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Helsinki, 01 July 2019

Substance name: O,O,O-triphenyl phosphorothioate
EC number: 209-909-9
CAS number: 597-82-0
Date of latest submission(s) considered[1]: 10 April 2019
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressee(s): Registrant(s)[2] of O,O,O-triphenyl phosphorothioate (Registrant(s))

## DECISION ON SUBSTANCE EVALUATION

Based on Article 46(1) of the REACH Regulation (Regulation (EC) No 1907/2006), you are requested to submit the following information on the registered substance:

1. Simulation testing on ultimate degradation in surface water: Aerobic mineralisation in surface water – simulation biodegradation test, test method EU C.25. / OECD TG 309 with the registered substance. Test must be conducted as a pelagic test using EU representative surface water with a suspended solids concentration of approximately 15 $mg_{dw}/L$ (but not outside the range of 10 to 20 $mg_{dw}/L$) at a temperature of 12°C. Care must be taken that no test concentration used is above the aqueous solubility of the registered substance in the test media. Also transformation products must be identified and reasonable attempts must be made to quantify them. Radiolabelled substance must be used with the radiolabel being located in the most recalcitrant part of the molecule. A mass balance must also be provided.

If the registered substance is considered persistent (P) or very persistent (vP) according to the Annex XIII criteria, the following information must be provided:

2. Long-term toxicity testing on aquatic invertebrates: *Daphnia magna* reproduction test, test method EU C.20. / OECD TG 211 with the registered substance. All reasonable efforts must be made to achieve exposure concentrations up to the aqueous solubility of the registered substance in the test medium. As detailed in Appendix 1, sampling for exposure concentrations must be done regularly and exposure concentrations must be recalculated based on a time-weighted average.

Information requirement 2 may be waived if the currently generated mammalian toxicity data are available and allow a conclusion that the registered substance meets the T criterion according to the Annex XIII criteria. If the mammalian toxicity data and requirement 2 do not allow a conclusion that the registered substance meets the T criterion

[1] This decision is based on the registration dossier(s) on the day until which the evaluating MSCA granted an extension for submitting dossier updates, which it would take into consideration.

[2] The terms registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

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according to the Annex XIII criteria, the following information must be provided:

3. Long-term toxicity testing on fish: Fish, early-life stage (FELS) toxicity test, test method OECD TG 210, with rainbow trout (*Oncorhynchus mykiss*) with the registered substance. All reasonable efforts must be made to achieve exposure concentrations up to the aqueous solubility of the registered substance in the test medium. As detailed in Appendix 1, sampling for exposure concentrations must be done regularly and exposure concentrations must be recalculated based on a time-weighted average.

If the registered substance does not meet the T criterion based on information requirements 2, 3 and based on available data from the currently generated and ongoing mammalian toxicity tests, but is considered to be very persistent (vP) according to the Annex XIII criteria, the following information must be provided:

4. Bioaccumulation in fish: Bioconcentration flow-through fish test, test method EU C.13 / OECD TG 305, aqueous exposure with the registered substance. Care must be taken that no concentration used is above the solubility limit of the registered substance in the test medium. Excessive fish growth and lipid increases must be avoided and the results must be corrected for growth and normalised to 5% lipid content.

You must provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the chemical safety report by **01 April 2021** if the results of information requirement 1 do not allow to conclude that the registered substance is (v)P according to the Annex XIII criteria.

If the results of information requirement 1, 2 and 3 allow to conclude that the registered substance is P and T according to the Annex XIII criteria, you must provide an update of the registration dossier(s) by **03 January 2023**.

Information requirement 3 may be waived if information requirement 2 allows a conclusion that the registered substance meets the T criterion according to the Annex XIII criteria, in that case the deadline for the information requirements is **03 October 2022**. An update of the registration dossier(s) by **02 October 2023** must be provided when all information requirements are to be performed.

The full study report(s) have to be submitted for all information requests. The deadlines take into account the time that you may need to agree on which of the registrant(s) will perform the required tests.

The reasons of this decision and any further test specifications are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

**Who performs the testing?**

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the studies on behalf of all registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

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**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 a suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals

Authorised[3] by Christel Schilliger-Musset, Director of Hazard Assessment

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

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**Appendix 1: Reasons**

Based on the evaluation of all relevant information submitted on O,O,O-triphenyl phosphorothioate (TPPT) and other relevant available information, ECHA concludes that further information is required to enable the evaluating Member State competent authority (eMSCA) to complete the evaluation of whether the substance constitutes a risk to the environment.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested to clarify the concern for PBT and vPvB.

There are also other dossier- and substance evaluations under the REACH Regulation on TPPT and related substances ongoing, respectively. ECHA is coordinating these different processes to avoid any unnecessary data requests. TPPT is the main constituent of "a mixture of: triphenylthiophosphate and tertiary butylated phenyl derivatives", which will be assessed under substance evaluation by the Dutch Competent Authority in 2021.

The concern(s) identified, and what is the possible regulatory outcome

TPPT was placed on the Community rolling action plan (CoRAP) due to concerns that it could be a persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) substance. In the view of ECHA, TPPT is considered to screen as P/vP. Regarding bioaccumulation, ECHA considers that the available information is sufficient to assess if the substance is B, but insufficient to assess if it is vB. The human and environmental toxicological information available is considered insufficient to assess the toxicity in terms of the T criterion of Annex XIII of the REACH Regulation.

As the registered substance is supplied in volumes exceeding 100 tonnes per year, and applications include those with a wide dispersive use, the PBT/vPvB concern must be clarified.

A tiered approach will be followed where information on environmental degradation half-live in surface water is requested first. Toxicity data is only required if the registered substance meets the definitive (v)P criterion, and bioaccumulation data only if the registered substance meets the vP criterion, but not the T criterion.

The sequence of the tiered approach deviates from the standard PBT assessment sequence. The reason is that the available information is regarded as sufficient to assess bioaccumulation against the B criterion, but not sufficient to assess against the vB criterion. A new bioaccumulation study, which involves vertebrate animals, should only be done if the vP criterion, but not the T criterion, is met.

The T testing strategy starts with determining if the currently generated mammalian toxicity data allow to conclude on the mammalian T criterion, followed by testing on invertebrate animals, and only if this study does not allow to conclude that the T criterion has been met will a new test on vertebrate animals be conducted. This approach is considered a refinement of the testing strategy, and as such contributes to animal welfare.

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In reply to your general comment it is noted that the evaluating MSCA would be available for clarification/discussion with you after each step of the tiered strategy.

The requested information will allow to conclude if the registered substance is a PBT/vPvB substance, and if further regulatory risk management measures, such as identification as a SVHC substance and the subsequent authorisation or restriction of TPPT, will be required.

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1. **Simulation testing on ultimate degradation in surface water: Aerobic mineralisation in surface water – simulation biodegradation test;**

Why new information is needed

The registered substance is neither readily nor inherently biodegradable as shown by screening tests and, therefore, screens as (v)P. Simulation biodegradation data are not available to conclude on the definitive (v)P criterion.

A hydrolysis as a function of pH study according to OECD TG 111 is available for TPPT conducted at a concentration of 19 µg/L. The Registrant(s) extrapolated for TPPT half-lives ($t_{1/2}$) at 20°C of 34, 149 and 163 days at pH 9, 7 and 4, respectively, and reported phenol formation of up to 5.8 µg/L within 30 days of incubation. These $t_{1/2}$ correspond to 60, 278, and 287 days at the EU relevant environmental temperature of 12°C, which would indicate slow hydrolysis of TPPT under environmentally relevant conditions. However, re-evaluation of this study by the evaluating MSCA identified severe shortcomings. The most important shortcoming was that sterile conditions were not maintained during testing, i.e. measures were included to minimise the process of microbial degradation during incubation (buffer solutions were filtrated sterile, incubation vessels were rinsed with ethanol and dried under UV light prior to usage), but sterility tests (which were performed only for the solutions at 50°C) showed that at test end none of the vessels at 50°C remained sterile. Consequently, hydrolysis of TPPT may have been overestimated, as biodegradation could have contributed to the decrease in TPPT concentration, especially at physiological conditions. The non-sterile conditions also affect the usability of phenol formation as indirect measure of TPPT hydrolysis, as microbial activity could lead to increased phenol formation, but also to phenol disappearance (phenol is readily biodegradable, while it does not hydrolyse (ECB, 2006)). Considering the other shortcomings, i.e. test concentration might have exceeded half the saturation concentration of TPPT (water solubility is 20-38 µg/L at 20°C, pH 7), measured concentrations immediately after preparation were for several treatments outside the required range of 90-110% and in two cases even below 70%, and for the calculation of hydrolysis rates, you made arbitrary choices where measurements at start and those close to the limit of quantification were left out of the data set, it has to be concluded that this study does not allow to derive reliable $t_{1/2}$ values for hydrolysis of TPPT. This study does allow to conclude that TPPT is degraded to some extent at all three pH levels, but even under non-sterile conditions observed degradation was limited.

Two $CO_2$ evolution studies according to OECD TG 301B are available for TPPT with the study conducted at 0.26 µg $^{14}$C-TPPT/L reporting 17.8-19.3% degradation after 29 days based on $CO_2$ measurements, and the study conducted at 10 and 20 mg TPPT/L reporting 0-2% degradation after 28 days also based on $CO_2$ measurements. As the test concentrations used in the latter study greatly exceeded the water solubility of TPPT of 20-36 µg/L and an emulsifier was used, the data are considered supporting only. Considering degradation is below 60%, it can be concluded that TPPT is not readily biodegradable.

A Zahn-Wellens study according to OECD TG 302B is available for TPPT conducted at 0.26 µg $^{14}$C-TPPT /L that reported after 29 days of incubation a degradation of 59.5-66.8%

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based on total radioactivity measurements and not dissolved organic carbon (DOC) removal. You noted that the tested concentration was too low for DOC measurements, and DOC was not determined. Furthermore, the radioactivity in the application solution was too low for high performance liquid chromatography with radiometric detection (HPLC/RAM) analysis, thus only liquid scintillation count (LSC) measurements were performed. The degradation percentages refer thus to total radioactive residues (TRRs) in the test medium as measured by thin layer chromatography (TLC). Therefore, the results are rather difficult to interpret, as no distinction can be made between parent and/or metabolites, and if losses are due to $CO_2$ formation or other processes such as adsorption. In any case, as 70% mineralisation was not reached within seven days, it can be concluded that TPPT is not inherently biodegradable.

The Registration dossier contained also one hydrolysis and two ready biodegradability studies that showed hardly any degradation and that were conducted with the multi-constituent substance named 'a mixture of triphenylthiophosphate and tertiary butylated phenyl derivates' (CAS 192268-65-8) to which you propose read-across. The source substance consists of several constituents with the major constituent [redacted]) being identical to the target substance TPPT. All three studies were conducted at concentrations greatly exceeding water solubility of TPPT and the other constituents. Furthermore, the measurements, i.e. phenol formation in the hydrolysis study and biological oxygen demand (BOD) in the closed bottle and Modified MITI tests, did not allow distinction between biodegradation of TPPT and the other constituents. In the latter study, lack of biodegradation (0%) was also demonstrated by measuring residuals. Overall, these studies support the conclusion that TPPT is not readily biodegradable, but they are of limited value for the P assessment of TPPT.

The screening studies showed that the registered substance is neither readily nor inherently biodegradable, but also that some primary degradation occurs under the prevailing testing conditions. Based on residual measurements, primary degradation amounted after 29 days of incubation to 39.2-48.5% in the $CO_2$ evolution study and 59.5-66.8% under the more favourable conditions of the Zahn-Wellens study. The degradation products were not identified, but the Pathway Prediction System of the University of Minnesota Biodegradation and Bioremediation Database, which is now hosted at EAWAG, Switzerland (EAWAG-BBD PPS) (Gao *et al.*, 2010), considers the removal of one or more phenol groups likely, while the reduction of the thiol group is considered neutral (and thus less likely). It should be kept in mind though that the data underlying these EAWAG-BBD PPS predictions were obtained from laboratory studies where bacterial strains were adapted to use organophosphorus insecticides as sole carbon and energy source (Munnecke *et al.*, 1976; Rani *et al.*, 2009; Yang *et al.*, 2011). Therefore, the EAWAG-BBD PPS predictions do not necessarily mean that TPPT will also be biodegraded in significant amounts in the environment where conditions are less favourable, and adapted microorganisms are rarely present. Since there are no simulation data on degradation in water, soil or sediment available, no conclusion can be drawn with regard to the persistence of the registered substance and if the (v)P criteria will be met. ECHA therefore considers it necessary to request a simulation study and to request the identification of transformation products.

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Considerations on the test method and testing strategy

Three simulation test methods are available that assess persistence in soil (OECD TG 307), sediment (OECD TG 308) or surface water (OECD TG 309). In order to determine which simulation test is the most appropriate method for addressing degradation of TPPT, the compartment of concern needs to be identified.

You did not provide an exposure estimation in the chemical safety report (CSR), stating that it is not necessary as no hazard with regard to physico-chemical properties, human health, or the environment was identified. From the CSR it is clear though that exposure to all environmental compartments can occur, both during the manufacturing phase, where closed and non-closed processes are used and where transfer takes place in dedicated and non-dedicated facilities, as during the usage phase, where lubricant additives, lubricants and greases containing the registered substance are used in vehicles, machinery and open systems by consumers, professionals and at industrial sites.

Assuming equal emissions to air, water and soil, the Estimation Program Interface EPI Suite™ Level III fugacity model predicts that 43% of the TPPT emitted in the environment will end up in soil, 51% in sediment and 5.6% in surface water. When emission is assumed to occur via water only or equally via air and water, close to 10% of the emitted TPPT ends up in water, and around 85-90% in sediment. This distribution pattern is supported by the physicochemical properties of the registered substance, i.e. the high adsorption coefficient log $K_{oc}$ of 5.0 (HPLC estimate) supports the high potential for adsorption to organic matter in sediment and soil, while the Henry's Law constant of 0.59 Pa*m³/mol at 25°C and the Henry Coefficient $K_{air,water}$ of 0.00028 (both calculated) indicate low volatility from the water surface. Overall, it appears that a large fraction of the emitted TPPT may end up in soil and sediment, but also that a significant amount may remain in the surface water compartment. Therefore, these three compartments are considered compartments of concern.

As there is no single compartment of specific concern, simulation testing on ultimate degradation in surface water (OECD TG 309) is the preferred method. Firstly, the aquatic compartment is by default considered a relevant compartment due to its large global volume, i.e. it receives significant amounts of emission directly and/or indirectly, and substances that have entered the compartment tend to reside there for long periods of time before reaching other compartments (sediment in the case of TPPT) and because water serves as an important medium for transport in contrary to soil and sediment. Secondly, interpretation of the surface water simulation test is more straightforward compared to the soil and sediment simulation studies, as formation of non-extractible residues (NERs) is minimised. Finally, the surface water simulation test is suitable to test lower, environmentally relevant, test concentrations. Considering all above, ECHA requests simulation testing on ultimate degradation in surface water.

The requested surface water simulation study shall be conducted as a pelagic test using EU representative surface water with a suspended solids concentration of approximately 15 $mg_{dw}/L$ (but not outside the range of 10 to 20 $mg_{dw}/L$). The test must be performed at

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the mean temperature of European surface waters which has been defined as 12°C in ECHA guidance (Chapter R.7.9.4.1, ECHA 2017). Care shall be taken that no test concentration used is above the aqueous solubility of the registered substance in the test medium. For an appropriate verification of the degradation kinetics and pathways, you must use radiolabelled substance and provide a mass balance. The radiolabel must be located in the most recalcitrant part of the molecule. You must make reasonable attempts to quantify the transformation products, and must document the analytical efforts made in the study report (which will be provided to ECHA). It should be noted that the available biodegradation screening studies that were conducted with low concentrations of radiolabelled registered substance confirm the technically feasibility of the current request.

Overall the test performed should meet the validity criteria of the OECD test guideline, and provide results suitable for comparison with the Annex XIII criteria of REACH.

If the simulation study results in the substance being not P/vP in the tested compartment and these results are sufficient to conclude on persistence in other environmental compartments, no additional simulation tests will be needed. If a concern on the persistence in some of the compartments remains, ECHA may consider whether further simulation testing needs to be requested in future substance evaluation (SEV) decisions.

You shall submit the full study report. Considering the complexity of the case as described above, access to all information available in the full study report (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties) is needed. This will allow ECHA to fully assess the provided information, including the statistical analysis, and to efficiently clarify the concern for PBT/vPvB.

Consideration of alternative approaches

The request for an OECD TG 309 study is suitable and necessary to obtain information that will allow to clarify whether the registered substance or one if its transformation products is persistent or very persistent. More explicitly, between different available alternatives it is the least onerous way to obtain the information. The possible alternatives of OECD TG 307 and OECD TG 308 can be equally informative, but their interpretation is generally less straightforward as discussed above.

Consideration of registrants' comments on the draft decision and proposals for amendment (PfAs) and of the PfAs

You agreed to conduct the simulation degradation testing in surface water if the study is technically feasible. ECHA notes that there are three available biodegradation screening studies that were conducted with low concentrations of radiolabelled registered substance, i.e. 0.26 µg $^{14}$C-TPPT/L, confirm the technical feasibility of the current request. If technical difficulties arise though, you must provide a justification in the study report that is supported by analytical information and that demonstrates that reasonable attempts were made to quantify the parent compound and to identify and quantify the transformation products. The analytical information must be detailed in the materials and methods section, as well as the results section of the report, and should include standard information such as the type of instruments, the chemicals/supplies used for the analysis,

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the chromatograms showing the limit of detection, the limit of quantification and the calibration curves.

You noted that OECD TG 309 allows a suspended solids or sediment concentration between 0.01 and 1 $g_{dw}$/L, and you proposed to elaborate the appropriate suspended solids concentration during the test. ECHA would like to note that the proposed setup where surface water is amended with suspended solids/sediment of 0.01 to 1 $g_{dw}$/L, corresponds to a suspended sediment type test as specified in paragraph 5 of OECD TG 309. However, paragraph 5 also clearly states that the aerobic mineralisation in surface water – simulation biodegradation test can be conducted as a pelagic type test with surface water only. The current request concerns a pelagic test using EU representative surface water without addition of suspended solids or sediment, and specifies EU representative surface water as having a suspended solids concentration of approximately 15 $mg_{dw}$/L, but not outside the range of 10 to 20 $mg_{dw}$/L. ECHA did not adapt the request.

You requested to extend the deadline for the simulation testing on ultimate degradation in surface water from 21 to 33 months. Considering that all relevant processes, e.g. preparations, experimental work, data analysis and update of the registration dossier(s), were taken into account while setting the deadline, ECHA considers that the current deadline provides sufficient time. The request was not adapted.

A PfA suggested to keep the request for a surface water simulation test (OECD TG 309), and extend the decision with an additional request for a sediment (OECD TG 308) or a soil (OECD TG 307) simulation test. The latter tests would only be required if no valid conclusions on persistency of the registered substance or of its degradation products could be drawn from the surface water simulation study, in which case the technical limitations would have to be substantiated by data of the failed surface water simulation study. The PfA and the decision do not differ from a scientific point of view. Both consider that based on substance properties a surface water simulation study should technically be feasible. In case this assumption proves to be wrong, the PfA suggests a safety net construction, whilst the current approach considers further simulation testing (e.g. sediment/soil) only as part of possible future SEV decisions.

ECHA prefers to limit the request to one simulation study at this stage based on the following reasons. The risk of a failed surface water simulation study due to technical limitations is not deemed high. In a biodegradation screening study you showed that testing is feasible at a test concentration as low as 0.26 µg $^{14}$C-TPPT/L using radiolabelled material, while in a hydrolysis study metabolite formation was followed at 19 µg/L using non-radiolabelled material. Therefore, it is anticipated that a surface water simulation study will yield usable persistence data. ECHA considers that you should have the possibility to argue that the degradation in one compartment (e.g. surface water) can be extrapolated to other compartments (e.g. sediment/soil) and that further testing might not be needed. In case you conclude that the substance is not persistent in surface water, a critical analysis of the data by ECHA would in any case be needed. ECHA could conclude from the same data that the substance does meet the (v)P criteria, or decide that a simulation test in another compartment might be required or that specific adaptations are

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required. To have an orderly discussion that is not hampered by the timelines set for testing, ECHA prefers to conduct this analysis after update of the dossier. Overall, a potentially slower, but a more structured and thorough approach is preferred. Therefore, considering this proposal the decision has not been amended.

You had several comments with respect to this PfA generally not considering it proportionate to perform additional simulation tests if the OECD TG 309 is considered valid and reliable, and supporting the consultation with the evaluating MSCA on the results. As indicated above, the PfA has been rejected, and no further simulation testing will be requested in this decision. All available environmental fate data will be evaluated following dossier update, after which it will be decided if further testing is deemed necessary. If during testing clarification/discussion is needed, the evaluating MSCA will be available.

Conclusion

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the registered substance subject to this decision: Simulation testing on ultimate degradation in surface water: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25. / OECD TG 309 with the registered substance. The test must be conducted as a pelagic test using EU representative surface water with a suspended solids concentration of approximately 15 $mg_{dw}/L$ (but not outside the range of 10 to 20 $mg_{dw}/L$) at a temperature of 12 °C; care must be taken that no test concentration used is above the aqueous solubility of the registered substance in the test media; transformation products must be identified and reasonable attempts must be made to quantify them down to 0.1% w/w; radiolabelled substance must be used, and a mass balance must be provided.

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2. **Long-term toxicity testing on aquatic invertebrates: Daphnia magna reproduction test**

3. **Long-term toxicity testing on fish: Fish, early-life stage (FELS) toxicity test**

Why new information is needed

For the registered substance to meet the T criterion it must exhibit chronic toxicity to aquatic organisms (NOEC/EC10 < 0.01 mg/L) and/or meet the criteria for classification according to the CLP Regulation as either carcinogenic (Carc. Cat. 1A or 1B), germ cell mutagenic (Muta. Cat. 1 or 1B), toxic for reproduction (Repr. Cat. 1A, 1B or 2), or specific target organ toxicity after repeated exposure (STOT RE Cat. 1 or 2). The available toxicity data, as discussed below, are insufficient to conclude on the T criterion.

*Long-term aquatic toxicity data*

The registration dossier contains long-term aquatic toxicity data for three trophic levels. However, only the algal effect data were derived from a study conducted with the registered substance. The respective static algal growth inhibition study was conducted as a limit test at a nominal concentration of 100 mg/L without application of a solvent. Following 3 days of stirring at room temperature and subsequent filtering, the undiluted filtrate was used as test medium. The validity criteria were met, and no inhibition of growth rate was observed. You stated that the water solubility of the registered substance under the prevailing test conditions was below the limit of quantification (LOQ) of 0.1 mg/L, and reported a 72h-EC10 of >100% of saturated solution. There was no analytical monitoring. Since no toxic effects were observed, the NOEC would ordinarily be expressed as equal to or above water solubility. However, in this case there are concerns that the procedure followed to dissolve the registered substance might have resulted in very low exposure concentration (far below the water solubility of 20-38 µg/L), as was observed in the *Daphnia magna* reproduction study discussed below.

Therefore, it is not possible to assess if the T criterion would be met. However, it does appear that algae are not the most sensitive species, and therefore at this stage a new algal growth inhibition study is not deemed necessary.

The long-term toxicity data for aquatic invertebrates and fish that are available in the registration dossier, were obtained from studies conducted with the multi-constituent substance named 'a mixture of triphenylthiophosphate and tertiary butylated phenyl derivates' (CAS 192268-65-8) to which you propose read-across. Interpretation of these studies is less straightforward, as the multi-constituent substance contains the registered substance, but also mono-, di-, tri- and tetra-butylated triphenylthiophosphates and impurities. The presence of more hydrophilic substances, - six of the eight reported impurities have log $K_{ow}$ values in the range of 1.5 to 4.8 (HPLC and QSAR estimates) and water solubilities ($S_w$) in the range of 1.3 to 971 mg/L at pH 6-7, 20-25°C (shake flask and QSAR estimates) - could have resulted in a reduced water solubility of the registered substance (log $K_{ow}$ = 4.8-6.5; $S_w$ = 20-38 µg/L at pH 7, 20-22°C) in the performed studies.

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Therefore, it is considered pivotal that the sampling for exposure concentrations has been done regularly and that the exposure concentrations have been calculated based on a time-weighted average. Even then, if a toxic effect is observed, it cannot be concluded with certainty that the observed toxicity is exerted by the registered substance, and not by one or more of the constituents/impurities. Comparison of chronic aquatic toxicity data of the individual substances (experimentally derived, or, if not available QSAR estimated data) would give some insight, but reservations would remain. Therefore, if a toxic effect is observed in a study conducted with the multi-constituent substance, it can merely serve as an indication of chronic toxicity of the registered substance, and a confirmatory study conducted with the registered substance would be deemed necessary.

For daphnia, two chronic studies conducted with the multi-constituent substance are available in the registration dossier. The more recent semi-static *Daphnia magna* reproduction study was conducted as a limit test at a nominal concentration of 5.5 mg/L without application of a solvent. Following 3 days of stirring at room temperature and subsequent filtering, the undiluted filtrate was used as test medium. The validity criteria were met, and no adverse effects were observed. You considered the data reliable without restrictions (Klimisch score of 1), and reported a NOEC for reproduction of >5.5 mg/L based on nominal test concentration.

ECHA reassessed the study and notes the following: Analytical monitoring (based on two main constituents, including TPPT) was conducted at test start, and subsequently once a week for freshly prepared, aged and control solutions, while renewal occurred daily. Test substance was detected in quantities below LOQ of 0.2 µg/L in two control samples. In the treatment, the actual concentrations were very low and varied strongly between batches. At test start, no reliable concentrations could be determined (1 of 6 samples was above LOQ), while subsequent measurements ranged 0.78 to 3.6 µg/L (expressed as multi-constituent substance), corresponding to 0.014 to 0.066% of nominal concentrations. In the study report, you argue that the low concentrations can be expected as the aqueous solubility of UVCB substances is highly dependent on the loading rate and test medium composition. ECHA agrees that the presence of other constituents could have reduced the water solubility of the registered substance (as discussed above). However, as the measured test concentrations were not within the range of 80-120% of nominal, the effect concentrations should have been based on time-weighted measured test concentrations, and not be expressed as nominal loading rates. If time-weighted measured test concentrations were to be derived they would clearly be far below the maximal water solubility of the registered substance. In any case, as other substances were present in the test medium, it is not possible to assess if the registered substance would meet the T criterion based on this study.

The second *Daphnia magna* reproduction study was conducted under flow-through conditions using five nominal test concentrations ranging from 6.4 to 250 µg/L. Dimethyl formamide (DMF) was used as solvent. The solvent concentration amounted to 0.08 mL/L, which is in accordance with OECD TG 211. Analytical monitoring of two major constituents (including TPPT) was conducted at least once a week. The mean measured concentrations,

expressed as multi-constituent substance, were reported to be 4.9, 11, 26, 46 and 150 µg/L. You reported NOECs for several endpoints with the most sensitive NOEC of 46 µg/L being for growth and mortality. Initially you regarded this study as reliable without restrictions (Klimisch score of 1), but following re-evaluation you disregarded it as unreliable because of major flaws related to improper preparation of the test solutions (Klimisch score of 3). ECHA assessed the study and notes the following: Validity criteria were met (except for dissolved oxygen saturation dropping for one day just below the criterion of 60% saturation). No effects were observed in the control or solvent control. Test solutions were prepared by delivering primary or secondary stock solutions in DMF to diluter mixing chambers where mixing with the test medium yielded the desired test concentrations. The report states that an oily film was observed in the diluter mixing chambers of the treatments, which indicates water solubility issues. Nevertheless, as the test solutions were clear and colorless in the test vessels at start and end of the test and the concentrations were weekly measured, this is not considered a major issue and the data do not support your conclusion to disregard the study as unreliable. It should be noted that the mean measured test concentrations amount to 1.2, 2.8, 6.6, 11.7 and 38.1 µg/L when expressed as TPPT. The highest concentration is likely to be above the water solubility of TPPT (20-38 µg/L). That said the NOEC would be 11.7 µg/L, which is just above the T criterion of 10 µg/L. Overall, ECHA considers this study reliable with restrictions (Klimisch score of 2), and the data indicate that the T criterion might be met for the registered substance. Nevertheless, as other substances were present in the test medium, it is not possible to assess if the registered substance would meet the T criterion. Therefore, a *Daphnia magna* reproduction test conducted with the registered substance is deemed necessary.

A FELS toxicity test conducted with rainbow trout (*O. mykiss*) and the multi-constituent substance is available in the registration dossier. This GLP compliant study was conducted under flow-through conditions using five nominal test concentrations ranging from 6.3 to 100 µg/L. DMF was used as solvent. The solvent concentration amounted to 0.1 mL/L, which is in accordance with OECD TG 210. Analytical monitoring of two major constituents (including TPPT) was conducted at least once a week, and the mean measured concentrations, expressed as multi-constituent substance, were reported to be 4.4, 8.7, 17, 29, and 66 µg/L. You reported NOECs for several endpoints with the most sensitive NOEC of 4.4 µg/L being for growth. Initially, this study was regarded as reliable without restrictions (Klimisch score of 1) by you, but following re-evaluation you disregarded it as unreliable (Klimisch score of 3) because of major flaws related to improper preparation of the test solutions.

ECHA assessed the study and notes the following: Validity criteria were met and no effects were observed in the control or solvent control. Test solutions were prepared by delivering primary or secondary stock solutions in DMF to diluter mixing chambers where mixing with the test medium yielded the desired test concentrations. The report states that an oily film was observed in the diluter mixing chambers of the treatments, which indicates water solubility issues. Nevertheless, as the test solutions were clear and colorless in the test vessels at start and end, and the concentrations were weekly measured, this is not

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considered a major issue and the data do not support your conclusion to disregard the study as unreliable. It also appears that the NOEC for growth was only derived after removal of one outlier from the control and one from the solvent control treatment, as the fish were apparently too large. ECHA does not agree with this approach. A Grubbs' test (extreme studentized deviate test) was performed by the evaluating MSCA, and for the control and the lowest test concentration a significant outlier was detected ($p<0.05$), but not for the solvent control. The question remains though, do these marginally higher values belong to a different Gaussian distribution, which would justify their removal, or are they just values from the tail of the same Gaussian distribution. The latter assumption is far more plausible, as there is no reason why the fish would differ as they were randomly allocated to the test vessels and kept under identical conditions. Therefore, all fish should have been used in the statistical analysis, and the NOEC for growth should have been reported as <4.4 µg/L expressed as multi-constituent substance, which corresponds to a NOEC of approximately <1.1 µg/L when expressed as TPPT. Overall, ECHA considers this study reliable with restrictions (Klimisch score of 2), and the data indicate that the T criterion would likely be met for the registered substance. Nevertheless, as other substances were present in the test medium, it is not possible to assess if the registered substance would indeed meet the T criterion. Therefore, a FELS toxicity test conducted with rainbow trout (*O.mykiss*) and the registered substance is deemed necessary.

*Mammalian toxicity data*

Regarding the mammalian T criterion, ECHA notes that the registered substance has not been classified according to the CLP Regulation neither as carcinogenic, germ cell mutagenic, toxic for reproduction or specific target organ toxic after repeated exposure. In the registration dossier, an Ames test (OECD TG 473) of less quality conducted with the registered substance is available that is negative. Other genotoxicity tests (OECD TG 471, 473, 476) conducted with the multi-constituent substance are also negative. In absence of effects in the genotoxicity studies, further carcinogenicity data are not required. A combined repeated dose toxicity and reproduction/developmental toxicity screening test (OECD TG 422) conducted with the registered substance is available that showed reproduction effects, i.e. decreased viability in offspring. In a reproduction/developmental toxicity screening study (OECD TG 421) conducted with the multi-constituent substance no reproduction effects were observed. Also no effects were observed in a repeated dose 28-day oral toxicity study (OECD TG 407) conducted with the multi-constituent substance. Based on these data you proposed to perform a sub-chronic 90-day oral toxicity study (OECD TG 443). ECHA evaluated the testing proposal and requested a sub-chronic toxicity (90-day) test: oral route with the registered substance. In January 2019 an abstract of a summary of the OECD TG 408 test was added to the registration dossier reporting a NOAEL of 39.5 mg/kg bw/d for male rats (corrected for the recovery values determined during concentration control analyses). Currently, additional mammalian testing is requested under a compliance check, comprising an *in vitro* gene mutation study in bacteria (OECD TG 471), *in vitro* cytogenicity study in mammalian cells (OECD TG 487), *in vitro* gene mutation study in mammalian cells (OECD TG 476/490), screening for reproductive/developmental toxicity in rats, oral route (OECD TG 421/422); and pre-natal developmental toxicity study in a first species (rat or rabbit), oral route (OECD TG 414).

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All tests are to be conducted with the registered substance, with deadline set at 20 March 2020. At this stage it is not deemed necessary to request further mammalian toxicity data under substance evaluation to clarify the PBT/vPvB concern.

*Conclusion on why new information is needed*
Considering all available data, it is likely that the T criterion will be met for TPPT based on long-term aquatic toxicity data. Fish appear more sensitive, but it cannot be excluded that the T criterion might also be met based on the mammalian toxicity data that is currently being generated and aquatic invertebrate data. Therefore, both a Daphnia reproduction study as well conditionally a FELS toxicity test with rainbow trout (*O. mykiss*) are deemed necessary.

Considerations on the test method and testing strategy
To avoid unnecessary testing a tiered testing strategy is followed. Testing is only required if the registered substance is considered persistent (P) or very persistent (vP) according to the Annex XIII criteria. Information requirements 2 and 3 may be waived if the currently generated mammalian toxicity data are available and allow a conclusion that the registered substance meets the T criterion according to the Annex XIII criteria. Furthermore, information requirement 3 may be waived if information from requirement 2 allows conclusion that the registered substance meets the T criterion according to the Annex XIII criteria.

The registered substance has a low water solubility ($S_w$ = 20-38 µg/L at 20-22°C, at pH 7), and is hydrophobic (HPLC and QSAR estimated log $K_{OW}$ = 4.8-6.5). The registered substance is not regarded as volatile as the Henry's Law constant of 0.59 Pa*m³/mol at 25°C is below 1 Pa*m³/mol. Nevertheless, TPPT is considered a difficult substance for aquatic toxicity testing as it fulfils the indicator values of $S_w$ <100 mg/L, log $K_{OW}$ >4 and H >0.1 Pa*m³/mol. Therefore you must consult guidance to help maintain or achieve the required exposure concentration (OECD TA 23).

All reasonable efforts must be made to achieve exposure concentrations up to the aqueous solubility of the registered substance in the test medium. Considering that the previously conducted semi-static daphnia study showed that stirring only yields very low and variable test concentrations, you should consider methods that will lead to more stable and higher exposure concentrations, e.g. solvent application, passive dosing or use of a generator column.

You must submit the full study report. Considering the complexity of the case as described above, access to all information available in the full study report (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties) is needed. This will allow ECHA to fully assess the provided information, including the statistical analysis, and to efficiently clarify the concern for PBT.

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*Specific considerations for the Daphnia reproduction study*

The water solubility of the registered substance is low. In the previously conducted semi-static daphnia study actual test concentrations were very low and variable. Therefore, additional focus should be on maintaining the exposure concentrations as constant as possible using a semi-static or a flow-through system. This must be monitored by verifying the exposure concentration at regular time intervals by analytical measurements throughout the experiment following the recommendations of OECD TG 211. Sampling for exposure concentrations must be done at minimum six times between t = 0 and 21 days (at start and end of renewal), evenly spread for semi static tests. For flow-through systems sampling must be done at minimum eight times between t = 0 and 21 days, with three sampling points in the first week to ensure stable test conditions. Exposure concentrations must be recalculated based on a time-weighted average, as described in Annex 6 of OECD TG 211.

*Specific considerations for the FELS toxicity test*

The study must be conducted with rainbow trout (*O. mykiss*) as this species was also used in the FELS toxicity test with the multi-constituent substance. The water solubility of the registered substance is low, and previous aquatic toxicity tests showed that actual test concentrations can be very low and variable. Therefore, the exposure concentrations must be maintained as constant as possible. During this test the organic carbon content of the test water should be kept as low as possible, and efforts must be made to establish the truly dissolved concentration, as recommended by the OECD TG 210, for example by taking measurements of particulate and dissolved organic carbon concentrations at appropriate time points and using an appropriate technique to enable the estimation of the bioavailable fraction if feasible (e.g. solid-phase microextraction). Furthermore, in addition to the test method, prior to initiation of the exposure period, proper function of the chemical delivery system across all replicates should be ensured by measuring the test concentrations. In addition, the actual test concentrations must be verified by analytical measurements, three times a week at regular time intervals throughout the experiment, changing systematically amongst replicates. Exposure concentrations must be recalculated based on a time-weighted average

Consideration of alternative approaches

No alternatives are available. The request for OECD TG 211 and OECD TG 210 are suitable and necessary to obtain information that will allow to clarify whether the registered substance meets the T criterion. More explicitly, there is no equally suitable alternative way available of obtaining this information. ECHA notes that there is no other experimental method available at this stage that will generate the necessary information. A testing strategy is followed where first invertebrate animals are tested to reduce testing with vertebrate animals.

Consideration of registrants' comments on the draft decision and PfAs and of the PfAs

ECHA acknowledges your commitment to conduct the requested tests on long-term toxicity on aquatic invertebrates and fish following the tiered testing strategy described above.

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You requested additional time for the aquatic toxicity tests, i.e. 18 months for the Daphnia reproduction tests and 36 months for the FELS toxicity test with rainbow trout (or alternatively 18 months with zebrafish or fathead minnow). You reasoned that in your in-house laboratory it is not possible to reserve capacity for sequential testing. ECHA would like to note that issues related to capacity of your in-house laboratory are no reason to adapt the requests or timelines. Furthermore, you reasoned that extensive preliminary testing is needed to achieve for this adsorptive and poorly water soluble substance stable exposure concentrations, ECHA considers that the standard timelines already account for these preparatory steps.

You indicated that greater flexibility in the choice of fish species should be allowed, as the requested species, i.e. rainbow trout for the FELS toxicity test and common carp for the BCF test, pose several practical difficulties, which are expected to result in increased time lines and a greater likelihood of failed tests. More specifically, you argued that your in-house laboratory only has seasonal availability of the necessary life stages of the requested fish species with fertilized rainbow trout eggs only available in winter and common carps of adequate size only available in late spring. You noted that tiered testing would require an additional year, and that test failure could result in an additional delay of a year, i.e. next spawning season. ECHA would like to note that, in contrast to your in-house laboratory, contract laboratories generally have year-round availability of relevant life stages of the requested fish species. Therefore, this is not considered a reason to adapt the request.

You further argued that the cold-water conditions needed to rear rainbow trout, will lower the solubility of TPPT making it even more challenging to maintain stable test concentrations. Indeed, rainbow trout is reared in colder water (10 °C) than other fish species specified in OECD TG 210 (22-26 °C), which will lower the water solubility of TPPT. However, this does not per se mean that it will be more difficult to maintain stable test concentrations, especially not, considered that in a flow-through test design the test water is continuously renewed and very low water concentrations can be maintained at constant levels. Furthermore, from the biodegradation screening study it is apparent that you can test at low test concentrations (0.26 µg $^{14}$C-TPPT/L). Therefore, testing at lower water temperature is not considered a reason to adapt the request.

Finally, you argued that the FELS toxicity test is not commonly performed with rainbow trout, even for plant protection products, because of the above given practical difficulties and because rainbow trout is not suited for higher tier testing. You propose to conduct the FELS toxicity test with zebrafish (*Dania rerio*) or fathead minnow (*Pimephales promelas*) to ease the challenges. ECHA would like to note that common practices in other frameworks are not relevant for this decision. OECD TG 210 specifies four freshwater fish species, including rainbow trout, indicating the suitability of this species for FELS toxicity testing. Therefore, the request was not adapted.

You noted that a reproduction screening study according to OECD TG 421 was ongoing in 2017 and that the final decision on a 90 day study proposal according to OECD TG 408 was pending. You argued that should the results of one of these two mammalian studies

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be available before the aquatic toxicity tests start and be sufficient to conclude that the substance fulfils the T-criterion according to Annex XIII of the REACH Regulation, no further long-term testing on aquatic organisms (invertebrates or fish) would be needed.

One PfA agreed with your approach and suggested to make the long-term toxicity testing on fish dependent on the outcome and review of the different mammalian testing. The PfA noted that only a summary of the OECD TG 408 test appears in the registration dossier, that the status of the OECD TG 421 test with the registered substance is unclear, and that additional mammalian testing (including reproduction toxicity testing) is requested under a compliance check with a deadline of March 2020. As the timescales in the present decision suggest that the new mammalian data would be available before the FELS test would commence, it was suggested in the PfA to resolve the classification of the registered substance based on the mammalian data before further vertebrate testing is to be performed. The PfA further noted that you do not consider the currently available reproduction data sufficient for classification as Repr. 2, but if you should decide that the registered substance is Repr. 2 based on the new data, you should not be required to perform the FELS test.

ECHA considered your comment, the PfA and your comments on the PfA, and notes the following: The available reproduction data, i.e. a reproduction/developmental toxicity screening study (OECD TG 421) conducted with the multi-constituent substance (conducted in 2011, report from 2011), and a combined repeated dose toxicity and reproduction/developmental toxicity screening test (OECD TG 422) conducted with the registered substance (conducted in 2008, report from 2010 and 2011), were not considered sufficient by you to classify the substance as Repr. 2. In contrast, 701 out of 875 notifiers did self-classify the registered substance as Repr. 2 in ECHA's C&L inventory based on these data (of which 356 of these notifiers further specified a concentration limit of 0 % ≤ C ≤ 4.1 % and indicated that the substance exerts developmental effects).

In your comments on the PfA you noted that the previously performed OECD TG 422 study that showed reproduction effects (poor implantation and poor F1 pup survivability starting at 125 mg/kg/day), was excluded for assessment of reproduction toxicity, as the test item used in that study was less pure (characterized as a yellow liquid, while the registered substance is a white solid), and out of the scope of the joint submission. You further noted that the reported effects were not observed in the newly performed OECD 421 study with no effects found on reproduction and pup survivability up to the highest dose level of 1000 mg/kg body weight. Regarding the new OECD TG 421 study you further noted that the study has been completed, but not yet submitted to ECHA (a dossier update is in preparation). You provided in your comments the following details on the study outcome: *"A potential delay in development of male pups in the high dose group (1000 mg/kg bw) was found. The percentage of male pups with nipple was statistically significantly increased (90.0 %, control: 44.7 %) and the mean number of nipples in males was increased (2.5, control: 1.2) on PND 13. No adverse findings were reported for the mid and low dose groups. Since both parameters of nipple development were dose-related, and statistically significantly altered at the highest dose level, a relation to treatment cannot be excluded. However, the increased number of nipples does not reflect a permanent damage. It is*

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*assumed that a few days later all pups will have equally progressed in their development. Since this effect is only observed at the highest dose level and since no other effects are reported, this slight delay is considered adverse but not severe enough for classification".* Based on these data you concluded that T criterion will not be met. You also concluded in your comments that the repeated dose 90-day oral toxicity test in rats (OECD TG 408) does not warrant a classification.

ECHA notes that the OECD TG 421 test conducted with the registered substance you referred to is not available in the registration dossier on the date of this decision. For the OECD TG 408 study only a summary is available on the date of this decision. ECHA can thus not evaluate your assessment and conclusions.

As you noted in your comments, other mammalian toxicity studies, including a prenatal developmental toxicity study (OECD TG 414) and genotoxicity studies, have also been requested under compliance check with the deadline set at March 2020. You note that the results from the OECD TG 414 study might lead to classification, as an OECD TG 414 conducted with the multi-constituent substance named 'a mixture of triphenylthiophosphate and tertiary butylated phenyl derivates' (CAS 192268-65-8) that contains the registered substance, showed developmental toxicity effects leading to classification. If the data allow to conclude that the mammalian T-criterion of Annex XIII is met, the daphnia reproduction study and the FELS study would indeed not be required.

In your comments on the PfA you requested support on how the new data relevant for substance evaluation should be reported to avoid unnecessary testing and unnecessary vertebrate testing. ECHA would like to note that considering the complexity of the case, access to all information available in the full study report (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties) is needed. This will allow to fully assess the provided information, including the statistical analysis, and to efficiently clarify the PBT concern with respect to the mammalian T criterion. Thus, considering your comments, the PfA, and your comments on the PfA, the decision has been amended making the daphnia reproduction study and the FELS test conditional dependent on the outcome of the requested/ongoing mammalian toxicity test.

PfAs were received suggesting that for both the invertebrate and vertebrate testing the requirements for establishing the truly dissolved concentration and analytical monitoring requirements for the test are not specified unless further reasoning is provided in the decision. ECHA notes the water solubility of the registered substance is low. In previously conducted aquatic toxicity tests, actual test concentrations were very low and variable. Therefore, ECHA considers it justified to request that the exposure concentrations are kept as constant as possible, and to verify this by sufficient analytical monitoring, as is recommended in the respective OECD test guidelines. The justifications have been extended in the respective sections. The requests were not modified.

Conclusion for the Daphnia reproduction study

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study

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using the registered substance subject to this decision: Long-term toxicity testing on aquatic invertebrates; test method: *Daphnia magna* reproduction test, EU C.20. / OECD TG 211 with the registered substance; all reasonable efforts must be made to achieve exposure concentrations up to the aqueous solubility of the registered substance in the test medium; sampling for exposure concentrations must be done regularly and exposure concentrations must be recalculated based on a time-weighted average.

Conclusion for Fish, early-life stage (FELS) toxicity test

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the registered substance subject to this decision: Long-term toxicity testing on fish: Fish, early-life stage (FELS) toxicity test, OECD TG 210 with rainbow trout (*Oncorhynchus mykiss*) with the registered substance; all reasonable efforts must be made to achieve exposure concentrations up to the aqueous solubility of the registered substance in the test medium; sampling for exposure concentrations must be done regularly and exposure concentrations must be recalculated based on a time-weighted average.

## 4. Bioaccumulation in fish: Bioconcentration: flow-through fish test

Why new information is needed

TPPT screens as (v)B based on HPLC and QSAR estimated log $K_{ow}$ values. The HPLC estimated log $K_{ow}$ values are 5.0 and 4.8, and were determined according to OECD TG 117 using the registered substance and the read-across multi-constituent substance, respectively. The QSAR estimated log $K_{ow}$ values are higher and amount to 5.45 (ACD/labs logP v14.03), 5.68 (ChemAxon MarvinSketch logP v16.10.24), 6.13 (Bioloom ClogP v1.7), and 6.47 (KOWWIN v1.67). Considering that all estimated log $K_{ow}$ values exceed the B screening criterion of log $K_{ow}$ ≥4.5, and that a fish bioconcentration test is available that reports BCF values for TPPT, it is not deemed necessary to request at this stage an experimentally determined log $K_{ow}$ using the slow stirring method (OECD TG 123) that would allow to definitely conclude on the B screening criterion.

In the registration dossier, a fish bioconcentration test with common carp (*Cyprinus carpio*) conducted with the multi-constituent substance named 'a mixture of triphenylthiophosphate and tertiary butylated phenyl derivates' (CAS 192268-65-8) is available. The multi-constituent substance contains the registered substance ([redacted]% w/w), mono-, di-, tri- and tetra-butylated triphenylthiophosphates as well as several impurities. The aqueous and tissue concentrations of several main constituents, including TPPT were measured. Therefore, ECHA considers the proposed approach acceptable for deriving BCF values for TPPT. The lipid normalised steady state BCF ($BCF_{ss}$) values for TPPT ranged from 1411-2551 and 1481-2871 L/kg for the low and high treatments, respectively. You evaluated this study as reliable with restrictions in the registration dossier of TPPT, and as reliable without restrictions in the registration dossier of the multi-constituent substance. Based on these $BCF_{ss}$ values, as well as QSAR estimated BCF

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values, you concluded in your PBT assessment that the registered substance meets the Annex XIII criterion for bioaccumulative (B) substances, but not the criterion for very bioaccumulative (vB) substances.

ECHA re-evaluated the fish bioconcentration test conducted with the multi-constituent substance, and noted that the study setup does not fully comply with OECD TG 305, i.e.: replicate aquaria were not included; two instead of four fish were sampled per time point; fish were sampled once instead of at least four times during the depuration phase; the replacement rate was too low; lipid content was only determined at the start and not at the end of the uptake phase; and relevant study details were not provided with regard to feeding and cleaning regime.

Furthermore, the reported measured aqueous concentrations are overestimates representing both the dissolved and suspended fraction, thus leading to an underestimation of the bioaccumulation potential. This is best illustrated by the high treatment, but also applies to the low treatment. The high treatment corresponds to a nominal test concentration of 188 µg TPPT/L and thus clearly exceeds the water solubility of TPPT by at least a factor 5 to 10, i.e. the water solubility of 20-38 µg/L (at 20-22°C, at pH 7) is most likely reduced by the presence of more hydrophilic substances (six of the eight reported impurities have log $K_{ow}$ values in the range of 1.5 to 4.8 (HPLC and QSAR estimates)) and water solubilities in the range of 1.3 to 971 mg/L at pH 6-7 and 20-25°C (shake flask and QSAR estimates)). Nevertheless, the measured aqueous TPPT concentrations in the high treatment remained during the 56 days of exposure within 95 to 97% of nominal. Apparently, the use of a dispersant resulted in a homogenous suspension. Thus, it can be concluded that the lipid corrected BCF values, ranging from 1411-2871 L/kg based on steady state calculations and from 1813-2472 L/kg based on kinetic fitting (recalculated by the evaluating MSCA), should be considered best-case estimates of the bioaccumulation potential of TPPT.

Consequently, the available information is regarded as sufficient to assess if the substance is bioaccumulative, but insufficient to assess if it is very bioaccumulative at this stage of the evaluation. ECHA therefore considers it necessary to request a bioconcentration study with the registered substance according to OECD TG 305 if the substance has been shown to meet the vP criterion, but not the T criterion.

Considerations on the test method and testing strategy

To avoid unnecessary vertebrate testing a tiered testing strategy is followed. This request is only required if the registered substance is considered very persistent (vP), but not toxic (T) according to the Annex XIII criteria.

Care must be taken that no concentration used is above the solubility limit of the registered substance in the test medium. You should consider to use radiolabelled test substance along with parent substance analysis as this will facilitate analytical analysis. The organic carbon content of the test water (e.g. from fish excreta and food residues) should be kept as low as possible, and, considering the low water solubility of the registered substance,

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efforts must be made to establish the truly dissolved concentration, for example by taking measurements of particulate and dissolved organic carbon concentrations at appropriate time points and using an appropriate technique to enable the estimation of the bioavailable fraction if feasible (e.g. solid-phase micro-extraction). Excessive fish growth and lipid increases must be avoided, since these might influence the results. The study report must be provided, so the raw data can be evaluated. The results must in any case be corrected for growth and normalised to 5% lipid content.

The registered substance has low water solubility ($S_w$ = 20-38 µg/L at 20-22°C, at pH 7), and is hydrophobic (HPLC and QSAR estimated log $K_{OW}$ = 4.8-6.5). The registered substance is not regarded as volatile as the Henry's Law constant of 0.59 Pa*m³/mol at 25°C is below 1 Pa*m³/mol. Nevertheless, TPPT is considered a difficult substance for bioaccumulation testing as it fulfils the indicator values of $S_W$ <100 mg/L, log $K_{OW}$ >4 and H >0.1 Pa*m³/mol, therefore guidance must be consulted to help maintain or achieve the required exposure concentration (OECD TA 23).

You shall submit the full study report. Considering the complexity of the case as described above, and access to all information available in the full study report (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties) is needed. This will allow ECHA to fully assess the provided information, including the statistical analysis, and to efficiently clarify the concern for vPvB.

Consideration of alternative approaches

The request for the OECD TG 305 is suitable and necessary to obtain information that will allow to clarify whether the registered substance is bioaccumulative or very bioaccumulative. More explicitly, there is no equally suitable alternative way available of obtaining this information. ECHA notes that there is no experimental study available at this stage that will generate the necessary information and testing on vertebrate animals is deemed necessary

Consideration of registrants' comments on the draft decision and PfAs and of the PfAs

ECHA acknowledges your commitment to conduct the requested bioaccumulation study.

You requested additional time for the bioaccumulation study i.e. 30 months when conducted with common carp (or 18 months with zebrafish or fathead minnow). In analogy to the discussion in section 3 on the FELS toxicity test: issues related to capacity of your in-house laboratory are no reason to adapt the requests or timelines; the flow through design allows testing at very low and constant water concentrations; and the standard timelines already account for all relevant steps, e.g. preparations, experimental work, data analysis and update of the registration dossier(s). Therefore, the request was not adapted.

You indicated that greater flexibility in the choice of fish species should be allowed, as the initially requested species, i.e. rainbow trout for the FELS toxicity test and common carp for the BCF test, pose several practical difficulties, which are expected to result in increased time lines and a greater likelihood of failed tests. As discussed in section 3

above, the availability of relevant life stages of the requested fish species in your in-house laboratory is no reason to adapt the request. With regard to the bioaccumulation study, you further argued that it is not possible to avoid growth and lipid changes when performing a bioaccumulation test with common carp (*Cyprinus carpio*), i.e. the juvenile fish required by OECD TG 305 are in a state of rapid growth (even when food is limited), and will grow during the estimated testing period of ≥75 days (50 days for uptake, and 25 days for depuration). You consider adult stages of small fish species (e.g. zebrafish or fathead minnow) more suitable. ECHA agrees that juvenile common carp will increase more in weight and lipid content than adult fish such as zebrafish. However, the initially specified setup with common carp would allow a direct comparison with previously generated bioaccumulation data in common carp to assess the potential influence of the solubility constraints and testing in a mixture related to the available study. Excessive fish growth and lipid increase can be avoided by following OECD TG 305, i.e. starting with common carp of 8.0 ± 4.0 cm, and reliable BCF values can be obtained after correction for growth and lipid increase.

In your comments you noted that the bioaccumulation study conducted with common carp and the multi-constituent substance is flawed and not sufficient to conclude on the B criterion. This study was not considered unreliable by you in the registration dossier, nor by ECHA in the decision, even though there are uncertainties associated with the study. From the study lipid corrected BCF values ranging from 1411-2871 L/kg when based on steady state calculations and from 1813-2472 L/kg when based on kinetic fitting were derived. These values are considered best-case estimates of the bioaccumulation potential of TPPT, as the measured aqueous concentrations of TPPT represent both the dissolved and suspended fraction thus underestimating the bioaccumulation potential of the registered substance.

In reply to your comments it is clarified that ECHA thoroughly re-evaluated the uncertainties of the available bioaccumulation study (without performing a new assessment) and considers that the available best-case BCF estimates are sufficiently robust to assess bioaccumulation in terms of the B criterion of Annex XIII of the REACH Regulation. These data, however, do not allow to conclude on the vB criterion.

Therefore, a new bioaccumulation study is requested if the registered substance meets the vP, but not the T criterion. Vertebrate testing is thus only requested if the vB concern needs to be substantiated. The request was adapted accordingly.

A PfA suggested not to specify the test species for bioaccumulation testing in the decision unless further reasoning is provided in the decision. In the PfA it was reasoned that since the registrant and ECHA have doubts about the validity of the previously generated bioaccumulation data in common carp, it is not clear what benefit there is in being able to compare the new results to the available BCF study. ECHA would like to note that the available study was neither considered unreliable by you in the registration dossier (only in the comments), nor by ECHA in the decision, even though there are uncertainties associated with the study. The best-case estimates (that underestimate bioaccumulation potential of the registered substance as measured aqueous concentrations of TPPT

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represent both the dissolved and suspended fraction) yield lipid corrected BCF values that exceed the B criterion. Therefore, a BCF study is only needed to determine if the registered substance is very bioaccumulative. A comparison between the studies would be facilitated when both were to be conducted with the same fish species. However, a bioaccumulation study conducted with another fish species will also allow to conclude if the substance should be regarded as very bioaccumulative._Therefore, while preference is given to the same species, it has been decided based on your comments, the PfA and your comments on the PfA, to no longer specify the fish species in the request. The decision has been adapted accordingly.

A PfA suggested that the requirement for establishing the truly dissolved concentrations for the test is not specified unless further reasoning is provided in the decision. ECHA notes the water solubility of the registered substance is low. In previously conducted aquatic toxicity tests, actual test concentrations were very low and variable. Therefore, ECHA considers it justified to request that the exposure concentrations are kept as constant as possible as recommended in OECD TG 305. The justification has been extended in the respective section. The request was not modified.

Conclusion

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the registered substance subject to this decision: Bioaccumulation in fish: Bioconcentration: flow-through fish test, EU C.13 / OECD TG 305, aqueous exposure with the registered substance; care must be taken that no concentration used is above the solubility limit of the registered substance in the test medium; excessive fish growth and lipid increases must be avoided; the results must be corrected for growth and normalised to 5% lipid content.

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## Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to suspected PBT/vPvB, O,O,O-triphenyl phosphorothioate CAS No 597-82-0 (EC No 209-909-9) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2016. The updated CoRAP was published on the ECHA website on 22 March 2016. The competent authority of the Netherlands (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

In accordance with Article 45(4) of the REACH Regulation, the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

The evaluating MSCA considered that further information was required to clarify the abovementioned concern. Therefore, it prepared a draft decision under Article 46(1) of the REACH Regulation to request further information. It subsequently submitted the draft decision to ECHA on 21 March 2017.

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

### Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took the comments from you, which were sent within the commenting period, into account and they are reflected in the reasons (Appendix 1). The request(s) or the deadline were not amended.

### Proposals for amendment by other MSCAs and ECHA and referral to Member State Committee

The evaluating MSCA notified the draft decision to the Competent Authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposals for amendment to the draft decision according to which the decision was amended.

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendments. Any comments on the proposals for amendment were taken into account by the Member State Committee and are reflected in the Reasons (Appendix 1).

### MSC agreement seeking stage

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-64 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

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**Appendix 3: Further information, observations and technical guidance**

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

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

3. In relation to the required experimental study/ies, the sample of the substance to be used ('test material') has to have a composition that is within the specifications of the substance composition that are given by all registrant(s). It is the responsibility of all the registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on the composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.

4. In relation to the experimental studies the legal text foresees the sharing of information and costs between registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who will carry out the study on behalf of the other registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at: https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx?CaseNumber=SEV-209-909-9-1
Further advice can be found at:
http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the registrants to perform the studies on behalf of all of them.