for the aquatic toxicity endpoints may not be conservative. ECHA also notes that your argumentation lacks a justification for reading across from one salt to the other one (e.g. from calcium to sodium), as there is remaining uncertainty related to this as, for instance, the calcium will bond to two acetate anions which can change the stereochemistry and, thus, bioavailability of the substance.

D. Conclusion on the read-across approach

ECHA considers that, for the reasons presented above, you have failed to explain as to how and why, in qualitative and quantitative terms, the (eco)toxicological properties of the registered substance can be accurately predicted by using the available infromation from the proposed source substances.

Pursuant to Article 41(1) of the REACH Regulation, ECHA concludes that the adaptation of the standard information requirements for the endpoints *In vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1.), *In vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.) or *In vitro* micronucleus study (Annex VIII, Section 8.4.2.), *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.), Screening study for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.), Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2), Pre-natal developmental toxicity study (Annex IX, Section 8.7.2), Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5) and Long-term toxicity testing on fish (Annex IX, Section 9.1.6) in the technical dossier based on the proposed read-across approach does not comply with the general rules of adaptation as set out in Annex XI, 1.5. Therefore, ECHA rejects all adaptations in the technical dossier that are based on Annex XI, 1.5.

4. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(d) and 13(4) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.

An "*In vitro* gene mutation study in bacteria" is a standard information requirement as laid down in Annex VII, Section 8.4.1. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided the following information:

i. Key study; Experimental result; on the registered substance, 1990, reliability 1 (reliable without restrictions); non-GLP; Guideline: according to OECD TG 471 (Bacterial Reverse Mutation Assay) with registered substance; Strains: *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100. Your conclusion: 'Negative with and without metabolic activation'.
ii. Supporting study; read-across from supporting substance (structural analogue or surrogate); 1985, reliability 2 (reliable with restrictions); non-GLP; ; Guideline: according to OECD TG 471 (Bacterial Reverse Mutation Assay); Test material: trisodium [{2-[bis(carboxylatomethyl)amino]ethyl}(2-hydroxyethyl)amino]acetate, CAS No 139-89-9; Strains: *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100. Your conclusion: 'Negative with and without metabolic activation'.

According to Article 13(3) of the REACH Regulation, tests required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods recognised by the Commission or ECHA.

Other tests may be used if the conditions of Annex XI are met. More specifically, Section 1.1.2 of Annex XI provides that existing data on human health properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3) may be used if the following conditions are met:

(1) Adequacy for the purpose of classification and labelling and/or risk assessment;
(2) Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3);
(3) Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3) if exposure duration is a relevant parameter; and
(4) adequate and reliable documentation of the study is provided.

ECHA notes that both studies provided above where conducted using the same four strains of *S. typhimurium*.

According to paragraph 13 of the current OECD TG 471 test guideline (updated 1997) at least five strains of bacteria should be used. These should include four strains of *S. typhimurium* (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by *E.coli* WP2 strains or *S. typhimurium* TA102 which have an AT base pair at the primary reversion site.

The key study you have provided is from the year 1990 according to OECD TG 471 and GLP with an assigned reliability score of 1. The test used four different strains of *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100. However, since the test was conducted, significant changes have been made to OECD TG 471 and this means that the key study does not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation. Because, the study was not conducted using one of the strains designed to detect cross-linking agents (*i.e. E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102) it cannot be cosidered to have adequate and reliable coverage of the key paramenters forseen to be investigated in the OECD TG 471.

Furthermore, ECHA notes that you have provided a supporting study, point ii) above, based on a read-across adaptation. As explained in the section '*Grouping of substances and read-across approach*' of this decision, your read-across approach cannot be accepted. Therefore, your adaptation of the information requirements cannot be accepted .

ECHA concludes that a test using *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 has not been submitted and that the test using one of these is required to conclude on *in vitro* gene mutation in bacteria.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471) using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.

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

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(d) and 13(4) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.

An "*In vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study" is a standard information requirement as laid down in Annex VIII, Section 8.4.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided the following information:

i. Key study; read-across from supporting substance (structural analogue or surrogate); 1984, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of the test Principle of test: 'in vitro mammalian chromosome aberration test'; 'NTP-Standard Protocol'); Test material: trisodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 150-38-9. Your conclusion: 'Negative'.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation. However, as explained in the section '*Grouping of substances and read-across approach*' of this decision, your adaptation of the information requirement cannot be accepted.

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

ECHA considers that the *in vitro* mammalian chromosome aberration test (test method EU B.10./OECD TG 473) and the *in vitro* micronucleus test (OECD TG 487) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.2. of the REACH Regulation.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* cytogenicity study in mammalian cells (test method: EU B.10./OECD TG 473) or *in vitro* mammalian cell micronucleus study (test method: OECD TG 487).

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

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(d) and 13(4) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.

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

In the technical dossier you have provided the following information:

i. Key study; read-across from supporting substance (structural analogue or surrogate); 1984, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of the test Principle of test: 'mammalian cell gene mutation assay'; 'NTP-Standard Protocol'); Test material: trisodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 150-38-9. Your conclusion: 'Negative'.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation. However, as explained in the section *'Grouping of substances and read-across approach'* of this decision, your adaptation of the information requirement cannot be accepted.

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

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian cell gene mutation test (test method: EU B.17./OECD TG 476) provided that both studies requested under 4. and 5. have negative results.

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

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(d) and 13(4) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.

"Screening for reproductive/developmental toxicity" is a standard information requirement as laid down in Annex VIII, Section 8.7.1. of the REACH Regulation if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from *in vitro* methods that the substance may be a developmental toxicant. No such evidence is presented in the dossier. Therefore, adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided the following information:

i. Key study; read-across from supporting substance (structural analogue or surrogate); 1963, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of test: 'In a 2 year feeding study on Wistar rats including reproductive and lactation experiments in four successive generations groups of 25 male and 25 female animals were exposed to CaNa2EDTA at dietary levels providing daily doses of approximately 50, 125, and 250 mg/kg bw '); Test material: calcium disodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 62-33-9. Your conclusion: NOAEL (P, F1, F2, F3) >= 250 mg/kg/day.

ii. Supporting study; read-across from supporting substance (structural analogue or surrogate); 1964, reliability 3 (not reliable); non-GLP; non-Guideline (Deficiencies: 'significant methodological deficiencies (small number of animals, no data on the second generation given, documentation insufficient, no results for the second generation reported'); Test material: disodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 139-33-3; N = 4 females and 2 males.

iii. Supporting study; read-across from supporting substance (structural analogue or surrogate); 1991, reliability 4 (not assignable); non-GLP; non-Guideline (Deficiencies: '*Groups of male mice were administered (oral) distilled water (control) or EDTA dissolved in distilled water for 5 consecutive days. The animals were killed at 1, 3, 5 and 7 wk after the end of treatment. After recording the fresh weights of the epididymides and testes, the tissues were fixed and processed for histological examination. The sperm count was determined and sperms were scored for abnormal sperm-heads. A minimum number of 1000 sperms were analysed per animal.*'); Test material: disodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 139-33-3. Your conclusion: no effects at highest dose tested (15 mg/kg/day).

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation. However, as explained in the section '*Grouping of substances and read-across approach*' of this decision, your adaptation of the information requirement cannot be accepted. Therefore, there is no need to assess the acceptability of the submitted studies further. ECHA has therefore not assessed if the provided studies meet the requiremnts of Annex XI, Section 1.1.2.

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

According to the test methods OECD TG 421/422, the test guideline is designed for use with the rat and the substance is administered orally unless other routes of administration are considered more appropriate. ECHA considers these default parameters appropriate and testing should be performed on the rat by the oral route.
Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit information derived with the registered substance subject to the present decision: Reproductive/developmental toxicity screening test (test method: OECD TG 421) in rats by the oral route; or Combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test (test method: OECD TG 422) in rats by the oral route.

Notes for your considerations

For the selection of the appropriate test, please consult ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, section R.7.5 and 7.6 (version 4.0, July 2015).

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

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(d) and 13(4) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.

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

In the technical dossier you have provided the following information:

i. Key study; read-across from supporting substance (structural analogue or surrogate); 1977, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of test: 'A bioassay for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats'); Test material: trisodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 150-38-9; Doses: 0; 250 and 500 mg/kg/day in feed; Duration: chronic 103 weeks. Your conclusion: NOAEL male/female >= 500 mg/kg/day (based on no effects).
ii. Key study; read-across from supporting substance (structural analogue or surrogate); 1970, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of test: '13 weeks feeding study on rats'); Test material: disodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 139-33-3; Doses: 0; 500, 2500 and 5000 mg/kg/day in feed. Your conclusion: NOAEL male/female >= 500 mg/kg/day.
iii. Supporting study; read-across from supporting substance (structural analogue or surrogate); 1977, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of test: 'A bioassay for possible carcinogenicity was conducted by administering the test material in feed to B6C3F1 mice); Test material: trisodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 150-38-9; Doses: 0; 469 and 938 mg/kg/day in feed; Duration: chronic 103 weeks. Your conclusion: NOAEL male/female >= 938 mg/kg/day (based on no effects).
iv. Supporting study; read-across from supporting substance (structural analogue or surrogate); 1980, reliability 4 (not assignable); non-GLP; non-Guideline (Principle of test: '1 months feeding study on rats'); Test material: disodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo). Your conclusion: NOAEL male/female 1125 mg/kg/day.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation. However, as explained in the section *'Grouping of substances and read-across approach'* of this decision, your adaptation of the information requirement cannot be accepted. Therefore, there is no need to assess the acceptability of the submitted studies further. ECHA has therefore not assessed if the provided studies meet the requiremnts of Annex XI, Section 1.1.2.

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

ECHA has evaluated the most appropriate route of administration for the study under consideration according to REACH Annex IX, Section 8.6.2. and its column 2 provisions. Based on the information provided in the technical dossier and the chemical safety report the conditions for testing by the dermal route are not met. Furthermore, the properties of the registered substance, its uses and information on toxicity, indicate that human exposure by the inhalation route seems to be less likely. Hence, ECHA considers that the default oral route is the most appropriate route of administeration.

According to the test method EU B.26/OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Repeated dose 90-day oral toxicity study (test method: EU B.26./OECD TG 408) in rats.

9. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(d) and 13(4) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.

A "pre-natal developmental toxicity study" is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided the following information:

i. Key study; read-across from supporting substance (structural analogue or surrogate); 1981, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of test: '*EDTA and four of its salts, disodium, trisodium, calcium di-sodium, and tetrasodium edetate, were studied for teratogenic potential in rats. Equimolar doses based on 1000 mg/kg were given by gastric intubation on Days 7 to 14 of gestation. On day 21 of gestation the dams of each group were sacrificed and litter data for each dam collected*'); Test material: tetrasodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 64-02-8; Doses: 0; 250 and 500 mg/kg/day in feed. Your conclusion: LOAEL maternal toxicity 1374 mg/kg/day; NOAEL teratogenicity >= 1374 mg/kg/day.

ii. Supporting study; read-across from supporting substance (structural analogue or surrogate); 1977, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of test: subcutaneous, diet and gavage administration, rat, gestation day 7-14); Test material: disodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 139-33-3; Doses: 375 mg/kg/day (subcutaneous); 954 mg/kg/day (in diet); 1250 and 1500 mg/kg/day (gavage). Your conclusion: 'No NOAEL identified'.

iii. Supporting study; read-across from supporting substance (structural analogue or surrogate); 1971, reliability 2 (reliable with restrictions); non-GLP; non-Guideline (Principle of test: diet administration, rat, gestation day 0-21 in total 21, 8 or 15 days); Test material: disodium 2,2',2'',2'''-(ethane-1,2-diyldinitrilo)tetraacetate, CAS No 139-33-3; Doses: 1000 and 1500 mg/kg/day. Your conclusion: No NOAEL reported.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation. However, as explained in the section '*Grouping of substances and read-across approach*' of this decision, your adaptation of the information requirement cannot be accepted. Therefore, there is no need to assess the acceptability of the submitted studies further. ECHA has therefore not assessed if the provided studies meet the requiremnts of Annex XI, Section 1.1.2.

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

According to the test method EU B.31/OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default consideration, ECHA considers testing should be performed with rats or rabbits as a first species.

According to the test method EU B.31/OECD TG 414, the test substance is usually administered orally. On the basis of this default consideration, ECHA considers testing should be performed by the oral route.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a first species (rats or rabbits) by the oral route.

10. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(d) and 13(4) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.
"Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex IX, Section 9.1.5. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement. Column 2 of Annex IX, Section 9.1. specifies that long-term aquatic toxicity testing shall be proposed by the Registrant if the chemical safety assessment according to Annex I indicates the need to investigate further effects on aquatic organisms. The choice of the appropriate test(s) will depend on the results of the chemical safety assessment.
In the technical dossier you have provided an experimental study according to EEC Guideline XI/681/86, Draft 4: "Prolonged toxicity study with *Daphnia* magna: Effects on reproduction" performed with the analogue substance Na2-EDTA (CAS No 139-33-3) and a 21 day NOEC value of 25 mg/L (based on reproduction, was estimated.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation. However, as explained in the section *'Grouping of substances and read-across approach'* of this decision, your adaptation of the information requirement cannot be accepted. Furthermore, in the absence of quantitative exposure assessment and risk characterisation for the aquatic compartment, ECHA cannot, with the information currently available in the dossier, establish whether REACH Annex IX, 9.1, Column 2 specific rules for adaptation apply for the registered substance.

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

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *Daphnia magna* reproduction test (test method: EU C.20./OECD TG 211).

11. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(d) and 13(4) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.

In the technical dossier you have provided an experimental study according to OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test) performed with the analogue substance CaNa2-EDTA (CAS No 62-33-9) and a 35 day NOEC value of >= 25.7 mg/L was estimated.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation. However, as explained in the section *'Grouping of substances and read-across approach'* of this decision, your adaptation of the information requirement cannot be accepted. Furthermore, in the absence of quantitative exposure assessment and risk characterisation for the aquatic compartment, ECHA cannot, with the information currently available in the dossier, establish whether REACH Annex IX, 9.1, Column 2 specific rules for adaptation apply for the registered substance.

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

Regarding the long-term toxicity testing on fish pursuant to Annex IX, section 9.1.6.1, ECHA considers that the FELS toxicity test according to OECD TG 210 is the most sensitive of the standard fish tests available as it covers several life stages of the fish from the newly fertilised egg, through hatch to early stages of growth and should therefore be used (see ECHA Guidance on information requirements and chemical safety assessment (version 2.0, November 2014), Chapter R7b, Figure R.7.8-4). The test method OECD TG 210 is also the only suitable test currently available for examining the potential toxic effects of bioaccumulation (ECHA Guidance Chapter R7b, version 2.0, November 2014). For these reasons, ECHA considers the FELS toxicity test using the test method OECD TG 210 as appropriate and suitable.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Fish, early-life stage (FELS) toxicity test (test method: OECD TG 210).

Notes for your consideration

Before conducting any of the tests mentioned above in points 10-11 you shall consult the ECHA Guidance on information requirements and chemical safety assessment (version 2.0, November 2014), Chapter R7b, Section R.7.8.5 to determine the sequence in which the aquatic long-term toxicity tests are to be conducted and the necessity to conduct long-term toxicity testing on fish.

According to ECHA Guidance on information requirements and chemical safety assessment (version 2.0, November 2014), Chapter R7b (Section R.7.8.5., including Figure R.7.8-4), if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both. In such case, according to the integrated testing strategy, the *Daphnia* study is to be conducted first. If based on the results of the long-term *Daphnia* study and the application of a relevant assessment factor, no risks are observed (PEC/PNEC<1), no long-term fish testing may need to be conducted. However, if a risk is indicated, the long-term fish study needs to be conducted.

12. Exposure assessment and risk characterisation (Annex I, Sections 5. and 6.) for environment

Pursuant to Articles 10(b) and 14(1) of the REACH Regulation, the registration shall contain a chemical safety report (CSR) which shall document the chemical safety assessment (CSA) conducted in accordance with Article 14(2) to (7) and with Annex I of the REACH Regulation.

Pursuant to Article 14(4), if the substance fulfils the criteria for any of the hazard classes listed in that provision or is assessed to be a PBT or vPvB, the CSA shall include exposure assessment and risk characterisation.

Annex I section 5 of the REACH Regulation requires the Registrant to generate exposure scenarios and exposure estimations for the registered substance. The exposure assessment shall consider all stages of the life-cycle of the substance resulting from the manufacture and identified uses and shall cover any exposures that may relate to the identified hazards.

Annex I section 6 of the REACH Regulation requires the Registrant to characterise the risk for each exposure scenario and shall consider the human population (exposed as workers, consumer or indirectly via the environment and if relevant a combination thereof) and the environmental spheres for which exposure to the substance is known or reasonable foreseeable, under the assumption that the risk management measures described under exposure scenario in the Section 5 have been implemented. In addition, the overall environmental risk caused by the substance shall be reviewed by integrating the results for the overall releases, emissions and losses from all sources to all environmental compartments.

ECHA's Guidance on information requirements and chemical safety assessment, Part B: Hazard Assessment, Section B.8.4. (pages 47 to 48) (version 2.1, December 2011) states that "*if no adverse effects have been observed in studies at the highest recommended concentration/doses tested, this would normally indicate that no hazard has been identified and no DNEL or PNEC can be derived and hence exposure assessment for that route of exposure, type of effect or protection target would not be needed*".

In the CSR you provided, the exposure assessment for the environment is missing. You claimed that no exposure assessment is necessary for the environment by stating that "*The substance is not classified as hazard to the aquatic environment, i.e. hazard phrases such as H412, H411, H410, H400 or H413 are not assigned (refer to section 3 "Classification and Labelling" and section 7 "Environmental Hazard Assessment" of this CSR). Furthermore HEDTA is not a PBT or vPvB (refer to section 8 "PBT and vPvB Assessment" of this CSR).*

With respect to the terrestrial compartment, due to the ionic structure under environmentally relevant pH conditions, no adsorption onto the organic fraction of soil or sediments is expected (refer to chapter 4.2 "Environmental distribution" of this CSR). Concerning exposure of the atmospheric compartment, there would be no emission into the atmosphere because of the very low vapour pressure of the substance (1.39E-013 Pa) [refer to section 4 of this CSR ("Environmental Fate Properties").

HEDTA is considered inherently biodegradable and its bioaccumulation is not expected (due to an estimated Log Kow of -11.35 and BCF of 3.2 [refer to section 4 "Environmental Fate Properties" of this CSR). As a consequence biomagnification via the food chain is very unlikely to occur. Furthermore, the substance is not classified as H373, H372, H361, H362. Therefore exposure assessment regarding secondary poisoning is not required. Consequently risks to the environment during the entire life cycle of the substance are considered to be controlled."

ECHA notes that you have classified the substance as Acute Tox. 4 (H302), Eye Damage 1 (H318) and Eye Irrit. 2 (H319) and thus, fulfilling the criteria set out in Article 14(4) of the REACH Regulation to require an exposure assessment and a risk characterisation in the chemical safety assessment.

Additionally, ECHA notes that effects were observed in some environmental toxicity studies. In particular, in the toxicity to aquatic algae and cyanobacteria IUCLID endpoint (6.1.5) a 48h EC50 value of 4.4 mg/L (act. ingr. at 41%) based on growth rate was obtained for the registered substance.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to generate an environmental exposure assessment for all relevant exposure scenarios and revise the risk characterisation accordingly.

Appendix 2: Procedural history

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

The compliance check was initiated on 5 October 2015.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation

ECHA notified you of the draft decision and invited you to provide comments. ECHA did not receive any comments by the end of the commenting period.

ECHA notified the draft decision to the competent authorities of the Member States for proposal(s) for amendment(s).

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

Appendix 3: Further information, observations and technical guidance

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

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will eventually result in a notification to the enforcement authorities of your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new test(s) must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants. It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new test(s) is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new test(s) must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the test(s) to be assessed.