SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Trixylyl Phosphate

EC No 246-677-8

CAS RN 25155-23-1

Evaluating Member State: Italy

Dated: 28 February 2022

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2014

Before concluding the substance evaluation a Decision to request further information was issued on: 29 June 2018.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].
Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the Registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

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Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance, Trixylyl Phosphate (EC number 246-677-8; TXP) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB
- Wide dispersive use
- Exposure of environment
- Exposure of workers
- High RCR
- High (aggregated) tonnage

During the evaluation, an additional concern was identified:

- Potential risk for soil compartment

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

The substance was discussed by PBT expert group during 5-PBTEG meeting and 8-PBTEG meeting.

Trixylyl phosphate was included on 16 December 2013 in the Candidate List (Toxic for reproduction, Article 57(c)).

Trixylyl phosphate was further included in the Annex XIV to REACH Entry No. 47, with a Sunset Date of 27 May 2023, and latest application date of 27 November 2021. Intrinsic property(ies) referred to in Article 57:Toxic for reproduction (Article 57(c).

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

The evaluating Member State (eMSCA) agrees with the approach taken by the Registrant(s) in performing the exposure and risk assessment for human health. Nevertheless, it should be noted that, as follow up of the evaluation and considering the corrected DNELs calculated by the eMSCA, some scenarios (PROCs) may present RCR(s) above 1. Therefore, it is recommended that the Registrant(s) update their CSR with the inclusion of appropriate RMM whereas a potential risk is identified.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

On the basis of effects noted in *Daphnia magna*, the eMSCA agrees that the substance is deemed to be classified with Aquatic acute 1 M=10 and with STOT Rep. Exp. 2 H373 - Affected organs: adrenals, testes, epididymides, ovaries, heart and liver on the basis of the effects of the Repeated oral toxicity study.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable.

4.1.3. Restriction

Not applicable.

4.1.4. Other EU-wide regulatory risk management measures

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

5.2. Other actions

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the eMSCA. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Initiate CLP Annex VI dossier	---	---

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance, Trixylyl Phosphate (EC number 246-677-8; TXP) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB
- Wide dispersive use
- Exposure of environment
- Exposure of workers
- High RCR
- High (aggregated) tonnage

During the evaluation, an additional concern was identified:

- Potential risk for soil compartment

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Persistence	Concern confirmed: Request on Simulation testing on ultimate degradation in surface water fulfilled by the Registrant(s). Persistence is confirmed. No further action is needed.
Bioaccumulation	Concern unresolved: Due to significant uncertainties, the read-across and the weight of evidence proposed by the Registrant(s) cannot be considered acceptable at this stage, pending further investigation.
Acute aquatic toxicity	Concern confirmed: On the basis of effects noted in *Daphnia magna*, eMSCA agrees that the Substance is deemed to be classified with Aquatic acute 1, M=10.
Chronic aquatic toxicity	Concern confirmed: Due to the lack of long-term studies on the registered substance for the most sensitive invertebrate *Daphnia magna*, applying the surrogate approach, the Substance fulfills the environmental classification of Aquatic chronic 1 according to the CLP Regulation.
Effects on terrestrial organisms-Long-term toxicity to terrestrial invertebrates	Concern refuted: Request fulfilled by the Registrant(s). No further action is needed.
Effects on terrestrial organisms – Effects on soil microorganisms	Concern refuted: Request not addressed by the Registrant(s). Newly submitted read across data provided sufficient and suitable evidence of no concern for soil microorganisms. No further action is needed.
Wide dispersive use	Request fulfilled by the Registrant(s). No further action is needed.
Exposure of environment	Request fulfilled by the Registrant(s). No further action is needed.
Exposure of workers	Exposure for workers has been correctly addressed in the SEV.
High RCR	Concern confirmed.

	The eMSCA agrees with the approach taken by the Registrant(s) in performing the exposure and risk assessment for human health. Nevertheless, it should be noted that, as follow up of the evaluation and considering the corrected DNELs calculated by the eMSCA, some scenarios (PROCs) may present RCR(s) above 1. Therefore it is recommended that the Registrant(s) updates their CSR with the inclusion of appropriate RMM whereas a potential risk is identified.
Repeated dose toxicity	Concern confirmed. Read-across and weight of evidence applied by Registrant(s) in the Repeated dose toxicity study is considered acceptable by the eMSCA. Substance is deemed to be classified with STOT RE 2 H373 - Affected organs: adrenals, testes, epididymides, ovaries, heart and liver.

7.2. Procedure

The evaluation of Trixylyl Phosphate started in April 2014.

The eMSCA considered that further information was required to clarify the above mentioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of REACH to request further information. It submitted the draft decision to ECHA on 26 March 2015.

A unanimous agreement of the Member State Committee on the draft decision was reached on 23 May 2016 in a written procedure launched on 13 May 2016. ECHA took the decision on pursuant to Article 51(6) of REACH, requesting further information to clarify the concerns for PBT/vPvB and potential risk for the soil compartment.

Subsequently the Registrant(s) updated the dossier with the requested information.

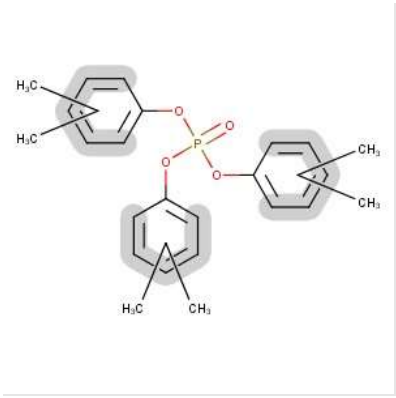
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Trixylyl phosphate
EC number:	246-677-8
CAS number:	25155-23-1
Index number in Annex VI of the CLP Regulation:	015-201-00-9
Molecular formula:	C24H24O4P to C24H27O4P
Molecular weight range:	---
Synonyms:	

Type of substance ☐ Mono-constituent ☐ Multi-constituent ☒ UVCB

Structural formula:



7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Liquid
Vapour pressure	Based on the results, the vapor pressure of the compound is calculated as being equal to 1.6 x 10-6 Pa (1.6 x 10-8 mbar) at 20 °C, and 9.2 x 10-5 Pa (9.2 x 10-7 mbar) at 50 °C.
Water solubility	Considering the tenfold concentration of the samples before the analysis, the above results correspond to a water solubility of less than 2x10-5g/l at 20 °C ± 1°C applying the column elution method. The substance is considered as insoluble in water.
Partition coefficient n-octanol/water ($LogK_{ow}$)	The estimated $LogP_{ow}$: > 6.2 The extrapolated $LogP_{ow}$ value calculated for Kronitex TXP: 6.38
Flammability	--
Explosive properties	Non explosive
Oxidising properties	The substance to be registered is deemed not to be potentially oxidising, based on the chemical structure and an oxygen balance value of -224.14
Granulometry	The study does not need to be conducted because the substance is marketed or used in a non solid or granular form
Stability in organic solvents and identity of relevant degradation products	---
Dissociation constant	---

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☒ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

This substance is used by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

Table 7

USES	Use(s)
Uses as intermediate	---
Formulation	This substance is used in lubricants and greases, hydraulic fluids and metal working fluids. Release to the environment of this substance can occur from industrial use: formulation of mixtures.
Uses at industrial sites	Release to the environment of this substance can occur from industrial use in processing aids at industrial sites, of substances in closed systems with minimal release, manufacturing of the substance and formulation of mixtures.
Uses by professional workers	This substance is used in metal working fluids, hydraulic fluids, lubricants and greases and heat transfer fluids. This substance is used for the manufacture of: machinery and vehicles. Release to the environment of this substance can occur from industrial use: in processing aids at industrial sites and of substances in closed systems with minimal release. Other release to the environment of this substance is likely to occur from: indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters), indoor use as processing aid and outdoor use in close systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids).
Consumer Uses	---
Article service life	Other release to the environment of this substance is likely to occur from indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters) and outdoor use in close systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids). This substance can be found in complex articles, with no release intended: vehicles and machinery, mechanical appliances and electrical/electronic products (e.g. computers, cameras, lamps, refrigerators, washing machines).

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

The substance is currently listed on Annex VI of CLP Regulation ((EC) No 1272/2008). According to the harmonised classification and labelling (ATP03) approved by the European Union, this substance may damage fertility.

Table 8

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
015-201-00-9	trixylyl phosphate	246-677-8	25155-23-1	Repr. 1B	H360F		

7.6.2. Self-classification

- In the registration(s)*:*

STOT Rep. Exp. 2 H373
Aquatic Acute 1 H400 M=10
Aquatic Chronic 1 H410

7.7. Environmental fate properties

7.7.1. Degradation

Trixylyl phosphate is not expected to hydrolyse under normal environmental conditions. According to OECD guideline 111, a key study with reliability 1 (Kiss, 2010) demonstrated that the substance was stable to hydrolysis at environmentally relevant pH, with half lives at pH 4, 7 and 9 greater than 1 year. Studies on direct phototransformation in water are not available but it is assumed on the basis of chemical structure that the substance is not degraded by direct photolysis. It is concluded, therefore, that abiotic processes would not contribute significantly to the depletion of the substance within the environment.

Concerning biotic degradation, a ready biodegradation study with reliability 1 (Sipos, 2010) was performed according to a standard test protocol (OECD test guideline 301D, Ready Biodegradability Closed Bottle test). With an initial test substance concentration of 2.6 mg/l, 14% of TXP was biodegraded after 28 d. The Registrant(s) concluded that the substance is not readily biodegradable and based on the available information, the eMSCA can support this conclusion.

As requested in the Substance Evaluation Decision, the Registrant(s) submitted a simulation testing on ultimate degradation in surface water with reliability 1 (Coleman, Schaefer, 2018), performed with the registered substance according to the OECD guideline 309 (Aerobic Mineralisation in Surface Water - Simulation Biodegradation Test) and in compliance with GLP. The study was run for 60 days using natural water collected from Tuckahoe Lake, Queen Anne MD. The aerobic transformation of 14C-labeled Trixylyl Phosphate at concentrations of ~10 and ~200 µg/L (actual concentrations were 10 µg/L and 220 µg/L) was studied at 12 °C. In order to assess viability of the microbial population,

benzoic acid at a concentration of 10 µg/L was used as reference substance. HPLC/β-RAM analyses of samples from the live and sterile 200 µg/L treatment groups showed that the parent compound Trixylyl Phosphate remained substantially intact in all live and sterile vessels throughout the course of this study. Mineralization was minimal throughout the course of the study, with 14C gas production of 0.48% AR in the 10 µg/L group, 0.14% AR in the live 200 µg/L group, and 0.01% AR in the sterile 200 µg/L group. Mineralization in the reference substance group reached >60% by Day 28, and both vessels exceeded the 60% acceptance criteria by Day 35, indicating that the microbial population was viable over the course of the study. No non-parent peaks were detected in any of the sterile vessel samples, or in any of the acetonitrile water extract samples from the live vessels. Minor non-parent peaks were detected in a few of the tetrahydrofuran cap extract samples from the live vessels at Days 35 and 60, and these accounted for mean maximum of 2.5% AR at Day 60.

The DT50 and DT90 values are reported as >60 days, the duration of the study, confirming that the substance does not undergo mineralization in water.
Concerning sediment and soil simulation tests the Registrant(s) proposed a data waiving since the substance is found to meet the Persistent/very Persistent (P/vP) criteria in water.

7.7.2. Environmental distribution

The substance has a high adsorption to soil ($Logk_{oc}$=5.08 and koc=119941) and a low volatility.

In the CSR, the Registrant(s) declare that the substance is a liquid under all environmental conditions with low volatility. As such any environmental release will result into soil and water compartment with little release directly to atmosphere. The high adsorption to soil indicates that the majority of substance will partition to soil and sediment rather than water.

Data from distribution modelling studies (Level III, Fugacity model) show that only a small amount of TXP released to the environment will be in the air compartment at steady state. When the substance is released to air it distributes mainly to the soil compartment, presumably by atmospheric deposition. When it is released to soil, the substance generally remains in soil, with only a small fraction distributing to the water and sediment compartment. When released to water, the substance is likely to distribute mainly to the sediment phase at steady state, but a small fraction is also predicted to remain in water. In conclusion, the substance will preferentially be distributed into soil and sediment ("Environmental Risk evaluation report: Trixylenyl phosphate (CAS RN 25155-23-1)" August 2009, UK Environment Agency; "Screening-level hazard characterization: Trixylenyl Phosphate (CAS RN 25155-23-1)" June 2010, U.S. Environmental Protection Agency).

7.7.3. Bioaccumulation

The $LogK_{ow}$ value of TXP is > 6.2 (OECD TG 117), therefore TXP fulfills the screening criteria for B.

The initially provided information did not allow to exclude the B criterion. As a consequence, the substance was considered potentially B/vB.

In the updated CSR, for the aquatic bioaccumulation endpoint, the Registrant(s) reported QSAR estimations, read-across and experimental studies that were already submitted in the previous CSR.

Additionally, in order to clarify the B/vB concern, the Registrant(s) proposed a new read-across approach based on an analogous substance Phenol, isopropylated, phosphate (3:1), CAS number: 68937-41-7 (OECD TG 305, GLP study, 2015).

eMSCA reports below the evaluation for Bioaccumulation of the dataset present in previous and in the current CSRs that triggered to the potential B/vB property conclusion:

The Registrant(s) reported two experimental studies (no guideline followed) where the product tested contained triphenyl phosphate, cresyl diphenyl phosphate (two main components), tri-cresyl phosphate (three main components) and trixylenyl phosphate (three main components). For these two studies the results are:

-first study results: bioconcentration factor BAF:1900; 1300 and 1500.

-second study results: only 0.017-0.14 per cent of the total amount of test substance fed to fish was found to be present in the fish at the end of the study. The bioaccumulation factors, based on the estimated concentration in fish and concentration in food, are all very much less than one. The substance is therefore proposed to not be bioaccumulative on the basis that it does not meet the threshold values listed in the Reach Regulation. In both studies the transformation products have not been analysed.

At last, the Registrant(s) reported a read-across from the substance tricresyl phosphate (TCP). This substance does not demonstrate a propensity towards bioaccumulation, based on the measured values within the studies. The Registrant(s) reported in IUCLID dossier a justification for read-across.

In conclusion, the Registrant(s) admitted that it was not possible to provide a definitive BCF value due to the variation in the results obtained (none of these above exceed the threshold values of 2000 or 5000).

In order to derive a BCF value, the Registrant(s) considered appropriate to utilize a geometric mean across the data set. On the basis of a weight of evidence approach, the Registrant(s) concluded that the substance is not bioaccumulative because BCF is <2000 (geometric mean: 669,24 L/kg ww). In reference to the experimental data on bioaccumulation reported by the Registrant(s) and for the first experimental study (Bengtsson et al. 1985) eMSCA noted that: the study used shorter uptake durations than what is recommended for the OECD TG 305 method; no guideline followed; the test was carried out at 10°C; Steady state was reached within the 14-day exposure period for triphenyl phosphate, the cresyl diphenyl phosphate components and two of the tricresyl phosphate components of the mixture. Steady-state bioconcentration factors (BCFs) were determined as 400 l/kg, 100- 220 l/kg and 800 l/kg for these components respectively. For the other components, steady state was approached, but had not been reached by the end of the 14-day uptake period and the non-steady state BCFs estimated at 14 days were 400 l/kg for the remaining tricresyl phosphate component and 1,300-1,900 l/kg for the three trixylenyl phosphate components; and the values obtained of BCF have not been lipid normalized content. Moreover, in reference to the second experimental study (World Health Organisation) the results are not comparable with any thresholds in Annex XIII and they could be considered only for supporting analysis.

In reference to the experimental study on TCP (read-across -RA), eMSCA noted that this experimental test is proposed to be acceptable in the context of a weight of evidence approach just for TCP; due to general level of uncertainty the data is not a support information in a weight of evidence approach for TXP.

In reference to QSAR estimations provided by the Registrant(s), eMSCA noted that these estimations could be used only as results to support reliable experimental studies in a weight of evidence approach.

In conclusion, eMSCA highlighted that: information suggest that the substance is close to fulfill the B criterion. However, data are not lipid normalised, the steady state is not reached in one of the study (Bengtsson et al., 1985) and the available dietary study is difficult to interpret. Moreover the UK Risk Evaluation Report, 2009 which included a PBT assessment of the substance, reports a measured BCF of ~1900.

As a conclusion, eMSCA considered that the provided information did not allow to exclude the B criterion. As a consequence, the substance was considered potentially B/vB.

As preannounced in the commenting phase, in their updated CSR, the Registrant(s) provided a new study (Schneider, SZ, Siddiqui, AI, Martin KH, Gallagher, SP, 2015, OECD TG 305, GLP) as read-across approach, based on an analogous substance Phenol, isopropylated, phosphate (3:1), CAS RN 68937-41-7 (IPTPP).

The new OECD TG 305 GLP study, seems to clarify that Phenol, isopropylated, phosphate (3:1) is not considered to be bioaccumulative. Registrant(s) provided documents justifying the read-across approach for the Bioaccumulation property, which however is considered not acceptable by eMSCA at this stage. In particular, the read-across justification is quite limited and includes only statements on RMM for health hazards, description of the category members and a data matrix introducing the source data. However, bridging data or elaboration linking the $\log K_{ow}$ of the substance constituents to that of the target constituents are completely missing.

The eMSCA assessed the updated information on IPTPP as follow: the Registrant(s) provided a robust study summary of the study. The fish species tested *was Lepomis macrochirus* (Bluegill). The organisms were exposed to IPTPP at 3.1 and 24 µg/L (mean measured water concentration). Total exposure/uptake duration: 23 days. Total depuration duration: 10 days.

Steady-state BCF values for the low treatment group were 225, 773 and 512 in edible, non-edible and whole fish, respectively. Steady-state BCF values for the high treatment group were 293, 922 and 634 in edible, non-edible and whole fish tissue, respectively.

Kinetic BCFK values for the low treatment group were 281, 733 and 516 for edible, non-edible and whole fish tissue, respectively. Kinetic BCFK for the high treatment group were 311, 776 and 559 for edible, non-edible and whole fish tissue, respectively. It is demonstrated that the outcomes were based on lipid normalization and growth dilution correction.

According to the available information eMSCA noted that the test was adequate. The validity criteria of OECD 305 TG seem to be satisfied and the results indicate that the tested substance is not bioaccumulative, because all the BCF values are less than 1000.

However, according to the available information, eMSCA noted that the substance tested in the BCF study (Schneider et al., 2015) is a UVCB, and the components measured for the calculation of the BCF are not specified. Therefore, without further knowledge on the BCF related to the individual components, it is not appropriate to extrapolate a BCF value <2000 for TXP from the BCF study on IPTPP.

Based on the above considerations, the eMSCA cannot accept the Registrant(s) proposal of a weight of evidence approach covering a combination of QSAR techniques, literature data and read across. Indeed, the eMSCA considered that each result is not sufficient to conclude on B property, and therefore also the study on IPTPP could not be considered conclusive, alone. In particular, there is not sufficient justification to accept the read-across on IPTPP to estimate the bioaccumulative potential of TXP.

In conclusion, the eMSCA considers that the weight of evidence approach supported by the new OECD TG 305 GLP study on Phenol, isopropylated, phosphate (3:1), cannot be considered as sufficient to conclude on the B potential of TXP, making this endpoint unresolved.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

One short-term toxicity study on registered substance was provided by the Registrant(s): a key study, under flow-through conditions, with reliability 1 (Palmer SJ, Chafey KW, Krueger HO, 2003a), on *Pimphales promelas* according to EPA OPPTS 850.1075 (Freshwater and Saltwater Fish acute Toxicity Test) and OECD TG 203 (Fish, Acute Toxicity test), using a solvent (DMF).

The 96-hour LC50 value of the test item (registered substance, Phosflex TXP CAS RN 25155-23-1) was greater than 1119 µg/L (mean measured concentration). The 96-hour NOEC were 1119 µg/L.

The study is adequately described and is in accordance with the conditions for the validity of the test.

The Registrant(s) provide a justification for waiving a long-term toxicity test, claiming that no toxicological effects in the acute test on fish at the limit of water solubility is noted. The Registrant(s) also point out that the substance is self-classified for environmental effects based on toxicity to aquatic invertebrates rather than fish.

The eMSCA doesn't agree with the waiving justification. The substance is poorly water soluble (WS 0.02 mg/L) so the long-term toxicity testing must be considered. Moreover, self-classification is not a basis to adapt the information requirement.

In conclusion, there is a data gap on chronic toxicity to fish. However, data suggest that invertebrates are likely to be more sensitive than fish and algae, therefore no further action is needed.

7.8.1.2. Aquatic invertebrates

One short-term study was provided by the Registrant(s) on the registered Substance: a key study with reliability 1 (Palmer SJ, Chafey KW, Krueger HO, 2003), on *Daphnia magna* according to OECD TG 202 (Daphnia sp. Acute Immobilisation Test, 1984) and EPA OPPTS 850.1010 (Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids), using a solvent (DMF).

The 48-h EC50 of the test item (registered substance, CAS RN 25155-23-1) obtained from key study was calculated to be = 0.06 mg/L (arithmetic mean of measured concentrations). The study is adequately described and is in accordance with the conditions for the validity of the test.

Four long-term studies, all with reliability 2, were provided by the Registrant(s) based on a weight of evidence approach, utilizing read across to structural analogues.
Three studies investigated the effect of two commercial isopropylphenyl diphenyl phosphate products, Kronitex 200 and Phosflex 31P (CAS RN 68937-41-7) on the survival and reproduction of *Daphnia magna* and *Gammarus pseudolimnaeus*, and on the emergence of *Chironomus plumosus.*
One study, according to equivalent or similar as OECD TG 202, investigated the effect of tris(methylphenyl) phosphate (CAS RN 1330-78-5) on the reproduction and mortality of *Daphnia magna*.
The Registrant(s), as a weight of evidence approach, considered appropriate to utilise a geometric mean for data on *Daphnia magna* in order to derive an appropriate NOEC for use in hazard assessment (21-day NOEC = 0.033 mg/L).
The eMSCA considers that these studies have issues with poor reporting and with some uncertainties (i.e., control mortality).

The provided read-across justification is quite limited since it includes only statements on RMM for health hazards, description of the category members and a data matrix introducing the source data.

There are no chronic data for the registered substance and thus there is no bridging data to support the predictions. The acute invertebrate EC_{50} for this substance is 0.06mg/L, while the source CAS n. 68937-41-7 is less toxic to invertebrates (48-h LC_{50} 2.44 mg/L). Thus, the prediction may underestimate the toxicity.

Due to significant uncertainties, the eMSCA considers that the read-across applied by the Registrant(s) is not acceptable. In conclusion, there is a data gap regarding long-term toxicity to aquatic invertebrates, which have to be filled if the chemical safety assessment indicates the need to investigate further the effects on aquatic organisms.

7.8.1.3. Algae and aquatic plants

One study was provided by the Registrant(s) on the registered substance: a key study with reliability 1 (Desjardins D, Chafey KW, Krueger HO, 2003), static on *Selenastrum capricornutum* (new name: *Pseudokirchnerella subcapitata*), according to OECD TG 201 (Alga, Growth Inhibition Test), EU Method C.3 (Algal Inhibition test) and EPA OPPTS 850.5400 (Algal Toxicity. Tiers I and II), using a solvent (DMF).

EC50 and NOEC (72-96h) of the test item (registered substance. CAS RN 25155-23-1) resulted > 1011 µg/L and 112 µg/L respectively, based on initial measured concentrations (effects observed based on cell density, area under the growth curve and growth rate). The study is adequately described and is in accordance with the conditions for the validity of the test.

In conclusion, the eMSCA agrees with the Registrant(s) that the substance is acutely toxic to invertebrates (48-h EC50=0.06mg/L).

7.8.1.4. Sediment organisms

The Registrant(s) provided a data waiving for toxicity to sediment organisms with a justification based on exposure pattern and RMMs applied for the registered substance. In accordance with REACH Annex IX, the Registrant(s) argued that toxicity study on sediment organisms does not need to be performed as any significant direct and indirect exposure to sediment compartment can be considered unlikely. In any of identified uses, the registered substance is not intended to be directly released to the aquatic environment and the results from RMMs indicate that toxicity study on sediment organisms can be waived claiming exposure considerations.

Although the majority of the substance would distribute into sediment if released to aquatic compartment (results from Level III Fugacity Model), eMSCA notes that in view of the currently available data, exposure of aquatic organisms, including sediment organisms, is expected as negligible. As reported in the registration dossier, levels of releases of the registered substance to aquatic environments (surface water and sediment) are not relevant.

Thus, at this stage, in view of the provided arguments, eMSCA can support the Registrant(s)' justifications for data waiving on this endpoint.

However, eMSCA points out that, under the follow up of this evaluation, some hazard assessment conclusions, including the related PNEC derivation, for aquatic compartment have been revised, considering as not acceptable the 21-day NOEC value for *D. magna* from read across study (see related sections at paragraphs 7.8.1.2 and 7.8.4). Therefore, eMSCA considers that a refinement of exposure and risk characterization including an

update of related RCRs is recommended to the Registrant(s) with the aim of finalizing the conclusions on this environmental compartment accordingly.

7.8.2. Terrestrial compartment

In order to clarify the potential concern for soil compartment, the Registrant(s) provided toxicity data on all three terrestrial taxonomic groups (soil macroorganisms, soil microorganisms and terrestrial plants). As requested under Substance Evaluation Decision, the Registrant(s) submitted a long term toxicity testing on soil macroorganisms with the registered substance, while read across studies on Phenol, isopropylated, phosphate-IPTPP (CAS RN 68937-41-7), a structural analogue of Trixylyl phopsphate (TXP), were used to assess the effects on terrestrial plants and soil microorganisms.

Based on the outcome of the revised CSA, eMSCA can support the Registrant(s)' conclusion on hazard assessment for soil compartment, indicating no concern for the toxicity to soil organisms.

7.8.2.1. Toxicity to soil macroorganisms

As requested under Substance Evaluation Decision, the Registrant(s) submitted a long term toxicity study on soil macroorganisms (Earthworm Reproduction Test – Eisenia fetida/Eisenia andrei) performed with the registered substance TXP according to OECD TG 222 and under GLP. The toxic effects of TXP on survival, growth and reproduction of earthworms Eisenia fetida were assessed during an 8-week exposure period in artificial soil substrate. In this reliable test, adult earthworms were exposed for the first 28 days to a geometric series of five concentrations (nominal) of 62.5,125,250,500 and 1000 mg/kg soil dw, then removed to evaluate the mortality and growth. An additional 28 days-exposure period was used to determine the effects on reproduction. There was no mortality of adult earthworms exposed to tested concentrations of TXP for 28 days. Based on body weight and survival data of adult earthworms, the NOEC was determined to be 1000 mg/Kg soil dw, the highest concentration tested. No significant reduction was observed for juvenile production in the treatment groups in comparison to the controls. The EC10 and EC50 for reproduction were each greater than 1000 mg/kg dry soil, the highest concentration tested. For body weight and juvenile reproduction, the test results used for CSA were respectively a NOEC of 1000 mg/kg soil dw and a LOEC greater than 1000 mg/kg soil dw.

Based on the experimental reliable data, no toxic effects of TXP were observed on soil macroorganisms.

Following the assessment, eMSCA can conclude that newly submitted data on soil macroorganisms as provided by the Registrant(s) are suitable and definitive for this endpoint, fulfilling the requested information under Substance Evaluation Decision; no further information is needed to clarify the hazard on soil macroorganisms and related concern under this substance evaluation.

7.8.2.2. Toxicity to terrestrial arthropods

The registration dossier does not contain data for this endpoint. The Registrant(s) have waived testing on terrestrial arthropods with a justification based on exposure considerations. The substance is not intended to be released directly to the environment and thus, exposure to soil compartment can be regarded as unlikely to occur. Moreover, available toxicity data set on other soil organisms indicates that these phosphates as a group do not show direct toxicity to terrestrial organisms.

Therefore, based on the currently available data, the eMSCA can support the Registrant(s)' conclusion on terrestrial arthropods. As such, the outcome of CSA indicates that further assessment of this endpoint is not required.

7.8.2.3. Toxicity to terrestrial plants

For short term toxicity, in absence of data on registered substance, the Registrant(s) provided the results from an OECD TG 208 study with the structural analogue substance DURAD 310M (trade name of Phenol, isopropylated, phosphate 3:1-IPTPP; CAS RN 68937-41-7) as a read across approach for the effects on terrestrial plants. Short term toxicity of IPTPP was examined at concentrations of 100, 10, 1 and 0 mg/Kg on the tested endpoints: emergence and growth of seedling of wheat (*Triticum aestivum*), radish (*Raphanus sativus*) and mung bean (*Phaseolus aureus*) were determined over a periods of 19, 18 e 19 days respectively, representing 14 days after at least 50 % emergence of control seedling.

In the reliable test, no toxicity to all tested terrestrial plants species was observed on all endpoints examined (rate of emergence and growth) over the exposure time. The LC50 for emergence and EC50 for growth rate result to be both greater than the highest concentration tested, 100 mg/Kg soil dw for all tested species. This study was performed in accordance with OECD TG 208 (Terrestrial Plants Test: Seedling emergence and Seedling Growth test) with all validity criteria fulfilled.

For long term toxicity to terrestrial plants, the registration dossier does not contain data. In accordance with REACH Annex IX, the Registrant(s) have waived toxicity testing, claiming that available short term toxicity test on soil plants (Read across with the analogue substance IPTPP) and implemented RMMs are appropriate to cover the information requirements, indicating no need for long term toxicity testing on this endpoint.

eMSCA notes that the data from the read across studies provided by the Registrant(s) can be considered as reliable and well documented information to be used for addressing the conclusions on short term toxicity to terrestrial plants. The validity and applicability of the proposed read across have been demonstrated and adequately justified by the Registrant(s). Thus, eMSCA considers that the read across approach as provided by the Registrant(s) is sufficient to enable as adaptation for prediction of toxicity to soil plants for the registered substance, being fulfilled all conditions set out in Annex XI of REACH Regulation.

Therefore, based on available information from registration dossier, the eMSCA can support the Registrant(s)' conclusion, indicating no concern for this endpoint as well as no further information needs to be required under this substance evaluation.

7.8.2.4. Toxicity to soil microorganisms

As indicated under Substance Evaluation Decision, the Registrant(s) did not address the requested OECD TG 216 long term toxicity study on soil microorganisms using the registered substance. As announced, the Registrant(s) used a read across approach and existing data to evaluate the hazard on soil microorganisms and related concern. For this endpoint the Registrant(s) Registrant(s) applied a read across approach using test results from long term toxicity testing with an analogue substance, REOFOS 35 (trade name for Phenol isopropylated, phosphate (3:1) -IPTPP; CAS RN 68937-41-7). In this reliable study, toxicity effects of the analogue substance on nitrogen transformation activity of soil microorganisms were examined according to OECD TG 216 and GLP compliant at concentrations of 10, 32, 100, 318 and 1010 mg/Kg dry soil over a 28 days exposure. The study was conducted according to the procedure outlined in the protocol Reofos 35: Soil Microorganisms Nitrogen Transformation Wildlife International Protocol. No statistically significant effects on Nitrogen Transformation Activity of soil microorganisms were observed at tested concentrations. The EC10 was calculated to be 582,7 mg/Kg dry soil using linear interpolation between concentrations 318 and 1010 mg/kg dry soil. The EC25 and EC50 were estimated to be ≥ 1010 mg/Kg dry soil, the highest concentration tested.

Moreover, in addition to this OECD TG 216 study with structural analogue, the Registrant(s) note that further reliable data set available from read across studies on this category of phosphates also indicates a lack of toxicity to aquatic microorganisms (with no inhibition of microbiological activity in STP) as well as negligible effects on anaerobic bacteria. Thus,

the data set from Activated Sludge Respiration Inhibition and biodegradation studies supports the results from OECD TG 216 read across study and the conclusions that this substance is unlikely to pose hazardous effects on microorganisms, including soil microorganisms.

With regards to the OECD TG216 study with IPTPP, structural analogue of the registered substance, as provided by the Registrant(s) to clarify the potential concern for this endpoint, the eMSCA notes that the applied read across approach is adequate, reliable and well documented to substantiate the Registrant(s)' conclusion under this substance evaluation.

Phenol isopropylated, phosphate (3:1), as source substance used for read across, and TXP belong to the category of Phosphinated Flame Retardants and can be considered as structural analogues with similar physico-chemical and ecotoxicological profile.

The validity and reliability of the proposed read across have been demonstrated and adequately justified; all conditions set out in Annex XI of REACH Regulation are fulfilled.

Therefore, with the currently available information from registration dossier, the eMSCA can support the read across approach and the related data used by the Registrant(s) to address this endpoint. Thus, the eMSCA also notes that the outcome of revised CSA using currently available results from read across studies and the refined environmental assessment, including the applied RMMs, indicates no hazard to soil microorganisms.

Following the assessment, the eMSCA concludes that the read across data can be considered as suitable and sufficient to clarify that there is no concern for toxicity to soil microorganisms for this category of substances.

7.8.3. Microbiological activity in sewage treatment systems

The Registrant(s) provided the study, static to microorganisms (Sipos K., 2010), according to OECD TG 209 (Activated Sludge, Respiration Inhibition Test), EU Method C.11 (Biodegradation: Activated Sludge, Respiration Inhibition Test) and EPA OPPTS 850.6800 (Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble). The Registrant(s) report the study with reliability 1. An EC50 (3 hour) > 100 mg/L (nominal) and NOEC (3h) = 1000 mg/l were reported.

The substance is not considered to pose a hazard to STP microorganisms. The eMSCA agrees with the Registrant(s) conclusion.

7.8.4. PNEC derivation and other hazard conclusions

Table 9

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	PNEC aqua (freshwater): 0.06 µg/L	Assessment factor: 1000
Marine water	PNEC aqua (marine water): 0.006 µg/L	Assessment factor: 10000
Intermittent release to water	PNEC: 0.6 µg/L	Assessment factor: 100

Sediments (freshwater)	PNEC value: 0.72 mg/Kg sediment dw	Extrapolation method:Equilibrium partitioning method In absence of sediment toxicity data for TXP, the PNEC sediment (freshwater) was derived using the equilibrium partitioning method with default values.
Sediments (marine water)	PNEC value: 0.072 mg/kg sediment dw	Extrapolation method: Equilibrium partitioning method. In absence of sediment toxicity data for TXP, the PNEC sediment (marine water) was derived using the equilibrium partitioning method, with default values.
Sewage treatment plant	PNEC STP: 100mg/L	Assessment factor: 10 Extrapolation method: assessment factor PNEC STP The PNEC for STP was based upon the 3 hour NOEC for Activated Sludge Respiration Inhibition with appropriate assessment factors as detailed in guidance document Part R.10 – Dose [Concentration]-Response Regarding Environment. The supporting studies for this endpoint within the dossier are of similar order of magnitude; hence the key study value has been utilised
Soil	PNEC value: 11.7 mg/kg soil dw	Assessment factor: 50 Extrapolation method: assessment factor According to ECHA Guidance R.10, PNEC soil was derived from the lowest NOEC result from long term toxicity testing to soil organisms and an assessment factor of 50. eMSCA can support these hazard assessment conclusions, including the related PNEC derivation.

The eMSCA highlights that the PNEC values for aquatic compartments provided by the Registrant(s) (PNECfreshwater = 0.66 mg/L and PNECmarine water = 66 ng/L) were derived using the lowest value (*D. magna* NOEC (21d) = 0.033 mg/L) of two long-term toxicity results from species representing two trophic levels (Daphnia and algae) and assessment factors of 50 and 500 for freshwater and marine water, respectively. Since the NOEC value on *Daphnia magna* is considered not acceptable by the eMSCA (see 7.8.1.2) and consequently the NOEC value from the algal growth inhibition test cannot be used if unsupported by long-term EC_{10} or NOECs of species of other trophic levels (ECHA Guidance on information requirements and chemical safety assessment, Chapter R.10), PNEC(s) have been estimated by eMSCA applying an assessment factor of 1000 for freshwater and 10000 for marine water on the lowest L(E)C50 of the relevant available toxicity data (*D. Magna* 48-h EC50=0.06mg/L). PNEC(s) should be reconsidered if long-term toxicity data for aquatic invertebrate will become available.

7.8.5. Conclusions for classification and labelling

On the basis of effects noted in *Daphnia magna*, the eMSCA agrees that the substance is deemed to be classified with Aquatic acute 1 M=10.
Moreover due to the lack of long-term studies on the registered substance for the most sensitive invertebrate *Daphnia magna*, applying the surrogate approach, the substance fulfills the environmental classification of Aquatic chronic 1 according CLP Regulation (EC50=0.06 mg/L and not rapidly degradable substance).

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

The Registrant(s) made a written assessment of toxicokinetic taking into account that organophosphorus compounds are usually esters, amides or thiol derivatives of phosphonic acid. They form a large family of ~50000 chemical agents with biological properties that have important and sometimes unique implications for human being. Toxicokinetics of these types of compounds are fairly well documented and understood from works on associated pesticides and industrial chemicals, and are widely available within the public domain literature. As such, further investigation of these types of effects via further experimental animal studies are not considered appropriate.

The absorption, distribution, metabolism, and elimination of organophosphates are therefore critical to the toxicological effects of these compounds.

The eMSCA agrees with the Registrant(s) approach and conclusions.

Mechanism of toxic effects

One mode of action of organophosphate compounds is the phosphorylation and inactivation of acetylcholinesterases. This causes an increase and accumulation of acetylcholine at nerve endings, stimulating neuro-effector junctions, skeletal neuro-muscular junctions, autonomic ganglia and in the brain. Overstimulation causes a depolarising block of neuromuscular junction receptors. This gives rise to a large number of clinical effects in the central nervous system, autonomic nervous system and leads eventually to paralysis.
After the initial organophosphate acetylcholinesterase bonds are formed a conformational change in the molecular structure of the organophosphate occurs which increases the binding and subsequently makes the organophosphate-acetylcholinesterase complex irreversibly bound. This process is called "ageing"and ishighly dependent upon the type of organophosphate such that significant aging varies between, 2-36 hours after initial binding. In addition to acetylcholinesterase inactivation and subsequent acetylcholine accumulation there is also central nervous system antagonism of γ-aminobutyric acid (GABA) and dopaminergic neurons.

Neurocognitive effects and late onset peripheral neuropathy are well described.

Mechanism of action

Most organophosphates are highly lipid-soluble agents and are well absorbed from the skin, oral mucous membranes, conjunctiva and gastrointestinal and respiratory routes. The onset, severity andvduration of toxicity is determined by the dose, route of exposure, physicochemical properties of the organophosphate (e.g. lipid solubility), rate of metabolism (whether transformation in the liver is required before the compound becomes toxic). and whether the organophosphorylated cholinesterase degrades rapidly.
When inhibition of cholinesterases does occur, assays of plasma butyryl cholinesterase and red blood cell acetylcholinesterase (AChE) are widely used for confirming and assessing exposure.

Exposure to organophosphorus agents causes sequential toxic effects in human being. In most instances the earliest cholinergic phase may only be observed. This cholinergic phase

progresses to the intermediate syndrome in ~20% of subjects. Both the acute cholinergic phase and the intermediate syndrome are associated with a high risk of mortality. The final phase, organophosphate-induced delayed polyneuropathy, which does not carry the risk of death, sets in 7–21 days after exposure to an organophosphorus agent and may not be preceded by either the cholinergic phase or the intermediate syndrome.

The inactivation of the cholinesterases occurs in the blood and in a wide range of nerve, neuromuscular (skeletal, smooth and cardiac) and glandular tissues where these enzymes have a role in cell-to-cell communication and the hydrolysis of xenobiotics. These enzymes have possible (but as yet unidentified) roles such as cell development and growth. The inhibition of AChE leads to the accumulation of acetylcholine, the neurotransmitter at all ganglia in the autonomic nervous system and at many synapses in the brain, skeletal neuromuscular junctions, at some postganglionic nerve endings of the sympathetic nervous system and adrenal medulla. The role of butyryl cholinesterase in the body is yet to be fully identified, but it is known to be involved in the hydrolysis of many therapeutic agents (e.g. suxamethonium, esmolol, procaine and cocaine). There are many other roles speculated for butyryl cholinesterase and these include cellular differentiation and growth, as a scavenger in xenobiotic exposure and as a modulator in lipid metabolism. The consequences of inhibition of other enzyme systems by organophosphorus compounds are as yet uncertain. A variety of tissue carboxylesterases exist in the serum, liver, intestine and other tissues.

Although inhibition of one specific carboxylesterase (neuropathy target esterase) has toxic effects, no direct detrimental effects of inhibition of other carboxylesterases have been demonstrated. However, carboxylesterases may contribute markedly to the metabolic degradation of organophosphates and inhibition of these enzymes may increase the toxicity of organophosphorus compounds. The search for effects of inactivation or changes in other physiological systems is still currently under investigation. The following effects of organophosphorus agents have been demonstrated in animals and are theoretically possible effects in human being:

1. Inactivation by phosphorylation of other beta esterases.
2. Altering the release of neurotransmitters, (γ-aminobutyric acid (GABA) and glutamate).
3. Increasing the number of GABA and dopamine receptors.
4. Acting as agonists at M2/M4 muscarinic receptors.
5. Inhibition of mitochondrial enzymes, respiration and ATP generation.
6. Induction of mast cell degranulation, probably causing the release of histamine or histamine-like compounds.

Absorption

All organophosphates are known to be absorbed from the small intestine or dermal exposure. Peak concentrations may occur within a few hours, although rate of absorption is known to be dependant on the chemical structure of the organophosphate in question. Dermal and oral routes studies on organophosphates are available within the literature.

- Dermal

No specific studies were identified that investigated the dermal absorption of the organophosphates in humans.
It has been suggested that similarities with regard to structure and physical properties among the isomeric tricreysl phosphates (one form of organophosphate) make it likely that the isomers of this type of organophosphate could also be readily absorbed through the skin (NTP 1994). In the cat, 73% of the radioactivity from a 50-mg/kg dose of14C-tricreysl phosphate was no longer present at the application site (intrascapular region) after 12 hr. Maximum concentrations of radioactivity were reached in the examined tissues within 24 hr. By day 10, at least 48% of the dose was absorbed as indicated by urinary and fecal excretion data.
^{32}P- tricreysl phosphate (200 mg/kg) was poorly absorbed through dog abdominal skin. The absorption of 2 to 4 mg/kg tricreysl phosphate by human palm skin was approximately

100 times faster than through the dog abdominal skin based on urinary excretion and surface-area data.

- Oral

At least 41% of a single gavage dose of 7.8 mg/kg14C-labeled tricresyl phosphate in rats was excreted in the urine over 7 d following administration (Kurebayashi et al., 1985). About 12% of a single gavage dose of 89.6 mg/kg in rats was excreted in the urine. Most of the urinary excretion occurred within the 24 hr after administration.

Distribution

Distribution of the metabolites of an organophosphate substance is known to occur to a wide variety of tissues, although evidence suggests that these do not bioaccumulate on the basis of the excretion data. An organophosphate will undergo significant alteration following adsorption in the body to form a diverse group of compounds with a wide range of lipid/water solubility characteristics and variable volume of distribution.

- Dermal

Data available indicates that the distribution of radioactivity in the dog following a single 200-mg/kg application of 32P-tricresyl phosphate to the abdominal skin was highest in the liver followed by the blood, kidney, lung, muscle and spinal cord, brain and sciatic nerve at 24 hr post-exposure. In cats, the highest levels of radioactivity occurred in the bile, gall bladder, urinary bladder, kidney, and liver at 1–10 d after application of 50 mg/kg of ^{14}Ctricresyl Phosphate. In addition, low levels of radioactivity were found in the spinal cord and brain. Analysis showed that the parent compound was found primarily in the brain, spinal cord, and sciatic nerve, while metabolites were primarily found in the liver, kidney, and lung. It is not known if the patterns of distribution for tricresyl phosphate and metabolites can be generalized to other organophosphates; however given the likely mode of action within biological systems, this cannot be precluded.

- Oral

Twenty-four hr after 89.6 mg/kg of ^{14}C- tricresyl phosphate was administered by gavage to rats, the highest concentrations of radioactivity were found in the intestine (including contents), followed by the stomach, adipose tissue, liver, and kidneys (4–13-fold higher than blood concentrations). The lowest concentrations were found in heart, muscle, and brain (lower than blood concentrations). In rats, 14C-organophosphates were rapidly distributed to muscle and liver following intravenous administration. This was followed by a redistribution of radioactivity to adipose tissue and skin. The parent compounds were rapidly cleared rapidly from the tissues and did not bioaccumulate.

Metabolism

It is understood that some organophosphates are metabolised in the liver to much more active metabolites (-oxons). These poisons are also usually highly lipid soluble. Thus the slow conversion of these substances, which are widely distributed into fat, may lead to delayed and/or prolonged cholinesterase inhibition and toxic effects. This slow redistribution and/or activation may have implications for treatment: longer treatment and late commencement may be of benefit in these patients.

In rats, metabolism of tricresyl phosphate following oral gavage of 7.8 or 89.6 mg/kg was found to involve successive oxidations and hydrolysis resulting in the production ofp-hydroxybenzoic acid. The major urinary metabolites identified werep-hydroxybenzoic acid, di-pcresyl phosphate, andp-cresylp-carboxyphenyl phosphate. The main biliary metabolites were di-p-cresyl phosphate,p-cresylp-carboxyphenyl phosphate, and the oxidized triesters, di-p-cresylp-carboxyphenyl phosphate, andp-cresylp-carboxyphenyl phosphate. Fecal metabolites were similar to the biliary metabolites. $^{14}CO_2$ was found in expired air following administration and appeared to be formed probably through decarboxylation ofp-hydroxybenzoic acid by intestinal microbes.

Elimination

Elimination of the substance via excreted fluids is known to happen with the majority of the metabolites excreted within a short period of time. Dermal and oral route studies are available and considered.

- Dermal

About 48% of a single dermal application of a 50 mg/kg dose of organophosphate was excreted by day 10 post-exposure with 28% of the dose excreted in the urine while 20% of the dose was excreted in the feces.
Approximately 40–60% of an intravenous injection of 2 or 20 mg/kg of a radiolabelled organophosphate underwent biliary excretion within 6 hr of administration (NTP, 1994). It was determined that biliary excretion increased with increasing dose from 2–20 mg/kg resulting in a doubling of biliary excretion.
For a number tricresyl phosphates, the percentage of administered radioactivity excreted in the feces was less than the percentage excreted in bile suggesting that the isomers underwent enterohepatic recirculation.

- Oral

Excretion of radioactivity following oral administration of ^{14}C- tricresyl phosphate in rats at doses of 0.5 2, 20, and 200 mg/kg was investigated by NTP (1994). Radioactivity from tricresyl phosphate was excreted primarily in the feces at all dose levels. Radioactivity was excreted primarily in the urine at 0.5 and 2 mg/kg and primarily in the feces at 20 and 200 mg/kg. Radioactivity from tricresyl phosphate was excreted primarily (70%) in the urine at all doses tested.
Rats that received14C- tricresyl phosphate as a single gavage dose of 7.8 mg/kg excreted 41% of the dose of radioactivity in the urine, 44% in the feces, and 18% in the expired air within 7 days. A majority of the excretion occurred within 24 hr. Rats with cannulated bile ducts excreted 28% of the administered radioactivity in the bile during the first 24 hr. Rats treated in a similar manner with 89.6 mg/kg of14C- tricresyl phosphate excreted 12% of the administered radioactivity in the urine, 77% in the feces, and 6% in the expired air. The radiolabeled material excreted in urine and bile was identified as metabolites of tricresyl phosphate in high dose rats. Parent compound was the dominant isomer excreted in the feces with some lesser amounts of metabolites present.

Conclusions

Depending on the compound, metabolism and absorption route, the peak excretion might be reached at different times after exposure. Absorption after dermal exposure is generally slower than after ingestion or presumably inhalation.

Toxicological effects are very dependent on the type of organophosphate ingested, the mode of that ingestion and the type and amount of the dose. It is not possible to determine exactly the toxicokinetics of the substance subject to the registration specifically. However given the overall data available in the literature, it is proposed that the modes of action within this assessment are appropriate for the assessment of the potential toxicokinetic actions of the substance.

Value used for CSA:

Bioaccumulation potential:	no bioaccumulation potential
Absorption rate - oral (%):	100
Absorption rate - dermal (%):	100
Absorption rate - inhalation (%):	100

The eMSCA agrees with the Registrant(s) conclusion.

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated.

7.9.3. Sensitisation

Not evaluated.

7.9.4. Repeated dose toxicity

Registrant(s) submitted several studies for repeated dose toxicity all by oral route. The study conducted by gavage in rats for three months is considered the one evaluable by the eMSCA.

In this study rats were dosed with 30, 100, 300 and 1000 mg/kg bw/day six days a week continuously (rest period on Sundays) with Tricresyl phosphate which is considered an appropriate structural analogue.
The registant(s) stated that treatment effects (not shown) were noted in all dose levels and that these are considered to be comparatively slight, with increasing severity as dose level increases. Registrant(s) stated that the lowest dose level (30 mg/kg bw/day) it is to be considered as the NOAEL, as minimal effects were noted at this level.
Nevertheless the eMSCA consider that 30 mg/kg bw/day is to be considered the LOAEL of repeated dose toxicity by oral route (gavage).

This findings are confirmed in the Combined Oral Repeated Dose and Reproductive/Developmental Toxicity Screening study conducted with TXP on rats dosed at 25, 200 and 1000 mg/kg bw/day. The study revealed effects effects at all dose levels (treatment related effect observed on body weight and weight changes dose, organ weight findings - adrenals, testes, epididymides, ovaries, liver (females only) -including organ/body weight ratios and non-neoplastic histopathological).

On the basis of effects noted in the repeated dose toxicity by oral route (gavage), the eMSCA agrees that the substance is deemed to be classified as STOT Rep. Exp. 2 H373 - Affected organs: adrenals, testes, epididymides, ovaries, heart and liver.

7.9.5. Mutagenicity

Not evaluated.

7.9.6. Carcinogenicity

Not evaluated.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Not evaluated.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

The following DNELs are derived by eMSCA according to ECHA Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health.

Value used for CSA:

Bioaccumulation potential:	no bioaccumulation potential
Absorption rate - oral (%):	100

Absorption rate - dermal (%): 100
Absorption rate - inhalation (%): 100

Table 10

CRITICAL DNELS/DMELS					
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**DNEL/ DMEL**	**Justification/ Remarks**
Workers Inhalation	Systemic effects-Long-term	Repeatet dose toxicity (Oral)	LOAEC* (52,5 mg/m^3)	DNEL 0.7 mg/m^3	AF for dose response relationship: 3 AF for dfference in duration of exposure: 2 AF for interspecies differencies (allometric scaling): 1 AF for other interspecies differences: 2.5 AF for intraspecies differences: 5 AF for quality of the whole database: 1 Overall Assessment Factor: 75
Workers Dermal	Systemic effects-Long-term	Repeatet dose toxicity (Oral)	LOAEL*(30 mg/kg bw/day)	DNEL 0.1 mg/kg bw/day	AF for dose response relationship: 3 AF for dfference in duration of exposure: 2 AF for interspecies differencies (allometric scaling): 4 AF for other interspecies differences: 2.5 AF for intraspecies differences: 5 AF for quality of the whole database: 1 Assessment Factor: 300

General Population Inhalation	Systemic effects-Long-term	Repeatet dose toxicity (Oral)	LOAEC* (26,3 mg/m^3)	DNEL 0.058 mg/m^3	AF for dose response relationship: 3 AF for dfference in duration of exposure: 6 AF for interspecies differencies (allometric scaling): 1 AF for other interspecies differences: 2.5 AF for intraspecies differences: 10 AF for quality of the whole database: 1 Assessment Factor: 450
General Population Dermal	Systemic effects-Long-term	Repeatet dose toxicity (Oral)	LOAEL* (30 mg/kg bw/day)	DNEL 0.017 mg/kg bw/day	AF for dose response relationship: 3 AF for dfference in duration of exposure: 6 AF for interspecies differencies (allometric scaling): 4 AF for other interspecies differences: 2.5 AF for intraspecies differences: 10 AF for quality of the whole database: 1 Assessment Factor: 1.800
General Population Oral	Systemic effects-Long-term	Repeatet dose toxicity (Oral)	LOAEL* (30 mg/kg bw/day)	DNEL 0.017 mg/kg bw/day	AF for dose response relationship: 3 AF for dfference in duration of exposure: 6 AF for interspecies differencies (allometric scaling): 4 AF for other interspecies

					differences: 2.5 AF for intraspecies differences: 10 AF for quality of the whole database: 1 Assessment Factor: 1800

* LOAEC derived from NOAEL according to Guidance R8

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

On the basis of effects noted in the Repeated oral toxicity study eMSCA agrees that the substance is deemed to be classified with STOT Rep. Exp. 2 H373 - Affected organs: adrenals, testes, epididymides, ovaries, heart and liver.

7.10. Assessment of endocrine disrupting (ED) properties

Not evaluated.

7.11. PBT and VPVB assessment

1) *Persistence:* Based on evaluation of the simulation study on ultimate degradation in surface water, Trixylyl phosphate fulfills the criteria of Annex XIII. Therefore the substance is considered to be Persistent (P) and very Persistent (vP).

2) *Bioaccumulation:* Unresolved. In a weight of evidence approach updated with the OECD 305 GLP study on Phenol, isopropylated, phosphate (3:1), all BCF's are less than 1000. However, due to uncertainties related to the composition of the IPTPP and the specific bioaccumulative potential of its constituents, the eMSCA considers that at this stage the read-across on IPTPP cannot be accepted to reach a conclusion on the B properties of Trixylyl phosphate. .

3) *Toxicity:* T (Harmonised classification in Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation) ATP03 COMMISSION REGULATION (EU) No 618/2012 of 10 July 2012: Repr. 1B H360F)

Overall conclusion: The substance fulfils the criteria for Persistency and Toxicity, while it is unresolved whether the substance fulfills the criteria for Bioaccumulation, as specified in REACH Annex XIII.

7.12. Exposure assessment

7.12.1. Human health

Worker

In general, eMSCA agrees with the approach taken by the Registrant(s) in performing the exposure and risk assessment for human health.

To be noted that some scenarios (PROCs) have been estimated applying Tier2 models, i.e., RISKOFDERM for the dermal and ART model for the inhalation route since the Tier1 model ECETOC TRA is expected to be over-conservative.

Consumer

Not applicable. There are no consumer uses of this substance.

7.12.2. Environment

The Registrant(s) revised the CSR using ATIEL-ATC SPERCs in the derivation of emissions to all the environmental compartments. In general, eMSCA agrees with the approach taken by the Registrant(s) for the exposure assessment refinement.

Aquatic compartment (incl. sediment)

For the Exposure Scenario 1 (Manufacture), no emission is permitted. Site bunding and engineering measures ensure that no emissions are undertaken.

Emissions to Sewer take place only from one site following significant treatment and utilising BAT. All site waste waters from processes and maintenance are contained and treated at the site WWTP. No emissions is permitted to groundwater. The site is obliged to use BAT to minimise future emissions.

For all the Exposure Scenarios the environmental release estimated are adequate, confirming a controlled risk.

Terrestrial compartment

For the Exposure Scenario 1 (Manufacture), no emissions is permitted to land.
For all the Exposure Scenarios the environmental release estimated are adequate confirming a controlled risk.

Atmospheric compartment

Emissions to air, where permitted, are in accordance with BAT (best available technology) for release of HCl from process reaction.
Some site is obliged to use BAT to minimise fugitive emissions, and general monitoring confirmed negligible releases to air from the manufacturing process.

7.12.3. Combined exposure assessment

For the human health, combined risk assessment – by summing up exposure levels of different tasks – has not been carried out since the majority of the PROCs have been estimated considering the maximum shift hours. This should be taken into account when the exposure scenarios will be implemented by downstream users.

7.13. Risk characterisation

In general, eMSCA agrees with the approach taken by the Registrant(s) in performing the exposure and risk assessment for human health. Nevertheless, it should be noted that, as follow up of the evaluation, it is possible that some scenarios (PROCs), considering the corrected DNELs calculated by the eMSCA may present RCR above 1. Therefore it is recommended that the Registrant(s) updates their CSR with the inclusion of appropriate RMM whereas a potential risk is identified.

To be noted that some scenarios (PROCs) have been estimated applying Tier2 models, i.e., RISKOFDERM for the dermal; and ART model for the inhalation route since the Tier1 model ECETOC TRA is expected to be over-conservative.

eMSCA points out that, under the follow up of this evaluation, some hazard assessment conclusions, including the related PNEC derivation, for aquatic compartment have been revised. Therefore a refinement of risk characterization including an update of CSR is recommended to the Registrant(s) with the inclusion of appropriate RMM whereas a potential risk is identified.

7.14. References

Aside from the registration dossier(s), no other additional sources were used. Registration dossier for Trixylyl Phosphate, European Chemicals Agency and Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health are available at: http://echa.europa.eu/

7.15. Abbreviations

AF	Assessment factor
BW	Body weight
CAS	Chemical abstracts service
C&L	Classification and labelling
CLP	Classification, labelling and packaging (Regulation (EC) No 1272/2008)
CMR	Carcinogenicity, mutagenicity and toxicity to reproduction
CSR	Chemical Safety Report
DNEL	Derived no effect level
eMSCA	Evaluating Member State Competent Authority
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
OECD	Organisation for Economic Co-operation and Development
PBT	Persistent, Bioaccumulative, Toxic
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
QSAR	Quantitative structure–activity relationship
RCR	Risk characterization ratio
RMMs	Risk Management Measures
STP	Sewage Treatment Plant
TXP	Trixylyl Phosphate
vPvB	Very Persistent and very Bioaccumulative