EUROPEAN CHEMICALS AGENCY

Helsinki, 14 February 2020

Addressee: [redacted]

Decision number: CCH-D-2114495838-25-01/F
Substance name: [[(phosphonomethyl)imino]bis[ethane-2,1-diylnitrilobis(methylene)]]tetrakisphosphonic acid
EC number: 239-931-4
CAS number: 15827-60-8
Registration number: [redacted]
Submission number: [redacted]
Submission date: 29/03/2011
Registered tonnage band: Over 1000

DECISION ON A COMPLIANCE CHECK

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

1. **Composition of the substance (Annex VI, Section 2.3.) as explained in Appendix 1, Section 1;**

2. **In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum using Sodium salts of [[(phosphonomethyl)imino]bis[ethane-2,1 diylnitrilobis(methylene)]]tetrakisphosphonic acid (5-7Na:1) (EC No. 701-216-4; hereafter DTPMP, 5-7 Na-salt);**

3. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2; test method: EU B.31/OECD TG 414) in rabbits, oral route with the DTPMP, 5-7 Na-salt;**

4. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the with the DTPMP, 5-7 Na-salt specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce systemic toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;**
   - **Cohorts 2A and 2B (Developmental neurotoxicity); and**
   - **Cohort 3 (Developmental immunotoxicity).**

You have to submit the requested information in an updated registration dossier by ***22 May 2023***. You shall also update the chemical safety report, where relevant. The deadline has been set to allow for sequential testing.

Appeal

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

Authorised[1] by Ofelia Bercaru, Head of Unit, Hazard Assessment

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

Appendix 1: Reasons

SUBSTANCE IDENTITY

1. 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 contain sufficient information for establishing the composition of the registered substance and therefore its identity.

In that respect, according to chapter 4.2 of the Guidance for identification and naming of substances under REACH and CLP (Version: 2.4, February 2017) – 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; and

- 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 and impurity, the typical, minimum and maximum concentration levels shall be specified regardless of the substance type.

The substance composition corresponds to the chemical representation of what the substance consists of and is therefore an essential part of substance identification and the corner stone of all the REACH obligations.

In the present dossier, you reported in the composition included in section 1.2 of the IUCLID dossier the following one main constituent, impurities and concentrations:

[redacted]

[redacted]

The minimum and maximum concentration levels of the impurities reported in section 1.2 have not been specified. This information is necessary to understand the variability in the composition of the registered substance for the purpose of determining substance identity.

ECHA therefore concludes that the compositional information has not been provided to the required level of detail.

You are accordingly requested to provide the missing information on the composition of the registered substance and especially the minimum and maximum concentration levels that are not reported.

The concentration range values must be representative for the registered substance as manufactured.

Regarding how to report the composition of the registered substance in IUCLID, the following applies: You shall report individually any constituent or 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 a description of the analytical methods for the identification and quantification of the constituents and impurities required to be reported, as required under Annex VI.2.3.7. of the REACH Regulation. The description shall be sufficient for the methods to be reproduced and shall therefore include details of the experimental protocol followed, any calculation made and the results obtained.

In your comments to the draft decision you acknowledged the need to update the compositional information of the Substance. You included in your comments a table describing the boundary composition of the Substance, as well as you own legal entity composition with concentration ranges. You indicated that revision of the analytical characterisation of the Substance is ongoing and the newly generated information will be submitted.

ECHA understands that you agree to the request in the decision and will provide the requested information. At this point ECHA cannot confirm whether the substance identity information submitted in the comments reflects the actual composition of the Substance. This will be verified once the revised analytical information becomes available.

TOXICOLOGICAL PROPERTIES

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information

specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

You seek to adapt the following standard information requirements with the adaptation arguments which are based on a grouping and read-across approach in accordance with Annex XI, Section 1.5. of the REACH Regulation:

- Mutagenicity (Annexes VII, VIII, and IX, Section 8.4.);
- Pre-natal developmental toxicity study (Annex X, Section 8.7.2); and
- Extended one-generation reproductive toxicity study (EOGRTS; Annex X, Section 8.7.2).

You have applied two different grouping and read-across approaches for the standard information requirements of mutagenicity and then of developmental and reproductive toxicity. ECHA has considered first the scientific and regulatory validity of your grouping and read-across approaches before addressing the individual endpoints (section 1 to 3).

Grouping of substances and read-across approach

General considerations

According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category.

Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach). ECHA considers that the generation of information by such alternative means should offer equivalence to prescribed tests or test methods.

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

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

The ECHA Read-across assessment framework foresees that there are two options which may form the basis of the read-across hypothesis[2, 3] - (1) (Bio)transformation to common compound(s)- the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed and (2) Different compounds have the same type of effect(s)- the read-across hypothesis is that the organism is exposed to different compounds which have similar (eco)toxicological and fate properties as a result of structural similarity (and not as a result of exposure to common compounds).

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

A. Scope of the category with regard to mutagenicity

A.1. Your description of the grouping

In your registration dossier you have formed a group (category) of 'DTPMP'. You identify the members of the DTPMP category on the front page of the CSR and have provided a read-across documentation in section 1.4.1. of the the CSR.

For the purpose of this decision, the following abbreviations are used for DTPMP category members you identified:

DTPMP acid	[[(phosphonomethyl)imino]bis[ethane-2,1-diylnitrilobis(methylene)]]tetrakisphosphonic acid (EC No. 239-931-4);
DTPMP acid, pH 1.5-3	Reaction products of diethylene triamine penta(methylene phosphonic acid) and sodium hydroxide at ph 1.5-3 (EC No. 244-751-4); [4]
DTPMP, 5 Na-salt	Pentasodium pentahydrogen[[(phosphonatomethyl)imino]bis[ethane-2,1-diylnitrilobis(methylene)]]tetrakisphosphonate (EC No. 263-212-4);
DTPMP, 7 Na-salt	Heptasodium trihydrogen [[bis[2-[bis(phosphonatomethyl)amino]ethyl]amino]methyl]phosphonate (EC No. 268-990-9); [5]
DTPMP, 1-3 Na-salt	Sodium salts of [[(phosphonomethyl)imino]bis[ethane-2,1-diylnitrilobis(methylene)]]tetrakisphosphonic acid (1-3 Na:1) (EC No. 701-215-9);
DTPMP, 5-7 Na-salt	Sodium salts of [[(phosphonomethyl)imino]bis[ethane-2,1-diylnitrilobis(methylene)]]tetrakisphosphonic acid (5-7:1) (EC No. 701-216-4).

You provide the following reasoning for the grouping the substances in DTPMP category: "*The category hypothesis is that all the members are various ionised forms of the same parent acid.*"

[2] Read-Across Assessment Framework (RAAF). 2017 (March) ECHA, Helsinki. 60 pp. Available online: Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across)
[3] Read-across assessment framework (RAAF) - considerations on multi-constituent substances and UVCBs. 2017 (March) ECHA, Helsinki. 40 pp. Available online: https://echa.europa.eu/publications/technical-scientific-reports
[4] The registration for this substance no longer exists. Due to the EC number adaptation it was split into registration for DTPMP, 1-3 Na-sat (EC No. 701-215-9) and for DTPMP, 5-7 Na-salt (EC No. 701-216-4).
[5] This substance is not yet registered.

You define the the structural basis for the grouping as all sodium, potassium and ammonium salts of diethylene triamine penta(methylene phosphonic acid).

A.2. ECHA's analysis of the grouping

According to the ECHA Guidance on information requirements and chemical safety assessment Chapter R.6.2, Section R.6.2.4.1, (version 1.0, May 2008) a category hypothesis should address "*the set of inclusion and/or exclusion rules that identify the ranges of values within which reliable estimations can be made for category members for the given endpoint. These rules, can be described as the applicability domain for an endpoint and provide a means of extending the category membership to chemicals not explicitly included in the current definition of a category.*"

Furthermore, according to the ECHA Guidance on information requirements and chemical safety assessment Chapter R.6.2, Section R.6.2.1.2, (version 1.0, May 2008) "*a category evaluation does not necessarily result in all the individual substances included in the category evaluation being registered to the Agency, although the data from these substances will be included in the category report in support of the registration.*"

Based on your description of the structural basis of your grouping/category approach, ECHA understands that all category members are sodium, potassium and ammonium salts of DTPMP acid.

ECHA considers your category as well defined with clear inclusion/exclusion criteria for category membership. The grouping approach is acceptable because the category members are various sodium, potassium and ammonium salts of DTPMP acid. ECHA assessed your proposed predictions on this basis.

B. Prediction of mutagenic properties

B.1. Your category hypothesis and information you provided

You have provided the following reasoning for the prediction of toxicological properties: "*The different salts are prepared by neutralising the acid to a specific pH and accordingly the constituents proportions and degree of ionisation are comparable between substances under similar conditions (in vivo and in the environment). All category members are based on the DTPMP structure. Data are available for the acid form and some salts. DTPMP category members are marketed as neutralized and acid aqueous solutions, and the acid is also available as a solid. The properties of the members of the category are consistent across all endpoints.*"

ECHA understands that you base your hypothesis on the fact that all substances will convert into the same DTPMP anion at physiological conditions, and as a result all substances will have the same toxicological properties.

You have provided the following genotoxicity studies in the technical dossiers of the category members:

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

(1) Key study ([redacted] 2003), reliability 1 (Reliable without restrictions), experimental result on DTPMP acid, non-GLP, non-Guideline (Principle of the test: similar to OECD 471; Deviations: only duplicate plates), *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and *E. coli* WP2 uvrA, Purity 11% of the substance. Your conclusion:

Negative with and without metabolic activation;

(2) Supporting study ([redacted] 1981), reliability 2 (Reliable with restrictions), experimental result on DTPMP acid, non-GLP, non-Guideline (Principle of the test: similar to OECD 471; Deviations: no strains to detect crosslinking agents), purity 50% of the substance, *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100. Your conclusion: Negative with and without metabolic activation;

(3) Supporting study ([redacted] 1977), reliability 4 (not assignable), experimental result on DTPMP acid, non-GLP, non-Guideline (Principle of the test: similar to OECD 471; Deviations: no strain capable of detecting cross-linking agents was included; test concentration intervals of x10 used; incomplete set of positive control substances used), Analytical purity: no data [but presumably not 100% of the substance], *S. typhimurium* TA 1538. Your conclusion: Negative with and without metabolic activation;

(4) Key study ([redacted] 2001), reliability 1 (Reliable without restrictions), experimental result on DTPMP acid, pH 1.5-3, GLP, Guideline (according to OECD 471), *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and *E. coli* WP2 uvrA, Purity 23.7% of the substance. Your conclusion: Negative with and without metabolic activation;

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

(5) Key study ([redacted] 2001), reliability 1 (Reliable without restrictions), experimental result on DTPMP acid, pH 1.5-3, GLP, Guideline (equivalent to OECD 473), Purity 23.7% of the substance. Your conclusion: Positive "*a dose related increase in the number of cells with aberrations was observed after 48 hours treatment*".

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

(6) Key study ([redacted] 1984), reliability 2 (Reliable with restrictions), experimental result on DTPMP acid, GLP, Guideline (equivalent to OECD Guideline 476; Deviations: "*Submission substance is [[(Phosphonomethyl)imino]bis[ethane-2,1-diylnitilobis(methylene)]]tetrakisphosphonic acid (DTPMP). Test substance for this study was Dequest 2060, but concentration of submission substance not given. Concentrations tested presumably refer to Dequest 2060 not to submission substance. Assuming a concentration of DTPMP of 14.5%, the highest tested concentration of 8 mg Dequest 2060/ml corresponds to 1.2 mg DTPMP/ml, which is below the maximum required by the guideline of 5 mg/ml.*"). Your conclusion: Negative with and without metabolic activation;

(7) Supporting study ([redacted] 1983), reliability 2 (Reliable with restrictions), experimental result on DTPMP acid, GLP, Guideline (equivalent to OECD Guideline 476; Deviations: "*not tested without metabolic activation*"), Purity 50% in water, Your conclusion: Positive with metabolic activation;

(8) Supporting study ([redacted] 1982), reliability 1 (Reliable without restrictions), DTPMP acid, GLP, Guideline (equivalent to OECD Guideline 476), Purity 50% in water. Your conclusion: Positive with metabolic activation;

(9) Supporting study [redacted] 1983), reliability 1 (Reliable without restrictions), experimental result on DTPMP acid, GLP, (equivalent to OECD Guideline 476; Deviations: no analytical data on purity). Conclusion: Negative with metabolic activation; and

(10) Key study ([redacted] 1997), Reliability 1 (Reliable without restrictions), experimental result on DTPMP acid, pH 1.5-3, GLP, Guideline (according to OECD 476; Deviations: "*The maximum concentration tested was 2200 µg/ml. Higher concentrations were claimed to give excessively high osmolality, although the*

values given for 4256 and 4242 µg/ml in subsequent tests only resulted in increases to 354 and 334 mOsm/kg respectively. Since no increases in mutant frequency were seen in the first test at a dose producing 330 mOsm/kg it could be argued that the dose of 4242 could have been tested. All concentrations below 5000 µg/ml are <10 mM, the upper limit defined by OECD for this assay. A toxicity limit was not reached in these tests - top levels had >75% survival. Therefore the upper limit defined for this assay was not reached."), Purity 46.9% of the substance. Your conclusion: Negative with and without metabolic activation.

In vivo mutagenicity study (Annex IX, Section 8.4, Column 2):

(11) Key study ([redacted] 1983), reliability 2 (Reliable with restrictions), experimental result on DTPMP acid, GLP, (equivalent to OECD Guideline 475; Deficiencies: "*insufficient cells scored for aberrations and for mitotic index*"), Purity 19.7 % of the substance, rats (N=6) were exposed for 6, 12, 24 and 48 hours; Doses: 0, 200, 660, 1970 mg active acid/kg bodyweight. Your conclusion: Negative.

The technical dossiers of two of the category members (DTPMP, 1-3 Na-sat, EC No. 701-215-9; and DTPMP, 5-7 Na-salt EC No. 701-216-4) do not currently contain all of the studies listed above. However, these studies were present in the technical dossier before the registration of DTPMP acid, pH 1.5-3 (EC No. 244-751-4) was split due to an EC No. adaptation. Additionally, as your intention is to read-across between the members of DTPMP category all of the information generated on those members is relevant for read-across assessment.

B.2. ECHA's analysis of your prediction of mutagenic properties in light of the requirements of Annex XI, Section 1.5.

Your read-across hypothesis assumes that all substances will have the same effects because they converge to the same DTPMP-anion species at physiological conditions. ECHA considers this a reasonable assumption and accepts that prediction of mutagenic properties can be made between the ammonium, potassium and sodium salts of DTPMP acid provided that the source study is adequate and reliable for the endpoint concerned.

According to Annex XI, Section 1.5., if the grouping concept is applied then in all cases the results to be read across should in particular:

- be adequate for the purpose of classification and labelling and/or risk assessment
- have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3).

ECHA have identified the following shortcomings in the *in vivo* study (study 11 above):

(a) The mitotic index is not determined/reported. Paragraph 39 of the OECD TG 475 requires that the mitotic index to be measured in at least 1000 cells per animal in all groups. You have not provided any information with regard to mitotic index.

(b) Too few metaphases analysed. Paragraph 40 of the OECD TG 475 requires 200 metaphases to be analysed from each animal. The current study have analysed between 280-600 metaphases in total from the 10-12 animals in the group; i.e. about 23-60 metaphases per animal. Furthermore, the test guideline specifies that if the background level of aberrant cells is <1% (i.e. in historical control database) then scoring additional cells should be considered. After 12 and 24 hours after treatment you report a background level of 0% aberrant cells in the negative control group. In addition, you have not reported the sub-types (breaks, exchanges) of the aberrations.

(c) Individual animal data not reported.

(d) Historical positive/negative control range and distribution not provided.
(e) The study does not meet criteria for an acceptable test as specified in paragraph 43 of the OECD TG 475 because the concurrent positive and negative control data cannot be assessed in relation to the laboratory historical control database; and as specified in point (a) and (b) the number of analysed cells is not appropriate.
(f) In addition, you claim that the maximum tolerated dose is 1970 mg/kg. ECHA notes that the reported mortality at the highest dose is inconsistent with what has been observed in 4 independent oral acute toxicity studies which report no deaths below 5836 mg/kg and establish LD_{50} to be >5836 mg/kg and <6881 mg/kg.

Due to the shortcomings listed above, ECHA does not consider the study as adequate and reliable because it does not cover the key parameters of the OECD TG 475.

C. Scope of the category with regard to developmental toxicity and toxicity to reproduction

C.1. Your description of the grouping

In section 1.4.2 of the the CSR, you expand the applicability domain of the category from ammonium, potassium and sodium salts of DTPMP acid to a larger group of structural analogues. You provide the following justification: *"The justification of the aminomethylenephosphonates as a group is primarily made on the grounds that the identified phosphonates share a common chemistry incorporating alkyl backbones with one or more tertiary amine centres and multiple methylphosphonate groups present"*

The common structural feature of this larger group is that they are all aminomethylene-phosonates as illustrated by Figure 1.1. in the CSR.

You have in Figure 1.1. in the CSR identified several smaller categories which together form the larger group of "aminomethylenephosonates". For the purpose of this decision, the following abbreviations are used for the category members:

DTPMP acid and its salts	as defined in Section A.1. above;
ATMP and its salts	nitrilotrimethylenetris(phosphonic acid), CAS No. 6419-19-8, and its salts;
ATMP-N-oxide acid and its salts	[nitrilotris(methylene)]trisphosphonic acid N-oxide, CAS No. 15834-10-3, and its salts;
HMDTMP acid and its salts	[hexane-1,6-diylbis[nitrilobis(methylene)]]tetrakisphosphonic acid, CAS No. 23605-74-5, and its salts;
EDTMP acid and its salts	[ethane-1,2-diylbis[nitrilobis(methylene)]]tetrakisphosphonic acid, CAS No. 1429-50-1) , and its salts;
CHDTMP acid and its salts	[cyclohexane-1,2-diylbis[nitrilobis(methylene)]]tetrakisphosphonic acid, CAS No. not provided, and its salts; and
BHMT acid and its salts	[[(phosphonomethyl)imino]bis[hexamethylenenitrilobis(methylene)]]tetrakisphosphonic acid, CAS No. 34690-00-1, and its salts.

C.2. ECHA's analysis of the grouping

According to the ECHA Guidance on information requirements and chemical safety assessment Chapter R.6.2, Section R.6.2.4.1, (version 1.0, May 2008) a category hypothesis should address "*the set of inclusion and/or exclusion rules that identify the ranges of values within which reliable estimations can be made for category members for the given endpoint. These rules, can be described as the applicability domain for an endpoint and provide a means of extending the category membership to chemicals not explicitly included in the current definition of a category.*"

Furthermore, according to the ECHA Guidance on information requirements and chemical safety assessment Chapter R.6.2, Section R.6.2.1.2, (version 1.0, May 2008) "*a category evaluation does not necessarily result in all the individual substances included in the category evaluation being registered to the Agency, although the data from these substances will be included in the category report in support of the registration.*"

You describe the applicability domain of the aminomethylenephosphonates as: "*registered phosphonates which share a common chemistry incorporating alkyl backbones with one or more tertiary amine centres and multiple methylphosphonate groups present*".

ECHA considers your applicability domain vague and poorly defined. The applicability domain as decribed include numerous other substances in addition to those you have identified. The applicability domain has to have clear an unambigious inclusion/exclusion criteria in order to be the basis of any meaningful predictions.

ECHA concludes that your applicability domain is unclear and thus cannot be the basis for any meaningful predictions.

D. Predictions of developmental toxicity and toxicity to reproduction

D.1. Your category hypothesis and information you provided

You have provided the following reasoning for the prediction of toxicological properties:
"*Several aminomethylenephosphonates [...] can be considered to be a group or family of structurally-analogous substances, within which many properties are generally consistent but in general do not follow predictable trends"; and "In most areas of mammalian toxicity the aminomethylenephosphonates have consistent properties. Irritation is driven by pH and salts in the neutral range are largely non-irritating while acid forms may be irritating. The substances generally have no acute toxic hazards. In repeated dose tests in the sub-chronic term the main effect is generally on haematology and ascribed to binding of dietary minerals within the gastrointestinal tract.*
In the chronic term, effects of aminomethylenephosphonates in mammals have not been tested in all phosphonates, and there is contradictory evidence for carcinogenicity in particular between substances and between studies".

To support your claim you have provided the studies listed below:

Developmental toxicity studies on source substance ATMP and its salts

(12) Reliability 2 ([redacted] 1979c), non-GLP, similar to OECD TG 414, rats were treated with 100, 500 and 1000 mg/kg/day of ATMP. The study reports a maternal NOAEL of 500 mg/kg/day based on marginally reduced body weight gain. The NOAEL for foetal toxicity and teratogenicity is reported as >1000 mg/kg/day.

(13) Reliability 2 [redacted] 1980), non-GLP, similar to OECD TG 414, mice were treated with 100, 500 and 1000 mg/kg/day of ATMP. The study reports a NOAEL of 1000 mg/kg/day for maternal toxicity (based on no effects), foetal toxicity and teratogenicity was greater than 1000 mg/kg bw/day (based on no treatment related effects).

Developmental toxicity studies on target substances DTPMP and its salts

(14) Reliability 2 ([redacted] 1982), non-GLP, similar to OECD TG 414, mice were treated with 500, 1000 and 2000 mg/kg/day of DTPMP, 7 Na-salt. The study reports a NOAEL of 1000 mg/kg/day for maternal toxicity (based on reduced body weight and soft stool), foetal toxicity and teratogenicity was greater than 2000 mg/kg bw/day (based on no treatment related effects).

Reproductive toxicity studies on source substance ATMP and its salts

(15) Reliability 2, [redacted] 1979a, non-GLP, non-guideline (three generation study), rats were treated with 300, 1000 and 3000 ppm of ATMP. The study reports NOAELs for general, reproductive toxicity and the offspring of 3000 ppm based on no effects.

Reproductive toxicity studies on target substances DTPMP and its salts

(16) Reliability 2, [redacted] 1979, non-guideline (one-generation study), rats were treated with 300, 1000 and 3000 ppm of DTPMP acid. The study reports NOAELs for general and reproductive toxicity of 3000 ppm. The NOAEL for the offspring is reported as 1000 ppm based on reduced pup weight.

D.2. ECHA's analysis of your prediction of developmental and reproductive properties in light of the requirements of Annex XI, Section 1.5.

ECHA understands that you base your prediction on an assumption that all substances within the aminomethylenephosphonates category will cause the similar effects. ECHA have assessed your predictions on this basis.

ECHA notes the following shortcomings with the proposed prediction for developmental toxicity and toxicity to reproduction:

i. Lack of consistency between the available data and the read-across hypothesis, no similar toxicity proven

According to the ECHA Guidance on information requirements and chemical safety assessment Chapter R.6.2, Section R.6.2.2.2, (version 1.0, May 2008) "a demonstration of consistent trends in the behaviour of a group of chemicals is one of the desirable attributes of a chemical category and one of the indicators that a common mechanism for all chemicals is involved".

You argue that DTPMP acid (and its salts) "*have similar toxicological properties*" when compared to the other members of the group. However, the information you provided does not prove it, instead it contradicts your assumption, as discussed below,

Firstly, ECHA notes that the documentation of the aminomethylenephosphonates is very limited, without an adequate and reliable documentation of the applied method, ECHA is unable to assess the validity of predictions made within the aminomethylenephosphonates category. Therefore ECHA have considered the information provided to the extent possible.

Secondly, ECHA notes that you do not compare the toxicity profile of DTPMP and is salts with all the other members of the group; you only provide data on ATMP. For the other

category members you provide a high level summary: "*Several aminomethylenephosphonates [...] can be considered to be a group or family of structurally-analogous substances, within which many properties are generally consistent but in general do not follow predictable trends*"; "*In most areas of mammalian toxicity the aminomethylenephosphonates have consistent properties*"; "*The substances generally have no acute toxic hazards*"; "*In repeated dose tests in the sub-chronic term the main effect is generally on haematology [...]*"; and "*In the chronic term, [...] there is contradictory evidence for carcinogenicity [...]*". ECHA considers that summary statements do not support your claim of similar toxicity among the category members.

Thirdly, you have not explained why you use ATMP, as a source substance, and not any of the other category members for predicting the outcome of pre-natal developmental toxicity studies and toxicity to reproduction studies for DTPMP acid and its salts. You have also not demonstrated that you have considered all relevant information within the aminomethylenephosphonates category for your predictions; nor have you demonstrated a consistent pattern of effect among the category members.

Finally, with regard to reading across from ATMP and DTMP (and its salts), ECHA has compared the toxicity profile of the two substances and does not agree with your claim that the toxicity profile of ATMP and DTPMP (or its salts) is similar.

This is because the general systemic toxicity of the source and target substances differs. ATMP has a NOAEL of >3000 ppm (>275-310 mg/kg/day; study 15 above) based on no effects. In addition, ATMP has NOAEL of >500 mg/kg/day (highest dose tested) from a two-year combined chronic toxicity study and carcinogenicity study (at the highest dose increased organ weights without histopathological findings in the adrenal glands, spleen, liver and pituitary). In contrast, DTPMP has NOAEL of 82-92 mg/kg/day from a sub-chronic toxicity study (based on increased red blood cell count, decreased haemoglobin and histopathological findings in the spleen; in addition bone is identified as a target organ). ECHA concludes that with regard to general toxicity the two substances appear to differ both in qualitative and quantitative terms.

Furthermore, with regard to developmental toxixity, the observed teratogenic effects in rats differ in qualitative terms between ATMP and DTPMP. For ATMP, ECHA notes that in the highest dose group six foetuses from a single litter had common multiple malformations that included flexed forepaws, shortened and thickened torso, abdominal distention and exaggerated flexure of the head. Soft tissue examination revealed two of these foetuses had a malformation defect of the heart. No skeletal abnormalities or malformations were observed. The other foetuses of the high group are described as "*generally unremarkable*". You dismiss these findings by stating "*Although a possible teratogenic effect could not be excluded, it was most likely that the effects were secondary to maternal toxicity*". ECHA does not agree with your dismissal of the observed effects because your explanation is not consistent with the overall general toxicity profile of the substance and if this truly is an effect of general maternal toxicity then one would expect more than one affected litter. In contrast, for DTPMP findings with regard to teratogenicity were a low number of vertebral abnormalities (but no malformations) in one foetus from one litter in the mid-dose (1000 mg/kg/day) and one foetus from two litters in the high dose group (2000 mg/kg/day).

Furthermore, you argue that deposition in bone is an intrinsic property of both ATMP and DTPMP, however, skeletal effects are only observed for DTPMP.

In summary, as explained above the general toxicity profile of ATMP and DTPMP appear to differ both in terms of identified target organs and the magnitude of the effects. In addition, the pre-natal developmental studies conducted in the rat show different types of teratogenic effects for the two substances. Given the observed differences in the toxicity profiles, ECHA concludes that you have no adequate basis for predicting prenatal developmental or reproductive toxicity for target substances DTPMP and its salts from ths source substance ATMP.

ii. Source study is not adequate

According to Annex XI, Section 1.5., if the grouping concept is applied then in all cases the results to be read across should in particular:

- be adequate for the purpose of classification and labelling and/or risk assessment
- have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3).

You have provided a three generation study (see study 15 above) conducted with ATMP. This study was not conducted according to GLP nor to an OECD test guideline and does not cover all the key parameters foreseen to be investigated in an extended one-generation reproductive toxicity study (OECD TG 443)

Specifically, an extended one-generation reproductive toxicity study (OECD TG 443) provides, in addition to information to general toxicity, information in particular on two aspects, namely on sexual function and fertility in P0 and F1 generations (further referred to as 'sexual function and fertility') and on development and toxicity of the offspring from birth until adulthood due to pre- and postnatal and adult exposure in the F1 generation (further referred to as 'effects on offspring'). Relevant elements for 'sexual function and fertility' are in particular functional fertility (oestrous cycle, sperm parameters, mating behaviour, conception, pregnancy, parturition, and lactation) in the parental generation after sufficient pre-mating exposure duration and histopathological examinations of reproductive organs in both P and F1 generations. Relevant elements for 'effects on offspring' are in particular peri- and post-natal investigations of the F1 generation up to adulthood including investigations to detect certain endocrine modes of action, and sexual development. Also the sensitivity and depth of investigations to detect effects on 'sexual function and fertility' and 'effects on offspring' needs to be considered.

ECHA concludes that with regard to reproductive toxicity no predictions can be made from ATMP because there is no adequate and reliable source study.

E. Conclusions on the grouping of substances and read-across approach

Predictions for mutagenicity: ECHA considers that your grouping of substaces into the category DTPMP, and its salts is acceptable and that the accurate predictions of mutagenicity can be made within the group provided that the source data is adequate and reliable..

Predictions for developmental toxicity and toxicity to reproduction: ECHA considers that your applicability domain for the Aminomethylenephosphonates is unclear and thus cannot be the basis for any meaningful predictions. Furthermore, ECHA considers that read-across from the source substance ATMP to the target substances DTPMP and its salts does not provide a reliable basis for prediction of the developmental toxicity and toxicity to reproduction. The main reason for this is that the toxicity profle of the substances differ. In

addition, there is no adequate and reliable source study available for toxicity to reproduction. Hence, this approach does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. of the REACH Regulation.

2. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2)

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "*If there is a positive result in any of the in vitro genotoxicity studies in Annex VII or VIII and there are no results available from an in vivo study already, an appropriate in vivo somatic cell genotoxicity study shall be proposed by the Registrant.*"

With regard to the information requirement described above, you have sought to adapt this information requirement by reading across within the DTPMP category. As explained above under 'Grouping of substances and read-across', ECHA accepts your read-across approach provided that there is reliable and adequate source data. ECHA considered all information available within the DTPMP category, as listed in section B.2. above and has the following observations:

- The results of the *in vitro* gene mutation studies in bacteria are negative with and without metabolic activation (studies 1-4);
- Both negative and positive results have been reported in the available *in vitro* gene mutation studies in mammalian cells. ECHA notes that all of the negative studies (studies 6, 9 and 10) have the same deficiencies, *i.e.* the highest test concentration is below the maximum concentration required by the test guideline; in addition, in the latter test the highest dose tested was not limited by cytotoxicity. Therefore, none of these tests can be considered fully conclusive with regard to gene mutation and cannot be used to dismiss the positive results (study 7 and 8). There are two tests available (study 7 and 8) which are positive with metabolic activation. The colony size was not assessed in any of these tests, thus, there is no information to support an argument that the positive result may be explained by a clastogenic effect of the substance.
- *The in vitro* cytogenicity study in mammalian cells (study 5) is positive. This positive result has been followed up *in vivo* (study 11). However, as explained in section B.2. this study is considered as not adequate and reliable and therefore cannot be used to dismiss the concerns for chromosomal aberrations and gene mutation raised by the other available *in vitro* tests (studies 5-10).

Based on the above, ECHA considers that the available *in vitro* data indicates concerns for gene mutations and chromosomal aberrations, and that an appropriate *in vivo* genotoxicity study to follow up the concerns is not provided. Consequently there is an information gap and it is necessary to provide information for this endpoint. As prediction possibility between DTPMP category members is approved for this endpoint it is sufficient to test either the registered substance or one of the other category members and apply the read-across and grouping approach for all other members. As there are no differences after absorption, at physiological conditions, between the category members the choice of the test substance is left to you.

In your comments to the draft decision, you agree that there are deficiencies in the existing *in vivo* micronucleus assay and that additional data is needed to clarify the concern. You propose to conduct an additional *in vitro* cytogenicity study in mammalian cells, using either OECD TG 473 or OECD TG 487. For *in vitro* mutagenicity you have re-assessed the available

information and concluded that there is no concern for *in vitro gene* mutation and proposed to repeat the gene mutation in mammalian cells to confirm this conclusion.

However, considering that an acceptable data set is available in the dossier to fulfil the information requirement for *in vitro* cytogenicity and *in vitro* gene mutation in mammalian cells, ECHA is not requesting any additional *in vitro* testing because this information is not likely to remove the identified concern arising from the information currently available. However, you may at your own discretion conduct additional *in vitro* testing.

In your comments you also indicated that you intend to test the DTPMP, 5-7 Na-salt (EC No. 701-216-4) to cover all the members of DTPMP category. ECHA agrees with this proposal and have amended the test material of the request accordingly.

Test selection

ECHA notes that in case there are positive results in both chromosomal aberration and gene mutation *in vitro* studies, the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.7.6.3 identifies that the following tests are options for a follow-up in vivo study. The mammalian erythrocyte micronucleus test ("MN test", OECD TG 474), the mammalian bone marrow chromosomal aberration test ("CA test", OECD TG 475) or the *in vivo* mammalian alkaline comet assay ("Comet Assay", OECD TG 489) are suitable to follow up a positive *in vitro* result showing chromosomal aberration. The MN test and CA test are able to detect chromosomal aberrations, whereas the comet assay is an indicator assay detecting putative DNA lesions. The *in vivo* comet assay is suitable to follow up a positive *in vitro* result showing gene mutation.

In your comments to the draft decision you indicate that "*in view of the lack of concern for mutagenicity as distinct from cytogenicity, the OECD TG 474 or OECD TG 475 would be more appropriate than the comet assay*".

However, in a proposal for amendment (PfA) submitted for this case, a Member State Competent Authority (MSCA) indicated that in the positive *in vitro* gene mutation in mammalian cells studies (OECD TG 476) there is no information on colony size. Therefore, without this information, the concern for gene mutations remains. Considering that the *in vitro* data indicates concerns for both gene mutations and chromosomal aberrations, the MN test and the CA test would not be suitable *in vivo* follow up tests, as these tests cannot detect gene mutations. The MSCA concludes that only the comet assay should be requested in the decision, as it is the only test that can cover both concerns.

In your comments on the PfA you agree with the MSCA.

ECHA agrees that the data reported for the OECD TG 476 ([redacted] 1982) lacks information on colony sizing, which means that neither gene mutation nor chromosomal aberration can be ruled out as a mechanism inducing the observed mutations. According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.7.6.3, the *in vivo* mammalian alkaline comet assay ("Comet Assay", OECD TG 489) is suitable to follow up positive *in vitro* results for gene mutation and for chromosomal aberrations. Therefore, ECHA considers this test to be most appropriate for the substance subject to the decision.

Test design

According to the test method OECD TG 489, the test shall be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

In line with the test method OECD TG 489, the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Outcome

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the 5-7 Na salt (EC No. 701-216-5):

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

Germ cells

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required under Annex X of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

Therefore, you may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*[6]) in addition to the other aforementioned tissues, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells, in accordance to Annex X, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal cells.

This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

3. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.)

Pre-natal developmental toxicity studies (test method OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

[6] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

You have adapted the information requirement for a pre-natal developmental toxicity study in a first species (Annex IX, Section 8.7.2.) by providing a pre-natal developmental toxicity study similar to OECD TG 414 ([redacted] 1982, GLP, Reliability 2). The study was conducted according to GLP in rats using 0, 1000 and 2000 mg/kg/day of DTPMP, 7 Na-salt. The study reports a maternal LOAEL of 2000 mg/kg/day based on 30% reduced body weight gain and soft stool. For teratogenicy the study reports a NOAEL of 2000 mg/kg/day.

With regard to the information requirement for a pre-natal developmental toxicity study in a second species, you have sought to adapt this information requirement by reading across from the source substance ATMP. However, as explained above under 'Grouping of substances and read-across', your adaptation of the information requirement is rejected. Consequently there is an information gap and it is necessary to provide information for this endpoint.

The test in the first species was carried out by using a rodent species (rat). According to the test method EU B.31/OECD 414, the rabbit is the preferred non-rodent species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbits as a second species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision you accept ECHA's current assessment of the endpoint. However, you indicate that there is ongoing work as a result of other ECHA decisions within other categories on aminomethylenephosphonates (ATMP) which may provide opportunities for read-across and/or explain the observed differences in the toxicological profiles of ATMP and DTPMP. You ask that the deadline for this request is set to allow sequential testing. The current decision already sets a deadline that allows sequential testing within the DTPMP category. The deadline of the decision is 36 months to allow sequential testing within the decision. The order in which to conduct the requested tests is up to you. ECHA considers this time sufficient to take into account the information which is currently being generated. Therefore, ECHA has not amended the deadline.

In addition, you also confirmed that the grouping approach applied for mutagenicity also applies for developmental toxicity. You indicated that you intend test the DTPMP, 5-7 Na-salt (EC No. 701-216-4) to cover all the members of DTPMP category. ECHA agrees with this proposal and has amended the test material of the request accordingly.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the DTPMP, 5-7 Na-salt (EC No. 701-216-4): Pre-natal developmental toxicity study (test method: OECD TG 414) in a second species (rabbit) by the oral route.

4. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3)

The basic test design of an extended one-generation reproductive toxicity study (test method OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as

laid down in column 1 of 8.7.3., Annex X. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information provided

You have provided a one-generation reproductive toxicity study (similar to OECD TG 415) conducted on DMPTP acid ([redacted] 1979) – listed as study 16 in section D.1.

ECHA notes that the study which you have provided in the technical dossier was not conducted according to GLP and not according to the OECD TG 443 or to OECD TG 416.

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) shall be considered equivalent to data generated by the corresponding test methods referred to in Article 13(3) 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.

However, a study which you have provided was conducted according to the OECD TG 415 and does not provide the information required by Annex X, Section 8.7.3., because it does not cover key parameters, exposure duration and life stages of an extended one-generation reproductive toxicity study. The main missing key aspect/element is an extensive postnatal evaluation of the F1 generation. In addition, the provided study has numerous deviations from the e OECD TG 415 *e.g. "No analytical confirmation of exposure", "no pre-mating exposure", "no assessment of oestrus cycle or sperm analyses"*, and *"no evaluation of sexual milestones in pup"*.

Therefore the conditions required in Annex XI, Section 1.1.2. are not met and ECHA concludes that the study does not provide the information required by Annex X, Section 8.7.3.

You have also sought to adopt this information requirement by reading across to a three-generation reproductive toxicity study (listed as study 16 in section D.1) conducted with ATMP. However, as explained above under 'Grouping of substances and read-across', your adaptation of the information requirement is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint. Thus, an extended one-generation reproductive toxicity study according Annex X, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) *The specifications for the study design*

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA Guidance, the starting point for deciding on the length of premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required because there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

The highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no relevant data to be used for dose level setting, it is recommended that a range-finding study (or range finding studies) is performed and that its results are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Species and route selection

According to the test method OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision you agreed to conduct the requested test. You also confirmed that the grouping approach applied for mutagenicity also applies for reproductive toxicity. You indicated that you intend to test DTPMP, 5-7 Na-salt (EC No. 701-216-4) to cover all the members of the DTPMP category. ECHA agrees with this proposal and have amended the test material of the request accordingly.

Cohorts 2A and 2B

Subsequent to a Proposal for Amendment from a Member State, the developmental neurotoxicity Cohorts 2A and 2B need to be conducted in case of a particular concern on (developmental) neurotoxicity.
Existing information on a substance structurally analogous to the Substance (you have applied read-across to a sodium salt of the Substance, i.e. "[[(phosphonomethyl)imino]bis[(ethylenenitrilo)bis(methylene)]]tetrakisphosphonic acid, sodium salt; sodium hydrogen [10,10-dihydroxy-10-oxido-2,5,8-tris(phosphonomethyl)-2,5,8-triaza-10-phosphadec-1-y... / 22042-96-2 / 244-751-4") derived from available *in vivo* studies ("r/a Key CTL, 1998/Repeated dose toxicity: oral", a 90-day study) shows evidence that total serum iron decreased in females only at 10000 ppm, i.e. iron deficiency. This was accompanied by an increase in red blood cell levels (polycythaemia) and decreased mean cell volume and mean cell haemoglobin in males and females at 10000 ppm. Haemoglobin and mean cell haemaglobin concentration were significantly decreased in females at top dose and there was decreased Perl's staining in spleen (a marker for iron complexes). You state in the dossier "*Thus, the findings noted in these haematological parameters and serum iron and binding capacity are likely to result from a perturbation of iron homeostasis, which is supported by the reduction of staining in the spleen. The effects are therefore due to the iron binding characteristics of Dequest 2066A, which is a chelating agent.*"

Iron deficiency is considered to be a specific mechanism(s)/mode(s) of action with an association to developmental neurotoxicity. It is known that severe iron-deficiency during pregnancy increases the risk of miscarriages, stillbirths, prematurity and low birth weight (World Health Organisation, 2014. Global nutrition targets 2025: anaemia policy brief (WHO/NMH/NDH/14.4). Geneva: World Health Organisation) and Markova et al. (Markova, V., Holm, C., Pinborg, A. B., Thomsen, L. L., & Moos, T. 2019. Impairment of the Developing Human Brain in Iron Deficiency: Correlations to Findings in Experimental Animals and Prospects for Early Intervention Therapy. Pharmaceuticals, 12(3), 120) reviews the essential role of iron in brain development, referring to studies which show that iron deficiency during pregnancy results in developmental neurotoxicity (e.g. Mihaila, C.; Schramm, J.; Strathmann, F.G.; Lee, D.L.; Gelein, R.M.; Luebke, A.E.; Mayer-Pröschel, M. Identifying a window of vulnerability during fetal development in a maternal iron restriction model. PLoS ONE 2011, 6, e17483). The responses seen in offspring subsequent to iron restriction are clearly adverse, e.g. increased auditory brainstem response conduction time.

In your comments on the Proposals for Amendment, you disagree on the need to trigger the DNT cohort. You argue that the DTPMP substances show a specific toxicity mechanism driven by the chelating property that causes a deficiency of different metals in the organism, such as iron, that this is threshhold based, is not related to the reproductive system and cannot be considered a specific concern to DNT. In respect of Markova *et al.*, you note the concern for publication bias and conclude that this publication alone should not be taken as concrete concern for neurodevelopmental effects of DTPMP as inferred in the PfA. You consider that there is not evidence of neurotoxicity seen with the Substance. You also consider that the iron deficiency caused by DTPMP may be caused by chelation of iron in the gastrointestinal tract, that DTPMP has low bioavailability and hypothesis that gavage dosing may prevent the iron deficiency.

ECHA considers that a specific concern for iron-deficiency mediated-neurotoxicity is not precluded if there are additionally other mechanisms and other sites affected. ECHA notes that your particular quotation about publication bias in Markova *et al.* comes from a section related to the analysis of human data, whereas the conclusions on small rodents from Markova *et al.* is that iron deficiency in pregnancy clearly shows dramatic effects on brain

development. ECHA considers that there is no information provided in the existing one-generation study to exclude the possibility of iron-deficiency mediated neurotoxicity; indeed organ weights and histopathology of the brain and nervous tissue of the offspring are not recorded in the robust study summary. Your hypothesis about iron deficiency being caused by gastrointestinal chelation, and that systemic DTPMP does not have toxic effects, is not supported by a direct experimental demonstration and remains hypothetical.

Based on the above, the developmental neurotoxicity Cohorts 2A and 2B need to be conducted.

Cohort 3

Subsequent to a Proposal for Amendment, the developmental immunotoxicity Cohort 3 needs to be conducted in case of a particular concern on (developmental) immunotoxicity.

Existing information on a substance structurally analogous to the Substance (you have applied read-across to a sodium salt of the Substance, i.e. "[[(phosphonomethyl)imino]bis[(ethylenenitrilo)bis(methylene)]]tetrakisphosphonic acid, sodium salt; sodium hydrogen [10,10-dihydroxy-10-oxido-2,5,8-tris(phosphonomethyl)-2,5,8-triaza-10-phosphadec-1-y... / 22042-96-2 / 244-751-4") derived from available *in vivo* studies ("r/a Key CTL, 1998/Repeated dose toxicity: oral", a 90-day study) shows evidence that total serum iron decreased in females only at 10000 ppm, i.e. iron deficiency. This was accompanied by an increase in red blood cell levels (polycythaemia) and decreased mean cell volume and mean cell haemoglobin in males and females at 10000 ppm. Haemoglobin and mean cell haemaglobin concentration were significantly decreased in females at top dose and there was decreased Perl's staining in spleen (a marker for iron complexes). You state in the dossier "*Thus, the findings noted in these haematological parameters and serum iron and binding capacity are likely to result from a perturbation of iron homeostasis, which is supported by the reduction of staining in the spleen. The effects are therefore due to the iron binding characteristics of Dequest 2066A, which is a chelating agent.*"

Iron deficiency is considered to be a specific mechanism(s)/ mode(s) of action with an association to (developmental) immunotoxicity. Aly et al., 2018 (Aly, S. S., Fayed, H. M., Ismail, A. M., & Hakeem, G. L. A. 2018. Assessment of peripheral blood lymphocyte subsets in children with iron deficiency anemia. BMC pediatrics, 18(1), 49) states "*Omara and Blakley, [13] proved a lower delayed hypersensitivity response (where CD4+ lymphocytes are the key marker) and decreased lymph proliferative activities in mice fed an iron-deficient diet than those fed a normal or supplemented iron diet.*" and also "*CD4+ lymphocytes are affected by iron deficiency which prevents the development of immune response against different pathologic challenges [15].*" Thus iron deficiency affects the function of the immune system as evidence by impaired contact sensitivity and delayed-type hypersensitivity ([13] Omara and Blakley, Br. J. Nutr. (1994) 72,899) and failure to respond to model antigens ([15] Grant et al., J. Nutr. (2003) 133, 2635).

In your comments on the Proposals for Amendment, you disagree on the need to trigger the DIT cohort for the reasons as set out under 'Cohorts 2A and 2B' above. You also set out that the Aly paper itself uses a small sample size, recorded potential bias issues, and stated that further work is needed to provide a more conclusive interpretation. You also state that the Aly paper looked at correlations between iron deficiency and lymphocytes, whereas the 90-day study did not show adverse findings relating to any white blood cell type.

ECHA considers that a specific concern for iron-deficiency mediated-immunotoxicity is not precluded if there are additionally other mechanisms and other sites affected. Your hypothesis about iron deficiency being caused by gastrointestinal chelation, and that systemic DTPMP does not have toxic effects, is not supported by a direct experimental demonstration and remains hypothetical. ECHA notes that the effects seen on lymphocytes in the Aly paper are comparatively small, and that, based on considerations of statistical power, it is not possible to conclude as to whether you could expect to see such an effect in the 90-day study, as relevant data (such as n, mean, s.d., normality) are not provided either by Aly et al or in the robust study summary. Moreover, the functional immune parameters measured by references [13] and [15] are not measured in the 90-day study and thus are not contradicted by information on the analogue substance.

Based on the above, the developmental immunotoxicity Cohort 3 needs to be conducted.

c) Outcome

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the DTPMP, 5-7 Na-salt (EC No. 701-216-4) : Extended one-generation reproductive toxicity study (test method OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce systemic toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;
- Cohorts 2A and 2B (Developmental neurotoxicity); and
- Cohort 3 (Developmental immunotoxicity).

Notes for your consideration

The conditions to include the extension of Cohort 1B are currently not met. However, you may expand the study by including the extension of Cohort 1B if new information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the available information, together with the new information shows triggers which are described in column 2 of Section 8.7.3., Annex X and further elaborated in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017). You may also expand the study to address a concern identified during the conduct of the extended one-generation reproduction toxicity study and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the expansion must be documented.

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 01/10/2015.

This draft decision replaces the previously issued draft decision with Communication number: CCH-D-2114311225-65-01/D.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

In your comments among others, you addressed the issues related to the endpoint: long-term toxicity on terrestrial invertebrates. This comment is addressed under the relevant testing proposal decisions issued for other members of the category of acid and salt forms of DTPMP.

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

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

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments 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.

In addition, you provided comments on the draft decision. These comments were not taken into account by the Member State Committee as they were considered to be outside of the scope of Article 51(5).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-67 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

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

2. Failure to comply with the requests in this decision will result in a notification to the enforcement authorities of your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.

4. If the required tests are conducted with an analogue substance in the context of a read-across approach, the identity of the test material used to perform the test should be specified in line with ECHA's Practical Guide on "How to use alternatives to animal testing to fulfil your information requirements" (chapter 4.4). This is required to show that the test material is representative of the analogue substance identified in the read-across approach and used to predict the properties of the registered substance.