Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

EC Number:	**215-245-0**
CAS Number:	**1314-85-8**
Index Number:	**015-012-00-1**

Contact details for dossier submitter:
Istituto Superiore di Sanità (ISS)
on behalf of the Ministry of Health
Viale Regina Elena, 299 - 00161 Rome, Italy.

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CONTENTS

1 PHYSICAL HAZARDS

The physical hazards of tetraphosphorus trisulphide were evaluated by considering:

- the available experimental data on flammability, self-heating and ability of the substances to emit flammable gases when in contact with water (obtained according to the UN RTDG, Manual of Tests and Criteria: Test N.1, Test N.5 and Test N.4, respectively). Those data formed the basis for the proposed revision of the classification as a Flammable solid Category 1 instead of 2, for the proposed addition of the classification as a Self-heating substance Category 1, as well as for the proposed removal of the classification as a Water-reactive substance Category 1;
- the molecule structure, to check for structural alerts that would trigger the need to further investigate the explosive, self-reactive and oxidising properties;
- the experience of the applicant in the manufacturing and handling of the substance, to address spontaneous ignition when in contact with air at normal temperatures;
- the physical state of the substance.

More details are presented as follows.

1.1 Explosives

1.1.1

Data waived: study scientifically not necessary.

According to the CLP Regulation (EC n.1272/2008, Annex I, Part 2, paragraphs 2.1.4.2 and 2.1.4.3):

A substance or mixture shall not be classified as explosive:

> (a) When there are no chemical groups associated with explosive properties present in the molecule. Examples of groups which may indicate explosive properties are:
>
> - C-C unsaturation (e.g. acetylenes, acetylides, 1, 2-dienes),
> - C-Metal, N-Metal (e.g. Grignard reagents, organo-lithium compounds),
> - Contiguous nitrogen atoms (e.g. azides, aliphatic azo compounds, diazonium salts, hydrazines, sulphonylhydrazides)
> - Contiguous oxygen atoms (e.g. peroxides, ozonides)
> - N-O (e.g. hydroxyl amines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles)
> - N-halogen (e.g. chloramines, fluoroamines)
> - O-halogen (e.g. chlorates, perchlorates, iodosyl compounds)
>
> or

(b) When the substance or mixture contains chemical groups associated with explosive properties which include oxygen and the calculated oxygen balance is less than -200.

or

(c) When the organic substance or a homogenous mixture of organic substances contains chemical groups associated with explosive properties but the exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500 °C (the temperature limit is to prevent the procedure being applied to a large number of organic materials which are not explosive but which will decompose slowly above 500 °C to release more than 500 J/g). The exothermic decomposition energy may be determined using a suitable calorimetric technique.

or

(d) For mixtures of inorganic oxidizing substances with organic material(s), the concentration of the inorganic oxidising substance is:

- less than 15% by mass, if the oxidizing substance is assigned to Categories 1 or 2;

- less than 30% by mass, if the oxidizing substance is assigned to Category 3.

As regards tetraphosphorus trisulphide, there are no chemical groups associated with explosive properties in the molecule. As a result, condition (a) is satisfied, whereas condition (b) is not applicable. Condition (c) is not applicable, because tetraphosphorus trisulphide is not an organic substance and does not contain chemical groups associated with explosive properties; in addition, no exothermic decomposition was observed during Differential Thermal Analysis (study reference: M. Leonardi and U. Zucchelli, Internal test report: Relative density, melting point, boiling point and flammability of P_4S_3, 2017). Condition (d) is not applicable, either, being tetraphosphorus trisulphide a substance, not a mixture.

Since there are no indications from the screening procedure that the substance may possess explosive properties, no further testing is deemed necessary.

Conclusion

Tetraphosphorus trisulphide fulfils criterion (a) of the screening procedure provided for explosive properties (no chemical groups associated with explosive properties). It can be concluded that tetraphosphorus trisulphide is not to be classified as explosive, according to the CLP Regulation (EC n.1272/2008).

1.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable, due to the physical state of the substance evaluated in this dossier (solid).

1.3 Oxidising gases

Hazard class not applicable, due to the physical state of the substance evaluated in this dossier (solid).

1.4 Gases under pressure

Hazard class not applicable, due to the physical state of the substance evaluated in this dossier (solid).

1.5 Flammable liquid

Hazard class not applicable, due to the physical state of the substance evaluated in this dossier (solid).

1.6 Flammable solids

1.6.1

Study reference

M. Leonardi and U. Zucchelli, Internal test report: Relative density, melting point, boiling point and flammability of P_4S_3, 2017.

Test type

The flammability of the substance was tested according to the UN Recommendations on the Transport of Dangerous Good (RTDG), Manual of Tests and Criteria: Test N.1 (Test method for readily combustible solids). No GLP compliance.

Test material

The test material used in the study is equivalent to the substance identified in the C&L dossier.

Test item: P_4S_3. Batch number: 040017. Purity: 55.8% as phosphorous.

Detailed study summary and results

The flammability of the substance was tested according to the UN RTDG, Manual of Tests and Criteria: Test N.1 (Test method for readily combustible solids).

The screening test was positive (the burning of a 200 mm length of the powder train occurred in 39 s) and, therefore, the full burning rate test was performed. The burning times of the test item were less than 45 s: 30, 25, 27, 26, 31, and 29 s. In each and any of the six test runs, the wet zone did not stop the propagation of the flame. Based on the observed burning behaviour, tetraphosphorus trisulphide should be classified as Flammable solid Category 1 with hazard statement H228 (Flammable solid), retaining the pictogram “GHS02: Flame” with the signal word “Danger”, according to the CLP Regulation (EC n.1272/2008). Moreover, division 4.1 packaging group II is assigned, in accordance with the UN RTDG, Manual of Tests and Criteria.

Conclusion

Under the experimental conditions of the UN RTDG, Manual of Tests and Criteria: Test N.1, the burning times of the test item were less than 45 s. In each and any of the six test runs, the wet zone did not stop the propagation of the flame. It can be concluded that tetraphosphorus trisulphide should be classified as Flammable solid Category 1 with hazard statement H228 (Flammable solid), retaining the pictogram “GHS02: Flame” with the signal word “Danger”, according to the CLP Regulation (EC n.1272/2008).

1.7 Self-reactive substances

1.7.1

Data waived: study scientifically not necessary.

According to the CLP Regulation (EC n.1272/2008, Annex I, Part 2, paragraph 2.8.4.2):

Substances and mixtures must be considered for classification in this hazard class as a self-reactive substance or mixture unless:

(a) There are no chemical groups present in the molecule associated with explosive or self-reactive properties; examples of such groups are given in Tables A6.1 and A6.2 in Appendix 6 of the UN Recommendations on the Transport of Dangerous Good (RTDG), Manual of Tests and Criteria.

Tetraphosphorus trisulphide does not contain any functional group that might trigger the classification as a self-reactive substance. In addition, no exothermic decomposition was observed during Differential Thermal Analysis (study reference: M. Leonardi and U. Zucchelli, Internal test report: Relative density, melting point, boiling point and flammability of P_4S_3, 2017). No further testing is, therefore, deemed as necessary.

Conclusion

There are no chemical groups or any other indications associated with explosives or self-reactive properties in the molecule. It can be concluded that tetraphosphorus trisulphide is not to be classified as a self-reactive substance, according to the CLP Regulation (EC n.1272/2008).

1.8 Pyrophoric liquids

Hazard class not applicable, due to the physical state of the substance evaluated in this dossier (solid).

1.9 Pyrophoric solid

1.9.1

Data waived: study scientifically not necessary.

According to the CLP Regulation (EC n.1272/2008, Annex I, Part 2, paragraph 2.10.1):

Pyrophoric solid means a solid substance or mixture which, even in small quantities, is liable to ignite within five minutes after coming into contact with air.

Experience in manufacturing and handling of tetraphosphorus trisulphide demonstrates the stability of the substance in air at room temperature for prolonged periods of time. No further testing is, therefore, deemed as necessary.

Conclusion

Based on the applicant’s experience in the manufacturing and handling of the substance, it can be concluded that tetraphosphorus trisulphide is not to be classified as pyrophoric solid, according to the CLP Regulation (EC n.1272/2008).

1.10 Self-heating substances

1.10.1

Study reference

Younis S, Final report: Flammability Testing on a Sample of Phosphorus Sesquisulphide P_4S_3, 2020.

Test type

The self-heating properties of the substance were tested according to the UN Recommendations on the Transport of Dangerous Good (RTDG), Manual of Tests and Criteria: Test N.4 (Test method for self-heating solids). GLP compliance. No deviations or amendments during the experimental phase of the study.

Test material

The test material used in the study is equivalent to the substance identified in the C&L dossier.

Test item: P_4S_3. Batch number: CS9-6287. Purity: 55.8% as phosphorous.

Detailed study summary and results

The cubic sample containers of 25 mm and 100 mm side lengths are employed. They are open at the top and manufactured from stainless-steel net, with a mesh opening of 0.053 mm. Each container is housed in an outer cubic container cover, manufactured from a stainless-steel net with a mesh opening of 0.595 mm which is slightly larger than the sample container, so that the container fits in this cover. In order to minimise the effects of air circulation, another stainless-steel cage, manufactured with a mesh opening of 0.595 and 150 x 150 x 250 mm in size, houses both these baskets.

The test material, in its commercial form, is filled to the brim of the sample basket, which is then tapped several times. If the sample has been observed to settle, then more material is added. The container is housed in the covers and placed in the centre of the oven at the required test temperature.

A hot air, circulating type oven with an inner capacity of approximately 120 litres and capable of controlling the internal temperature at 140±2 °C is used.

In the test, the oven temperature is raised to 140°C and held isothermally for a test period of 24 hours. The temperature of the sample and the temperature of the oven are continually monitored/recorded with inconel sheathed thermocouples (K-type), to provide accurate temperature measurements. One thermocouple is placed in the centre of the sample and the other between the sample container and the oven wall.

Due to concerns regarding the self-heating properties of the test item raised by the sponsor prior to the initiation of the study, testing was started with the 25 mm cube basket. On completion of the test, no sample remained. Exothermic activity and ignition were observed in the 25 mm basket at 140°C, with a temperature rise of ca. 340°C. Based on these results, tetraphosphorus trisulphide is classified as a Self-heating substance Category 1 with hazard statement H251 (Self-heating: may catch fire), with the pictogram “GHS02: Flame” and with the signal word “Danger”, according to the CLP Regulation (EC n.1272/2008). In addition, division 4.2 packaging group II is assigned, in accordance with the UN RTDG, Manual of Tests and Criteria.

Conclusion

Under the experimental conditions of the UN RTDG, Manual of Tests and Criteria: Test N.4, a positive test result (i.e dangerous self-heating) was observed in a 25 mm sample cube at 140°C, where spontaneous ignition occurred with the sample temperature exceeding the oven temperature by more than 60°C during the 24-hr period. It can be concluded that tetraphosphorus trisulphide is classified as a Self-heating substance Category 1 Self-heating: may catch fire H251 (Self-heating: may catch fire), with the pictogram “GHS02: Flame” and with the signal word “Danger”, according to the CLP Regulation (EC n.1272/2008).

1.11 Substances which in contact with water emit flammable gases

1.11.1

Study reference

Younis S, Final report: Flammability Testing on a Sample of Phosphorus Sesquisulphide P_4S_3, 2020.

Test type

The ability of the substance to emit flammable gases on contact with water was tested according to the UN Recommendations on the Transport of Dangerous Goods (UN RTDG), Manual of Tests and Criteria, Test N.5. (Test method for substances which in contact with water emit flammable gases). GLP compliance. No deviations or amendments during the experimental phase of the study.

Test material

The test material used in the study is equivalent to the substance identified in the C&L dossier.

Test item: P_4S_3. Batch number: CS9-6287. Purity: 55.8% as phosphorous.

Detailed study summary and results

The ability of the substance to emit flammable gases on contact with water was tested according to the (UN RTDG), Manual of Tests and Criteria, Test N.5. (Test method for substances which in contact with water emit flammable gases), by bringing it into contact with water under a variety of conditions. The method consists of three preliminary steps to determine if a violent reaction with water occurs and a final test to determine the quantity of gas generated. If it is known that the sample does not react violently with water the preliminary steps need not be conducted. No further tests are required if a spontaneous ignition occurs at any stage.

Step 1: A small quantity of the sample (ca. 2 mm diameter) is placed in a trough of distilled water at 20°C. It is noted if any gas is evolved and whether spontaneous ignition of the gas occurs.

Step 2: A small quantity of the sample (ca. 2 mm diameter) is placed in the centre of a filter paper floated flat on the surface of distilled water at 20°C. The filter paper is to keep the sample in one place under which condition the likelihood of spontaneous ignition of the gas is greater. It is noted if any gas is evolved and whether spontaneous ignition of the gas occurs.

Step 3: The sample is made into a pile approximately 20 mm high and 30 mm in diameter with a hollow in the top. A few drops of water are added into the hollow. It is noted if any gas is evolved and whether spontaneous ignition of the gas occurs.

Step 4: The substance should be inspected for any particles of less than 500 µm diameter, if the powder constitutes more than 1% (mass) of the total, or if the substance is easily crumbled, then the whole of the sample should be ground to a powder before testing begins. The test should be performed 3 times at ambient temperature (20°C) and atmospheric pressure. The substance (up to a maximum of 25 g) weighed into conical flask to produce between 100 and 200 mL of gas is placed in the flask and a suitable quantity of water added to a dropping funnel. The tap of the dropping funnel is opened to let the water into the conical flask and the logging software is started. The volume of gas evolved is measured and timed using a specifically designed apparatus consisting of a digital thermocouple reader, glass burette, conical flask, molecular strain transducer and a solenoid valve connected to a counter. The time and volume of gas generated is logged using bespoke data acquisition software. By evaluating the logged data in graph form, the rate of evolution of gas is calculated over 7 hours at 1-hour intervals. If the rate of evolution is erratic or is increasing after 7 hours, the measuring time should be extended to a maximum time of 5 days. If, at any time, the rate of gas evolution exceeds 10 L kg^{-1} min^{-1} or spontaneous ignition occurs at any stage the test can be stopped. If the chemical identity of the gas is unknown it should be analysed by any suitable means or tested for flammability.

Three preliminary steps and a final step were conducted. The test item was not sieved prior to testing, due to concerns about potential reaction during the sieving process. No stirring was performed at any stage of the analysis.

No gas generation or spontaneous ignition was observed in the preliminary steps (1 to 3). During the final step, no spontaneous ignition was observed after 5 days for test number 1 and after 7 hours for test number 2-3. The generated gas was however flammable during test number 1, with a maximum rate of gas generation of 0.7 L kg^{-1} h^{-1}.

Final Step Results:

Test Number	**1**	**2**	**3**
Mass of sample (g)	10.7	10.2	10.0
Was a spontaneous ignition observed?	No	No	No
Maximum rate of gas generation (L $kg^{-1}h^{-1}$)	0.7	0.0	0.0
Is the generated gas flammable?	Yes	N/A	N/A
Test duration	5 days	7 hours	7 hours

After Step 4, test 1 the evolved gas was analysed using a mass spectrometer (serial no C1256) and an increased response was found on mass numbers 12, 14, 16, 17, 28, 32, 34, 40 and 44. The increase in mass numbers 32 and 34 is suspected to be due to hydrogen sulphide H_2S, which is classified as Flammable gas Category 1 with hazard statement H220 (Extremely flammable gas).

The maximum rate of gas generated for step 4 test 1 was observed between 99 and 100 hours.

Based on the above results, no classification as per the CLP Regulation (EC) No. 1272/2008 should be given to tetraphosphorus trisulphide. No assignment to Division 4.3 of the (UN RTDG), Manual of Tests and Criteria should be given, either.

Conclusion

Under the experimental conditions of the UN RTDG, Manual of Tests and Criteria: Test N.5, no gas generation or spontaneous ignitions were observed in the preliminary steps. During the final step, no spontaneous ignition was observed after 5 days for test number 1 and after 7 hours for test numbers 2-3. The generated gas was, however, flammable during test number 1, with a maximum rate of gas generation of 0.7 L kg^{-1} h^{-1}. It can be concluded that no classification as per CLP Regulation (EC) No. 1272/2008 should be given for tetraphosphorus trisulphide.

1.11.2

NOTE: As regards the ability of tetraphosphorus trisulphide to emit flammable gases on contact with water, an additional study is available in the REACH Registration dossier. This study was neither conducted under GLP nor conducted according to the UN Recommendations on the Transport of Dangerous Goods (UN RTDG), Manual of Tests and Criteria, Test N.5. (Test method for substances which in contact with water emit flammable gases), and is not considered for the purpose of classification revision. A short summary is presented as follows for information only.

Study reference

Nebuloni M., Study report: Effect of the water on the degradation of P_4S_3 (phosphorus sesquisulphide) CLP classification, 2017.

Study summary and results

The aim of this study was to investigate the effect of water on the degradation of tetraphosphorus trisulphide in relation to the emission of flammable gases.

The investigation was carried out by monitoring the hydrogen sulphide (H_2S) evolution when the sample (P_4S_3, batch 040017) was loaded in water, using a peculiar laboratory equipment according to the Dietrich-Fruhling method.

The equipment consisted of a glass container, in which the reaction between tetraphosphorus trisulphide and water took place. The produced gas was collected and measured by a device connected to the container. The volume of the produced gas depends on the sample amount; from the volume of the gas produced over time, the gas evolution rate can be calculated.

The test item (5 g) was dropped into the vessel connected to the equipment, containing 50 mL of water at 25°C. Then the suspension was periodically shaken over 24 hours and the evolved gas was collected into the graduate cylinder connected to the vessel.

No degradation with relevant gas evolution was detected after 24 hours.

1.12 Oxidising liquids

Hazard class not applicable, due to the physical state of the substance evaluated in this dossier (solid).

1.13 Oxidising solids

1.13.1

Data waived: study scientifically not necessary.

According to the CLP Regulation (EC n. 1272/2008), 2.14.4.1 section, for organic substances or mixtures the classification procedure for the oxidising substance class shall not apply if:

- the substance or mixture does not contain oxygen, fluorine or chlorine; or

- the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bound only to carbon or hydrogen.

For inorganic substances or mixtures, the classification procedure shall not apply if they do not contain oxygen or halogen atoms.

Tetraphosphorus trisulphide satisfies the screening criteria reported in the CLP Regulation, equivalent in principle to Section 6 of Appendix 6 to the UN RTDG, Manual of Tests and Criteria, since it is an inorganic substance which does not contain any oxygen or halogen atom. In addition, testing is not applicable to flammable solids.

Conclusion

Tetraphosphorus trisulphide does not contain any oxygen or halogen atoms. According to the screening procedure provided for the identification of oxidizing solids, it can be concluded from its structure that tetraphosphorus trisulphide is not to be classified as an oxidizing solid, according to the CLP Regulation (EC n.1272/2008).

1.14 Organic peroxides

Hazard class not applicable, the substance evaluated in this dossier is not an organic peroxide.

1.15 Corrosive to metals

Hazard class not applicable, due to the physical state of the substance evaluated in this dossier (solid).

There is no reference in the definition of corrosive to metals (as in the CLP regulation, Annex I, 2.16.1 section) to the physical state of the substances or mixtures that need consideration for potential classification in this hazard class. However, according to the classification criteria, only substances and mixtures for which the application of the UN Test C.1 (described in part III, Section 37.4.1.1 of the UN RTDG, Manual of Tests and Criteria) is relevant need to be considered.

Application of classification criteria in the UN RTDG, Manual of Tests and Criteria, Section 37.4 excludes solids, while ‘liquids and solids that may become liquids (during transport)’, should be considered for such a classification. It can be concluded that, based on its physical state (solid) and melting point (174°C / Study reference: M. Leonardi and U. Zucchelli, Internal test report: Relative density, melting point, boiling point and flammability of P_4S_3, 2017), this hazard class does not apply to tetraphosphorus trisulphide.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3 HEALTH HAZARDS

3.1 Acute toxicity

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3.2 Skin corrosion/irritation

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3.3 Serious eye damage/eye irritation

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3.4 Respiratory sensitisation

Not evaluated in this dossier.

3.5 Skin sensitisation

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3.6 Germ cell mutagenicity

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3.7 Carcinogenicity

Not evaluated in this dossier.

3.8 Reproductive toxicity

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3.9 Specific target organ toxicity – single exposure

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3.10 Specific target organ toxicity – repeated exposure

Not evaluated in this dossier. For further details refer to the REACH Registration Dossier.

3.11 Aspiration hazard

Not evaluated in this dossier.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

According to the REACH Regulation (EC n. 1907/2006), Annex VII, Column 2, the study does not need to be conducted if the substance is inorganic.

4.1.2 BOD$_5$/COD

Not available.

4.1.3 Hydrolysis

Study reference

Nebuloni M., Study report: Degradation of P4S3 (phosphorus sesquisulphide): Hydrolysis in function to the pH (OECD/OCDE 111 directives), 2017.

Test guideline

Test performed according to: OECD Guideline 111 (Hydrolysis as a Function of pH). No GLP compliance.

Detailed study summary and results

The aim of this study was to investigate the rate of hydrolysis of the test substance as a function of pH and to determine the identity and rate of formation of the hydrolysis products.

Method

This investigation was carried out by monitoring the hydrogen sulfide (H_2S) evolution when the sample was loaded in controlled buffer pH solutions (pH= 4,7 and 8) for 5 days. The gaseous product (H_2S) was collected and measured by a device connected to the container according to the Dietrich-Fruhling method. From the volume of the produced gas (H_2S) in relation to the sample amount and from the volume evolution on the time, it was possible to assess abiotic hydrolytic transformations of P_4S_3 in aquatic systems at pH values normally found in the environment. For the assessment, the solubility at room temperature of H_2S in water was also considered (0.385 g/100 g H_2O).

Results

No remarkable hydrolysis to H_2S in buffered solutions was observed and thus the substance is considered as hydrolytically stable. There was a minor decrease of the pH of the solutions that might be ascribed to a low amount of phosphates and to the H_2S developed during the 5-day test.

Conclusion

Tetraphosphorus trisulfide is stable to hydrolysis at room temperature in acid, neutral and basic conditions.

4.2 Bioaccumulation

Not available.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

Study reference

Anonymous 2017: Phosphorus sesquisulphide 96-hour Acute Toxicity to *Danio rerio* (Zebrafish) OECD 203 Commission Regulation (EC) No 440/2008.C.1. (study report), Report no: <confidential>. Owner company; <confidential>, Report date: Nov 9, 2017

Detailed study summary and results

The acute lethal toxicity of tetraphosphorus trisulphide to the Zebrafish *Danio rerio* was investigated according to test guideline OECD TG 203 and EU Mehod C.1., under static exposure conditions over a period of 96 h.

The only applied loading rate was 100 mg/L. Therefore, this study represents a limit test. This test concentration was chosen based on preceding acute toxicity tests carried out with *Algae* and *Daphnia*. A 72 h E_rC_{50} of >100 mg/L and a 48 h EC_{50} of >100 mg/L, based on loading rates, were determined in the *Algae* and *Daphnia* toxicity test, respectively.

The test item is solid, poorly soluble in water (29.3 mg/L at a pH 3.6 and ca. 20°C) and with a purity of 55.8% as phosphorous. Therefore, the test medium consisted of a saturated stock solution, prepared by addition of the test item to natural water, 96-h stirring and filtration.

The concentration of the substance was determined by phosphorus-analytics at the beginning and at the end of the exposure phase. These analyses confirmed the low solubility of the test item. The effective concentrations were assessed based on the geometric mean of the measured concentrations of the test item, as well as on the loading rates.

At the loading rate of 100 mg/L of tetraphosphorus trisulphide, none of a total of seven fish died after 96 h of exposure. No mortality was observed in the blank control.

Toxic effects other than mortality, such as loss of coordination, hypo- or hyperactivity and swimming on the back, were not observed.

The no-observed-effect concentration (NOEC) was 5.82 mg/L mean measured concentration (based on phosphorus-analytics) or 100 mg/L loading rate.

Based on the binominal theory, the absence of mortality in such a test design provides at least 99% confidence that the median lethal concentration (LC_{50}) of the test item to *Danio rerio* (Zebrafish) is greater than the measured concentration of 5.82 mg/L or 100 mg/L loading rate.

Materials and methods

Test guideline

The test was performed according to test guideline OECD TG 203 (1992) and EU Method C.1 in GLP compliance and with no deviation.

Test material

The test material used in the study is equivalent to the substance identified in the C&L dossier.

Test item: P_4S_3. Batch number: 040017. Purity: 55.8% as phosphorous.

Test organisms

- Test species: *Danio rerio* (Zebrafish)

  Details on test organisms:
- Acclimation period: the fish were gradually acclimatized to the test conditions and were held for at least an additional 12 days prior to testing.
- Age at study initiation: young fish
- Body length at study initiation: between 1 and 3 cm.

Main Study Design

Test conditions

- Test type: static.
- The temperature was kept between 21°C and 22°C during all study in the test solution and also in blank control (within recommended range 21-25 °C, controlled at ± 2°C). pH = between 6.7 and 8.1 for the test solution and between 7.2 and 8.4 for blank control (within the recommended range 6.0-8.5). Dissolved oxygen: between 7.3 and 8.2 mg O_2/L for test solution and between 7.7 and 8.2 mg O_2/L for blank control (above the required range > 60% of the air saturation value at 21°C, i.e. >5.34 mg O_2/L).
- Test duration: 96 h

Test concentrations

- Tested doses: limit test at 100 mg/L done on 7 fish.
- Nominal concentration: limit test at 100 mg/L (loading rate). After stirring for 96 h of mixture between test item and fish medium, the test solution was filtered and the test item concentration was measured. Measured concentration: 5.88 mg/L at t = 0 h and 5.75 mg/L after 96 h. Geometric mean: 5.82 mg/L.
- Monitoring of test concentrations: the determination of the test item concentrations was based on phosphorus-analytics and total P was determined by photometry after a chemical extraction step.
- Results used to determine the conditions for the definitive study: the concentration for main test (limit test) was chosen based on preceding acute toxicity studies with *Algae* and *Daphnia* where a 72 h E_rC_{50} and a 48 h EC_{50} (respectively) of >100 mg/L loading were determined.

Results

At the loading rate of 100 mg/L of tetraphosphorus trisulphide, none of a total of seven fish died after 96 h of exposure. No mortality was observed in the blank control. Toxic effects other than mortality, such as loss of coordination, hypo- or hyperactivity and swimming on the back, were not observed.

Other measurements throughout the test: all values regarding pH-values, temperature and oxygen concentration were within the acceptable range. The validity criteria of the study were fulfilled.

The measured concentration by photometry in test solution was 5.88 mg/L at t = 0 h and 5.75 mg/L at t = 96 h (geometric mean: 5.82 mg/L).

LC_{50} 96 h *Danio Rerio* >100 mg/L loading rate

4.3.2 Short-term toxicity to aquatic invertebrates

Study reference

Anonymous 2017: Phosphorus sesquisulphide- 48-hour Acute Toxicity to *Daphnia magna* OECD 202, Commission Regulation (EC) No 440/2008.C.2. (study report), Report no: <confidential>. Owner company; <confidential>, Report date: Nov 3, 2017.

Detailed study summary and results

The median effect concentration (EC_{50}) and the no-effect concentration (NOEC) of tetraphosphorus trisulphide to *Daphnia magna* were investigated under static exposure conditions over a period of 48 h, following guideline OECD TG 202 and EU Method C.2.

The test item tetraphosphorus trisulphide is solid, poorly soluble (about 3 mg/L in *Daphnia* medium) and with a purity of 55.8% as phosphorous. The test media was prepared by directly adding the test item to *Daphnia* medium in a glass vessel, stirring this mixture for 96 h and filtering it. The single investigated loading rate was 100 mg/L. Forty individual *Daphnia* divided into 2 test vessels were exposed to the test item, and 60 *Daphnia* divided into 3 test vessels were used as blank controls. The determination of the test item concentrations was based on phosphorus-analytics (photometry after a chemical extraction step). Total phosphorus (total P) was measured at the beginning and at the end of the test. These analyses revealed that the concentrations of test item were stable over the whole 48 h period and corresponded to 2.8% and 2.7% of the nominal loading rate at the beginning and end of the test, respectively. The effective concentrations EC_x were assessed based on the loading rates of the test item as well as on the measured concentration.

No significant effects (≤10% immobilization) were observed at 100 mg/L loading rate or in the blank controls, neither after 24 h of exposure nor after 48 h. Therefore, the median effect concentrations (EC_{50}) of tetraphosphorus trisulphide on *Daphnia magna* after 24 h as well as after 48 h of exposure were estimated to be >100 mg/L loading rate or >2.8 mg/L measured concentration.

The NOEC values after 24 h and after 48 h of exposure were 100 mg/L loading rate or 2.8 mg/L measured concentration. All validity criteria were fulfilled.

Materials and methods

Test guideline

The test was performed according to OECD Guideline 202 (2004) and according to EU method C.2. The study was performed in GLP compliance.

Test material

The test material used in the study is equivalent to the substance identified in the C&L dossier.

Test item: P_4S_3. Batch number: 040017. Purity: 55.8% as phosphorous.

Details on Test organisms

- Test species: *Daphnia magna* (Straus, 1820)
- Origin: derived from a healthy stock and not first brood progeny
- Species life stage: less than 24 h
- Acclimation period: the *Daphnia* were already acclimatized before the test. Parental and young *Daphnia* held in 4 L glass aquaria (3 L medium) at 20±2 °C. *Daphnia* were fed only during acclimation period using a suspension of *Desmodesmus subspicatus* in Elendt M4 medium with an optical density OD680 of about 15 units.

Main study design

Test conditions

- Test type: static
- pH: between 6.7 and 7.1 for test solutions and between 6.5 and 7.0 for blank controls. Variation for control replicates: 0.5 unit (required: not more than 1.5 unit).

- Temperature: the temperature was maintained between 21° and 22°C for all the study period (required range of 18-22 °C, controlled at ± 1°C).
- Dissolved oxygen: between 6.0 and 7.5 mg/L for test solutions and between 6.3 and 7.9 mg/L for blank controls (required ≥3 mg O_2/L).
- Conductivity: 695 µS/cm for test solutions and 685 µS/cm for blank controls.
- Photoperiod:16 h photoperiod a day.
- Light intensity: overhead white fluorescent tubes.
- Test duration/total exposure duration: 48 h.

Test concentrations

- Limit test at 100 mg/L.
- Preparation of test solutions: after stirring for 96 h of mixture between test item and medium, the test solution was filtered and the test item concentration was measured.
- Monitoring of concentrations: the determination of the test item concentrations was based on phosphorus-analytics (photometry after a chemical extraction step). Total phosphorus (total P) was measured at the beginning and at the end of the test.
- Measured concentration: 2.76 mg/L at t = 0 h and 2.74 mg/L after 48 h. Geometric mean: 2.8 mg/L.

Test system

- Test vessel: 100 mL beakers, all-glass, with 50 mL of test medium, covered with a glass plate to avoid evaporation and contamination of the test solutions with dust.
- Aeration: the *Daphnia* were not aerated during the test.
- No. of organisms per vessel: 40 individuals per test concentration, 20 per vessel
- No. of vessels per concentration (replicates): only one concentration (100 mg/L) with two replicates of 20 *Daphnia* each.
- No. of vessels per control (replicates): three replicates with 20 *Daphnia* each.
- Feeding during test: the *Daphnia* was not fed during the test and the test vessels were not aerated.
- Effects measured: Immobility: observations of immobile *Daphnia* were made after 24 and 48 h of exposure. Any abnormal behaviour or appearance was reported.

Results

No significant effects (≤10% immobilization) were observed at 100 mg/L loading rate or in the blank controls, neither after 24 h of exposure nor after 48 h.

Therefore, the median effect concentrations (EC_{50}) of the test item on *Daphnia magna* after 24 h as well as after 48 h of exposure were estimated to be >100 mg/L loading rate or >2.8 mg/L measured concentration.

The NOEC values after 24 h and after 48 h of exposure were 100 mg/L loading rate or 2.8 mg/L measured concentration.

Monitoring of test concentrations: the concentrations of test item were stable over the whole 48 h period and corresponded to 2.8% and 2.7% of the nominal loading rate at the beginning and end of the test, respectively. The effective concentrations EC_x were assessed based on the loading rates of the test item as well as on the measured concentration.

Other measurements throughout the test: all values regarding pH-values, oxygen concentrations, conductivity and temperature were within the acceptable range. The geometric mean between the measured concentrations at t = 0 h and at t = 48 h was 2.8 mg/L.

The validity criteria of the study were fulfilled.

EC_{50} 48 h *Daphnia magna* >100 mg/L.

4.3.3 Algal growth inhibition tests

Study reference

Anonymous 2017: Phosphorus sesquisulphide - Fresh water algal growth inhibition test with *Desmodesmus subspicatus* OECD 201, Commission Regulation (EC) No 761/2009.C.3. (study report), Report no: <confidential>. Owner company; <confidential>, Report date: Nov 3, 2017.

Detailed study summary and results

The growth inhibitory effects of tetraphosphorus trisulphide to the green alga *Desmodesmus subspicatus* were investigated according to test guideline OECD TG 201 and EU Method C.3, over a period of 72 h.

The test item is solid, poorly soluble (about 2-3 mg/L in algal medium) and with a purity of 55.8% as phosphorous. The test media was prepared by directly adding the test item to algal medium in a glass vessel, stirring this mixture for 96 h and filtering it. The only loading rate was 100 mg/L. Six parallel test vessels were used for the test item and six for the blank controls. The determination of the test item concentrations was based on phosphorus-analytics (photometry after a chemical extraction step). Total phosphorus (total P) was measured at the beginning and at the end of the test. These analyses revealed that the concentrations of test item were stable over the whole 48 h period and corresponded to 2.8% and 2.2% of the nominal loading rate at the beginning and end of the test, respectively. The effective concentrations EC_x were assessed based on the loading rates of the test item as well as on the measured concentration. With respect to both endpoints growth rate and yield (algal biomass), no significant effects were observed at 100 mg/L loading rate, as compared to the blank controls.

Therefore, 72 h E_rC_{50} and E_yC_{50} values of the test item on the green alga *Desmodesmus subspicatus* were estimated to be >100 mg/L loading rate or >2.5 mg/L measured concentration. The NOE_rC and NOE_yC values were both 100 mg/L loading rate or 2.5 mg/L measured concentration. All validity criteria were fulfilled.

Materials and methods

Test guideline

The test was performed according to OECD Guideline 201 (2006) and according to EU method C.2. The study was performed in GLP compliance.

Test material

The test material used in the study is equivalent to the substance identified in the C&L dossier.

Test item: P_4S_3. Batch number: 040017. Purity: 55.8% as phosphorous.

Test organisms

- Test species: *Desmodesmus subspicatus.*
- Initial cell concentration: 0.5-0.85 µg/mL with respect to dry weight corresponding to about 2-5*10^3 cells/mL (i.e. OD680 of about 0.005 units; all test solutions contain the same initial biomass of test alga).

Main study design

Test conditions

- Test type: static
- Exposure duration: 72 h
- Temperature: between 19.6°C and 23.5°C; Mean: 21.8 (required: 21 to 24°C, maintained at ± 2°C). The temperature during the test was out of range. Since the lower temperatures occurred only for a couple of hours at the beginning of the test, and since the test vessels were randomly re-placed each day, this deviation is not expected to have any significant influence on the outcome of the test.
- Lighting: continuous (2000–3000 lux) from Osram Fluora L18W77 and Osram Daywhite L18W840 (Osram AG, Winterthur, Switzerland). The light intensity amounts to about 3000 lux which corresponds to about 40 µE•m^-2•s^-2. The present light intensity is lower than the 60-120 µE•m^-2•s^-2 recommended by the OECD TG 201. However, based on laboratory experience, the simultaneous use of Osram Fluora L 18W77 (suitable for plant growth) and Osram Daywhite L 18W840 (Osram AG, Winterthur, Switzerland) enable optimal growth conditions at a light intensity of 40 µE•m^-2•s^-2 to fulfil the required quality criteria according to OECD while yielding the “fittest” algal cells. The light intensity is maintained within ±15% from the average light intensity over the incubation area. The test vessels are re-positioned every day within the incubation area to minimize variation.
- pH: between 7.4 (start) and 8.1 (end) for blank control and between 7.4 (start) and 8.2 (end) for the only test item concentration. Variation for control replicates: 0.7 unit (required: not more than 1.5 unit).
- Adjustment of pH: initially adjusted to 8 ± 0.2; the pH of the control medium should not increase by more than 1.5 units during the test.

Test system

- Test medium*:* Growth medium: the growth medium was the same of test medium.

  Recommended OECD medium: NH_4Cl 15 mg/L, $MgCl_2$ x 6 H_2O: 12 mg/L, $CaCl_2$ x 2 H_2O: 18 mg/L, $MgSO_4$ x 7 H_2O: 15 mg/L, KH_2PO_4: 1.6 mg/L, $FeCl_3$ x 6 H_2O: 0.064 mg/L, Na_2-EDTA x 2 H_2O: 0.1 mg/L, H_3BO_3:0.185 mg/L, $MnCl_2$ x 4 H_2O: 0.415 mg/L, $ZnCl_2$: 3*10^{-3} mg/L, $CoCl_2$ x 6 H_2O: 1.5*10^{-3} mg/L $CuCl_2$ x 2 H_2O: 10^{-5} mg/L, Na_2MoO_4 x 2 H_2O: 7*10^{-3} mg/L, $NaHCO_3$: 50 mg/L. pH = 8.0 ± 0.2.
- Test vessel: 250 mL flasks, all-glass, with 100 mL of test medium, shaken (130 rpm), capped with air permeable stoppers.
- Effects measured: Determination of cell concentrations: cell concentrations were determined every 24 h using a spectrophotometer at 680 nm wavelength.

Test Concentrations

- Range finding study: before the main test a range finding study was performed at the following nominal concentrations: undiluted, 10x, 100x (i.e. 100, 10, 1 mg/L) equivalent respectively to measured concentrations 2.99, 0.30, 0.03 at t = 0 h and to measured concentrations 2.60, 0.16, 0.02 at t = 72 h
- Results used to determine the conditions for the definitive study: in the range-finding test E_rC_{50} value (based on growth rate) was found to be >>100 mg/L loading so the definitive test was performed as limit test at 100 mg/loading rate.
- No. of vessels per concentration (replicates): limit test 1 concentration with six replicates.
- No. of vessels per control (replicates): six replicates.

Results

Effect Concentrations based on Growth Rate and on Yield

With respect to both endpoints growth rate and yield (algal biomass), no significant effects were observed at 100 mg/L loading rate, as compared to the blank controls. Therefore, 72h E_rC_{50} and E_yC_{50} values of tetraphosphorus trisulphide on the green alga *Desmodesmus subspicatus* were estimated to be >100 mg/L loading rate or >2.5 mg/L measured concentration. The NOE_rC and NOE_yC values were both 100 mg/L loading rate or 2.5 mg/L measured concentration.

Test Concentration

The determination of the test item concentrations was based on phosphorus-analytics (photometry after a chemical extraction step). Total phosphorus (total P) was measured at the beginning and at the end of the test. These analyses revealed that the concentrations of test item were stable over the whole 48 h period and corresponded to 2.8% and 2.2% of the nominal loading rate at the beginning and end of the test, respectively. The effective concentrations EC_x were assessed based on the loading rates of the test item as wel as on the measured concentration.

The validity criteria of the study were fullfilled.

E_rC_{50} 72 h *Desmodesmus subspicatus* >100 mg/L loading rate and E_yC_{50} 72 h *Desmodesmus subspicatus* >100 mg/L loading rate (Note: concentration as loading rate before 96 h stirring followed by filtration and this solution was inoculated with algal medium. The geometric mean between the measured concentrations at t = 0 h and at t = 72 h was 2.5 mg/L. This value confirms the low solubility of test item in the algal medium).

4.3.4 *Lemna* sp. growth inhibition test

Not available.

4.4 Chronic toxicity

No additional data.

4.5 Acute and/or chronic toxicity to other aquatic organisms

Not available.