ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 14 February 2020

Addressee: [redacted]

Decision number: CCH-D-2114499240-48-01/F
Substance name: pentasodium pentahydrogen [[(phosphonatomethyl)imino]bis[ethane-2,1-diylnitrilobis(methylene)]]tetrakisphosphonate
EC number: 263-212-4
CAS number: 61792-09-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 08/03/2013
Registered tonnage band: 100-1000

DECISION ON A COMPLIANCE CHECK

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

1. **Identity and composition of the substance (Annex VI, Sections 2.1 and 2.3.);**

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

3. **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 (5-7 Na:1) (EC No. 701-216-4; DTPMP, 5-7 Na-salt);**

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 testing within the DMPTP category.

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 INFORMATION

1. Identity and composition of the substance (Annex VI, Sections 2.1 and 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: 2.1, 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, the typical, minimum and maximum concentration levels shall be specified regardless of the substance type

According to the Guidance, chapter 4.2.2, a multi-constituent substance is a substance defined by its composition, for which more than one main constituent is present at a concentration ≥ 10% (w/w) and < 80% (w/w). On the other hand, a mono-constituent substance is a substance in which one constituent is present at a concentration of at least 80% (w/w) and which contains up to 20% (w/w) of impurities.

Each constituent of a well-defined substance shall be completely specified by all relevant identifiers, including structural information. Each impurity of a well-defined substance ≥1% or relevant for the classification and/or PBT assessment shall be specified by at least one of the following identifiers: chemical name, CAS number and 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.

In the present dossier, you identified the registered substance as the well-defined substance pentasodium pentahydrogen [[(phosphonatomethyl)imino]bis[ethane-2,1-diylnitrilobis(methylene)]]tetrakisphosphonate. You reported only one main constituent in section 1.2 and specified a minimum concentration level of [redacted] (w/w) for the main constituent. You also reported a group of impurities in section 1.2 as reference substance "Closely related structural analogues of the main constituent: [redacted] You specified a typical concentration of [redacted] (w/w) and concentration range [redacted] (w/w) for this reference substance.
You provided a ^{31}P-NMR spectrum on page 10 of the analytical report ([redacted]) attached in section 1.4 of the dossier. The results of

^{31}P NMR analysis reported in "TABLE 2" on page 9 of the analytical report indicate that the sum of unidentified impurities or constituents correspond to [redacted] of the total phosphorus in the substance composition. These unidentified impurities or constituents have been reported under reference substance "*Closely related structural analogues of the main constituent:* [redacted] in section 1.2. ECHA observes that the integral value of the largest unidentified signal in the ^{31}P NMR spectrum at 9.0 ppm (integral value 4.24) is approximately 29% of the signal that has been assigned to a single phosphorus atom of the main constituent at 16.5 ppm (integral value 14.40). Therefore, some unidentified signals in the ^{31}P-NMR spectrum correspond to impurities or constituents that could be present at ≥1% and have not been reported individually, as required according to the Guidance.

Accordingly, the reference substance "*Closely related structural analogues of the main constituent:* [redacted] is overly generic and may cover impurities or constituents that are required to be reported individually. The analytical data attached in the dossier would indicate the presence of constituents or impurities required to be reported in the dossier.

ECHA therefore concludes that up to [redacted] of the composition has therefore not been reported to a sufficient level of detail:

- It cannot be concluded whether the reference substance "*Closely related structural analogues of the main constituent:* [redacted] covers constituents present at ≥10%. Such constituents shall be identified and reported individually.

- In addition, it cannot be concluded whether some of the impurities are present above 1% in the registered substance. Such impurities shall be identified and reported individually.

Furthermore, you have justified the deviation from the 80% rule for your substance by providing a statement in the remarks field of a reference substance "*Closely related structural analogues of the main constituent:* [redacted] in section 1.2: "*Purity >60 %. Although this purity is below 80 % this is still considered to be a mono-constituent substance in REACH registration. The purity of the main constituent plus closely related structural analogues, which are considered to have very similar properties and functionality, exceeds 75 %. This approach is in line with the recommendation of the REACH phosphonates consortium. Typical values correspond to those measured in batch LE125101b.*" However, based on the limited qualitative and quantitative information provided in the dossier it cannot be concluded that the impurities or constituents covered by the reference substance are closely related structural analogues of the main constituent. Therefore, ECHA concludes that the justification provided for deviation from 80% rule for well-defined substances is not sufficiently supported by the qualitative and quantitative information provided in the dossier.

ECHA also observes that you have identified in section 1.1 of the IUCLID dossier the registered substance as a multi-constituent substance. However, the identifiers reported in section 1.1 of the IUCLID dossier are based only on one of the constituents reported in section 1.2. ECHA therefore considers that the type of substance reported in section 1.1 is not consistent with the information provided in section 1.2.

You are accordingly requested to correct the information provided on the composition of the registered substance and especially the part of the composition that is not reported to a sufficient level of detail, including constituents and impurities. You should provide a more detailed composition in section 1.2 by sub-dividing the group of constituents "*Closely related structural analogues of the main constituent:* [redacted] into individual constituents or impurities, or groups of constituents based on their structural similarity.

The composition reported in section 1.2 needs to be verifiable by the analytical information provided in section 1.4.

If the substance is manufactured such that the composition is highly complex, unknown or variable and as a consequence, the substance cannot be identified based on individual constituents, the substance shall be rather identified as UVCB substance (a substance of Unknown or Variable composition, Complex reaction products or Biological materials). However, you would need to provide supporting documentation as to why the substance registered is better identified as a UVCB rather than a well-defined substance. In addition, should the substance be identified as a UVCB substance, further information is required to appropriately identify the registered substance in accordance with section 4.3 of the Guidance. Section 4.3 specifically refers to UVCB substances.

Consequently, further information is required to clarify the identity of the registered substance.

- If you decide to identify the substance as a well-defined substance, you should provide more detailed composition in section 1.2 as indicated above and prove that the composition of the substance is known by providing appropriate analytical data in section 1.4 that would allow the identification and quantification of the constituents and impurities of the substance required to be reported.

- If the substance is manufactured such that the composition is highly complex, unknown or variable and, as a consequence, the substance cannot be identified based on individual well-defined constituents, the substance shall be rather identified as a UVCB substance. Should the substance be identified as a UVCB substance, you will need to specify a chemical name of the substance that is representative of the registered substance and provide details of the process used for the manufacturing of the registered substance in accordance with section 4.3 of the Guidance. The description of the manufacturing process shall be sufficiently detailed to allow ECHA to understand which starting materials are used, and how any other steps and process parameters may affect the substance composition and therefore its identity. It shall include, as appropriate, the ratio of reactants and any relevant operating parameters (*e.g.* temperature and pressure).

Regarding how to report the composition of the registered substance in IUCLID, the following applies. You shall report individually any impurity or constituent 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.

Where the substance manufactured is identified as a well-defined substance the name should be based on each constituent present at concentration ≥10%. The name shall be reported in the IUPAC name field in section 1.1 of IUCLID.

Where the substance manufactured is best identified as a UVCB substance:

- The description of the manufacturing process of the UVCB substance shall be included in the Description field in Section 1.1 of IUCLID;
- The revised name of the substance shall be reported in the IUPAC name field in section 1.1 of IUCLID;
- The composition reported in section 1.2 shall be revised taking into account the following:
    - All known constituents and all constituents present in the substance with a concentration of ≥10% shall be identified and reported individually, with their typical concentration and concentration range;
    - All constituents relevant for the classification and/or PBT assessment of the registered substance shall be identified and reported individually, independently from their concentration; and
    - Unknown constituents shall be identified by a generic description of their chemical nature.

You shall ensure to select the "type of substance" corresponding to the substance subject to this registration from the appropriate dropdown list in section 1.1 of the IUCLID dossier. You shall ensure that the correct identifiers are used throughout the registration whenever reference is made to the specific substance which is the subject of this registration.

You shall ensure in any case that the composition is verifiable and therefore supported by a description of the analytical methods for the identification and quantification of the constituents required to be reported, as required under Annex VI. 2.3.7. of the REACH Regulation.

ECHA notes that in the event you cover different compositions /grades of the registered substance in the present registration dossier, you shall report separately the compositional information of each grade. This means that if the substance covered by the present registration has two (or more) different compositions, then these must be presented separately. ECHA highlights that failure to report separately the compositional information of each grade of a substance may result in one or more grades not being covered by this registration.

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.

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

"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 notes that you have not provided sufficient information on the descriptions of the methods used to determine the identity and composition of the registered substance as required by Annex VI, Section 2.3.7. of the REACH Regulation.

ECHA notes that the analytical report ([redacted]) provided in IUCLID Section 1.4 does not give sufficient qualitative and quantitative information on the phosphonate constituents or impurities grouped under the reference substance "Closely related structural analogues of the main constituent: [redacted] in section 1.2. The analytical report states that "*These species have been grouped since due to the absence of standards it is not possible to distinguish them as single species. Its contribution in the percentage composition is estimated as 100 minus the sum of the other known components.*" The justification is not sufficient for not distinguishing between the different phosphonate impurities (or groups or phosphonate impurities). Suitable analytical techniques such as ^{31}P-NMR with standards are widely available. Furthermore, quantification of the phosphonate constituents or impurities by back-calculation is not acceptable.

You are therefore requested to provide a description and results of an appropriate analysis that would enable identification and quantification of the specific constituents required to be reported in section 1.2, as indicated in section 1 above. The analytical data must be consistent with the information reported in sections 1.1 and 1.2.

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.

The analytical data, including the description of the analytical methods and the actual results of analysis shall be reported in IUCLID section 1.4.

TOXICOLOGICAL INFORMATION

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

You seek to adapt the following standard information requirement 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.);

ECHA has considered first the scientific and regulatory validity of your grouping and read-across approache in general before addressing the individual endpoint (section 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.

[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

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.*"

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

[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.

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. Conclusion on the grouping of substances and read-across approach

ECHA considers that your grouping of substances 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.

3. 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 indicate 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 DTPMP, 5-7 Na-salt (EC No. 701-216-4):

- *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 IX 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 IX, 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.

[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

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 22 October 2015.

This draft decision replaces the previously issued draft decision with Communication number: CCH-D-2114311750-62-01/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.

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.

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 carrying out the tests required by the present decision, it is important to ensure that the particular sample of substance tested 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. If the registration of the substance covers different grades, the sample used for the new tests must be suitable to assess these.

   Furthermore, 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.