SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Substance name

Reaction products of phosphoryl trichloride and 2-methyloxirane

CAS No 1244733-77-4
List No 807-935-0

(formerly identified with EC No 237-158-7 and List No 911-815-4)

Evaluating Member State: Denmark

Dated: August 2023

Template Version 2.1
March 2015

Evaluating Member State Competent Authority

Danish Environmental Protection Agency
Tolderlundsvej 5, 5000 Odense C, Denmark
Tel: +45 72544000
Email: mst@mst.dk

Year of evaluation in CoRAP: 2022

The evaluating Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

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

DISCLAIMER

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

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Reaction products of phosphoryl trichloride and 2-methyloxirane, hereafter referred to as TCPP, was originally selected for substance evaluation to clarify concerns about:

- Carcinogenicity
- Reproductive toxicity
- Endocrine disruption (ED)
- Wide dispersive uses, consumer uses, cumulative exposure and high aggregated tonnage.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

TCPP was subject to a comprehensive compliance check (CCH) under REACH in 2016, which resulted in a decision requesting further information on the name or other identifiers of the substance, the composition of the substance (including identification of constituents) and a pre-natal developmental toxicity study in a second species. The deadline for submitting the requested information was set to March 2018. Following the CCH, the chemical identifiers were adapted since the former registered substance TCPP contained a mixture of isomers.

In 2018, a screening assessment performed by ECHA identified a cancer risk for children from exposure to TCPP and the similar substances tris(2-chloroethyl) phosphate (TCEP) and tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP) used as flame retardants in flexible polyurethane (PUR) foams in childcare articles and residential upholstered furniture (ECHA, 2018). A restriction proposal proposed by the Commission was withdrawn in July 2019 pending the availability of two carcinogenicity studies in rats and mice with TCPP conducted by the US National Toxicology Program (NTP).

ECHA has published an assessment of regulatory needs (ARN) on a group of chlorinated trialkyl phosphate flame retardants, including TCPP (ECHA, 2022a) as well as a Regulatory Strategy for Flame Retardants (ECHA, 2023). The ARN confirmed that there is a known or potential hazard for reproductive toxicity, carcinogenicity and endocrine disruption for TCPP, but also for several other members of the group. The last foreseen action was proposed to be restriction following the harmonised classification and labelling (CLH) for a group of flame retardants to manage the risks to workers and consumers and to avoid regrettable substitution. However, it was also suggested to await the publication of the full study report for the two US NTP carcinogenicity studies. The full study report has now been made available on the US NTP website (NTP, 2023).

3. CONCLUSION OF SUBSTANCE EVALUATION

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

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	X

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

4. FOLLOW-UP AT EU LEVEL

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

Following the substance evaluation of all available data, the concerns for carcinogenicity, reproductive toxicity and endocrine disruption have been confirmed. The eMSCA has identified the need to proceed to follow-up regulatory actions at EU level to address these concerns. Except for harmonised classification and labelling (CLH), the eMSCA has not yet identified the most appropriate additional follow-up risk management measures and will prepare a separate regulatory management option analysis (RMOA) in which different risk management options will be elaborated. Some preliminary considerations are provided below.

4.1.1. Harmonised Classification and Labelling

The Substance has currently no harmonised classification in Annex VI of the CLP Regulation. In the ECHA screening report of 2018, it was considered that CLH may be an appropriate action for TCPP depending on the outcome of the two US NTP carcinogenicity studies with TCPP. These studies confirm clear carcinogenic effects of the substance (see section 7.9.6). TCPP appears to be a multi-site carcinogen in two species (rats and mice) probably acting via several modes of action. Therefore, preparation of a CLH proposal for this endpoint is considered as a justified risk management option. A CLH as Carc. 1B triggers automatically a restriction entry 28 (consumer mixtures), registration dossiers updates, action at workplace under OSH and action under sectorial legislations.

Although the criteria for classification of substances as endocrine disruptors (ED) under CLP have been published (Commission Delegated Regulation (EU) 2023/707) and reproductive toxicity has also been confirmed in this evaluation, further considerations are needed before deciding on the most appropriate risk management to address these endpoints.

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

To address the confirmed endocrine disrupting properties of the Substance, a proposal to identify the Substance as an ED according to REACH Art. 57(f) could be developed. Subsequently, the Substance could be included in the Authorisation List. After CLH, the carcinogenic properties of the substance could also be addressed via identification as a substance of very high concern via REACH Art. 57(a) depending on the outcome of the classification proposal. However, as indicated above, it is considered by the eMSCA that it would be most appropriate to first submit a proposal for harmonised classification before possible subsequent SVHC identification.

4.1.3. Restriction

The possible need for a restriction of the Substance together with other similar flame retardants has been previously identified (ECHA, 2018, 2022a, 2023). At that time, there

were no carcinogenicity studies available for TCPP and it was concluded in an EU Risk Assessment Report (EU RAR) that quantitative read-across to other flame retardants was not considered sufficiently robust for this endpoint (EU RAR, 2008a). The intention to prepare a restriction proposal was subsequently withdrawn and it was concluded by ECHA that US NTP data, which was under generation at the time, should be considered if a restriction proposal was prepared. The results of the NTP carcinogenicity studies have now been made available permitting the evaluating Member State to conclude on the substance evaluation and to possibly prepare a restriction proposal. A restriction proposal for the Substance and other structurally similar flame retardants needs further considerations in the RMOA. Indeed, chlorinated trialkyl phosphates are used as flame retardants also in a variety of polymers and applications including plastics, foams, coatings, paper and textiles potentially ending up in a number of different article types.

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

Not applicable.

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 evaluating Member State. 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**
RMOA	2023	Denmark
CLH	2023	Denmark
SVHC/Restriction (depending on the outcome of the RMOA)	tbd	Denmark

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

TCPP was originally selected for substance evaluation in order to clarify concerns about:

- Carcinogenicity
- Reproductive toxicity
- Endocrine disruption
- Wide dispersive uses, consumer uses, cumulative exposure and high aggregated tonnage

Table 3

EVALUATED ENDPOINTS Endpoint evaluated	Outcome/conclusion
Carcinogenicity	Carcinogenicity confirmed. Harmonised C&L process to be initiated.
Reproductive toxicity - Developmental toxicity - Fertility and sexual function	Toxicity to reproduction confirmed. See also Endocrine disruption
Endocrine disruption for human health	Endocrine disruption confirmed. Appropriate risk management measures to be considered.
Uses and exposure	Not evaluated by the eMSCA. Exposure and risk confirmed elsewhere*. Appropriate risk management measures to be considered.

* Separate reports identified a cancer risk for children and adults from exposure to TCPP and other flame retardants in flexible polyurethane (PUR) foams in childcare articles, residential upholstered furniture and other articles (ECHA, 2023).

7.2. Procedure

TCPP was initially suggested for CoRAP inclusion in 2015. The evaluation was put on hold to await the outcome of the two NTP carcinogenicity studies and to await clarifications of the substance identity which led to a change in chemical identifiers. The latest CoRAP justification document was submitted in March 2022 and the Substance was included in CoRAP for evaluation in 2022.

The evaluation of TCPP was targeted towards the concerns identified in the CoRAP justification document (carcinogenicity, reproductive toxicity and endocrine disruption). The evaluation was based on the information available in the registration dossier and information in the full study reports of the relevant studies as provided by the registrant. In addition, a targeted literature search was conducted to identify additional data relevant for the endpoints of concern. Finally, the US NTP has recently published the results of two carcinogenicity studies on TCPP (NTP, 2023) which were also taken into account in this substance evaluation. TCPP was discussed at the 23rd meeting of the ECHA Endocrine Disruptor Expert Group where the experts considered that there was already sufficient data to demonstrate the link between endocrine activity and adversity for this substance (ECHA, 2022b).

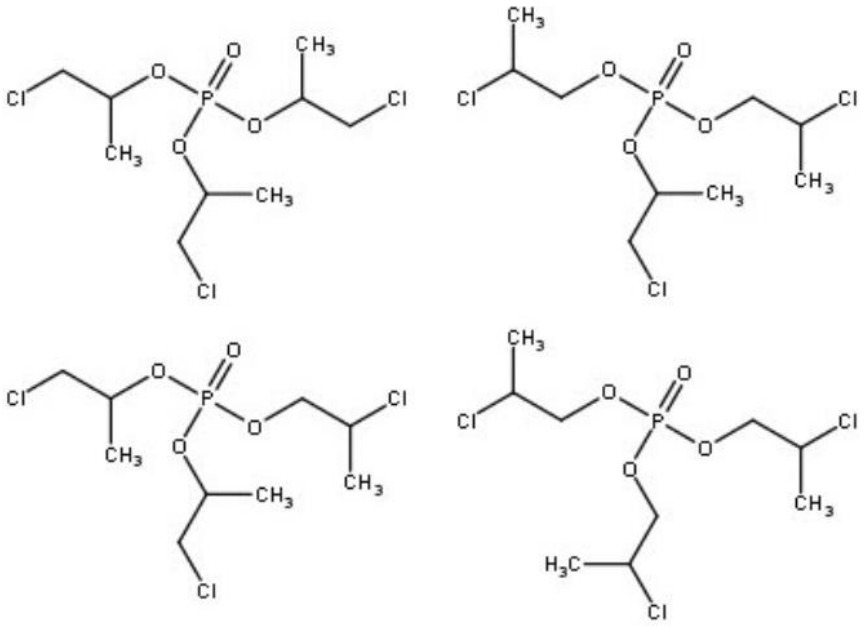
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Reaction products of phosphoryl trichloride and 2-methyloxirane
EC number:	807-935-0
CAS number:	1244733-77-4
Index number in Annex VI of the CLP Regulation:	Not applicable
Molecular formula:	C36H72Cl12O16P4
Molecular weight range:	327,57 g/mol
Synonyms:	TCPP

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

Structural formula:



TCPP is a UVCB produced as an isomeric mixture in a closed system by the reaction of phosphorus oxychloride and propylene oxide. The reaction generates a mixture of four main isomers.

Variations in manufacturing methods result in commercial formulations that contain different ratios of the four main isomers. The most abundant isomer in commercial products is tris(1-chloro-2-propyl) phosphate (TCIPP) as shown in Table 5. The TCPP mixture and commercial products are commonly referred to by TCIPP and by CAS RN 13674-84-5 (NTP, 2020).

It should also be noted that the test material composition in older studies varies beyond the ranges shown in Table 5. For example, in the *in vivo* studies included in the assessment of endocrine disruption for human health, it seems that the test material composition varies

between a study conducted in 1981 (Stauffer Chemical Co., 1981a) and studies conducted after 2000, which may explain differences in test results. In the study from 1981, there is approximately 23% 2-chloropropanol phosphate (identified as tris(2-chloropropyl) phosphate (CAS 6145-73-9) according to ChemIDPlus) whereas this isomer only constitutes <1 % of the composition of the test substance according to the table 5 below.

Table 5 (modified from (NTP, 2020))

Constituent					
Chemical name	**CAS RN**	**EC number**	**Concentration range**	**Other names**	**REACH Registration and CLP notification status***
Tris(1-chloro-2-propyl) phosphate (TCIPP)	13674-84-5	237-158-7	50-85 %	2-Propanol, 1-chloro-, 2,2',2"-phosphate Tris(2-chloro-1-methylethyl) phosphate Tris(2-chloro isopropyl)phosphate	Registered under REACH but manufacture has been ceased Notified C&L: 738
Bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate	76025-08-6	616-283-4	15-40 %	Bis(1-chloro-2-propyl) 2-chloro-1-propyl phosphate Bis(2-chloro isopropyl) 2-chloropropyl phosphate	Not registered under REACH No notified C&L
Bis(2-chloropropyl) 2-chloroisopropyl phosphate	76649-15-5	616-366-5	<15 %	2-Chloro-1-methylethylbis(2-chloropropyl) phosphate Bis(2-chloropropyl) 2-chloro-1-methylethyl phosphate Bis(2-chloro-1-propyl) 1-chloro-2-propyl phosphate	Not registered under REACH No notified C&L
Tris(2-chloropropyl) phosphate	6145-73-9	228-150-4	<1 %	1-Propanol, 2-chloro-, phosphate (3:1) Tris(2-chloro-1-propyl) phosphate	Not registered under REACH Notified C&L: 37

*ECHA substance entries accessed 23 June 2023

7.4. Physico-chemical properties

Table 6

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Liquid, colourless
Vapour pressure	0 hPa at 25 °C
Water solubility	1.08 g/L at 20 °C

Boiling point	288 °C at 1014 hPa)
Partition coefficient n-octanol/water (Log Kow)	2.68 at 30 °C and pH 7.1
Flammability	Non-flammable

7.5. Manufacture and uses

7.5.1. Quantities

Table 7

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

TCPP has a wide range of uses. It is used in polymers, adhesives and sealants, coating products, laboratory chemicals, textile treatment products, dyes, vehicles, machinery, mechanical appliances, electrical/electronic products (e.g. computers, cameras, lamps, refrigerators, washing machines), plastic (e.g. food packaging and storage, toys, mobile phones), fabrics, textiles, apparel (e.g. clothing, mattress, curtains or carpets, textile toys), wood (e.g. floors, furniture, toys) and metal (e.g. cutlery, pots, toys, jewellery).

Table 8

USES	Use(s)
Uses as intermediate	Not applicable
Manufacture	Environmental release category (ERC): ERC1: Manufacture of the substance Process category (PROC): PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) PROC 15: Use as laboratory reagent
Formulation	Environmental release category (ERC): ERC2: Formulation into mixture ERC3: Formulation into solid matrix

	ERC4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article) ERC5: Use at industrial site leading to inclusion into/onto article <u>Process category (PROC):</u> PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions PROC 4: Chemical production where opportunity for exposure arises PROC 5: Mixing or blending in batch processes PROC 6: Calendering operations PROC 7: Industrial spraying PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) PROC 10: Roller application or brushing PROC 13: Treatment of articles by dipping and pouring PROC 14: Tabletting, compression, extrusion, pelletisation, granulation PROC 15: Use as laboratory reagent PROC 19: Hand-mixing with intimate contact and only PPE available. PROC 21: Low energy manipulation of substances bound in materials and/or articles <u>Product category (PC):</u> PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 21: Laboratory chemicals PC 32: Polymer preparations and compounds PC 34: Textile dyes, and impregnating products
Uses at industrial sites	<u>Environmental release category (ERC):</u> ERC2: Formulation into mixture ERC3: Formulation into solid matrix ERC4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article) ERC5: Use at industrial site leading to inclusion into/onto article <u>Process category (PROC):</u> PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions PROC 4: Chemical production where opportunity for exposure arises

	PROC 5: Mixing or blending in batch processes PROC 6: Calendering operations PROC 7: Industrial spraying PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) PROC 10: Roller application or brushing PROC 13: Treatment of articles by dipping and pouring PROC 14: Tabletting, compression, extrusion, pelletisation, granulation PROC 15: Use as laboratory reagent PROC 19: Hand-mixing with intimate contact and only PPE available. PROC 21: Low energy manipulation of substances bound in materials and/or articles <u>Product category used:</u> PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 21: Laboratory chemicals PC 32: Polymer preparations and compounds PC 34: Textile dyes, and impregnating products <u>Sector of end use:</u> SU 5: Manufacture of textiles, leather, fur SU 12: Manufacture of plastics products, including compounding and conversion SU 18: Manufacture of furniture SU 19: Building and construction work SU 0: Other: all industrial use SU 0: Other: SU3: all industrial uses
Uses by professional workers	<u>Environmental release category (ERC):</u> ERC8a: Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor) ERC8b: Widespread use of reactive processing aid (no inclusion into or onto article, indoor) ERC8c: Widespread use leading to inclusion into/onto article (indoor) ERC8f: Widespread use leading to inclusion into/onto article (outdoor) ERC10a: Widespread use of articles with low release (outdoor) ERC11a: Widespread use of articles with low release (indoor) <u>Process category (PROC):</u> PROC 5: Mixing or blending in batch processes PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 10: Roller application or brushing PROC 11: Non industrial spraying PROC 13: Treatment of articles by dipping and pouring PROC 15: Use as laboratory reagent PROC 21: Low energy manipulation of substances bound in materials and/or articles <u>Product category used:</u> PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 21: Laboratory chemicals

	PC 32: Polymer preparations and compounds Sector of end use: SU 12: Manufacture of plastics products, including compounding and conversion SU 19: Building and construction work SU 0: Other: SU22 Public domain (administration, education, entertainment, services, craftsmen) SU 0: Other: Laboratory use
Consumer Uses	Environmental release category (ERC): ERC8c: Widespread use leading to inclusion into/onto article (indoor) ERC8f: Widespread use leading to inclusion into/onto article (outdoor) ERC10a: Widespread use of articles with low release (outdoor) ERC11a: Widespread use of articles with low release (indoor) Product category (PC): PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 32: Polymer preparations and compounds
Article service life	Environmental release category (ERC): ERC8c: Widespread use leading to inclusion into/onto article (indoor) ERC8f: Widespread use leading to inclusion into/onto article (outdoor) ERC10a: Widespread use of articles with low release (outdoor) ERC11a: Widespread use of articles with low release (indoor) Article category (AC): AC 1: Vehicles AC 2: Machinery, mechanical appliances, electrical/electronic articles AC 4: Stone, plaster, cement, glass and ceramic articles AC 5: Fabrics, textiles and apparel AC 7: Metal articles AC 11: Wood articles AC 13: Plastic articles AC 0: Other: Clothing (all kind of materials), towel (AC 5) AC 0: Other: Bedding, mattress (AC 5) AC 0: Other: Toys (cuddly toy) (AC 5) AC 0: Other: Car seat, chair, flooring (AC 5) AC 0: Other: Furniture (chair) (AC 11) AC 0: Other: Walls and flooring (also applicable to non-wood materials) (AC 11) AC 0: Other: Small toys (car, train) (AC 11) AC 0: Other: Toys, outdoor equipment (AC 11) AC 0: Other: Plastic, larger articles (plastic chair, PVC-flooring, lawn mower, PC) (AC 13) AC 0: Other: Toys (doll, car, animals, teething rings) (AC 13) AC 0: Other: Plastic, small articles (ball pen, mobile phone) (AC 13) Process category (PROC): PROC 14: Tabletting, compression, extrusion, pelletisation, granulation PROC 21: Low energy manipulation of substances bound in materials and/or articles PROC 24: High (mechanical) energy work-up of substances bound in materials and/or articles

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

There are no harmonised classifications for TCPP or any of its isomers.

7.6.2. Self-classification

TCPP is self-classified as Acute Tox. 4; H302 and Aquatic Chronic 3; H412. There are 2 aggregated notifications and 85 notifiers[2].

The previous identifier (EC 237-158-7; CAS RN 13674-84-5) is self-classified as Acute Tox. 4; H302, Aquatic Chronic 3; H412, Eye Irrit. 2; H319 and Skin Irrit. 2; H315. There are 6 aggregated notifications and 738 notifiers[3].

7.7. Environmental fate properties

The environmental fate properties have not been evaluated by the eMSCA in this substance evaluation.

7.8. Environmental hazard assessment

An environmental hazard assessment has not been performed by the eMSCA in this substance evaluation.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Available data on absorption, distribution, metabolism and excretion is summarised in the NTP study report (NTP, 2023). Available evidence shows that TCPP is readily absorbed and excreted by male Wistar rats following oral gavage administration. Approximately 98% of the administered dose is recovered during the 168 hours after dosing. Within 48 hours, 67%, 22%, and 7.7% TCPP was recovered in urine, faeces and expired air, respectively. TCPP was rapidly distributed to tissues with tissue to blood ratios highest in the liver and kidney followed by lung and spleen during the first 12 hours after administration. The elimination half-life in blood was estimated to be approximately 59 hours. Biliary excretion studies showed that approximately 45% of the administered dose was excreted in bile within 48 hours and that TCPP excreted in faeces is likely from biliary excretion (NTP, 2023).

The metabolism of TCPP has previously been investigated in an EU Risk Assessment report from 2008 (EU RAR, 2008a). According to this report, TCPP is extensively metabolised prior to excretion in the urine and faeces. Unchanged TCPP represented less than 2% of the administered dose. 0,0-[Bis(1- chloro-2-propyl)]-0-(2,proprionic acid)phosphate was identified as a major metabolite in both urine and faeces, accounting for over 50% of the dose. At the low dose, this metabolite was excreted approximately equally in the urine and

[2] ECHA classification inventory accessed 29 June, 2023. Link: https://echa.europa.eu/da/information-on-chemicals/cl-inventory-database/-/discli/details/245118

[3] ECHA classification inventory accessed 29 June, 2023. Link: https://echa.europa.eu/da/information-on-chemicals/cl-inventory-database/-/discli/details/12076

faeces in males, whereas at the higher dose, it was excreted predominantly in the urine in both males and females. The dose-dependent excretory pattern of this metabolite in the urine and faeces corresponds well with the dose-dependent changes in excretion of total radioactivity observed at both dose levels, suggesting that this metabolite is responsible for the dose-dependent excretory pattern noted at these dose levels. Other metabolites isolated and identified were bis(1-chloro-2-propyl)monophosphoric acid which accounted for 12% of the total radiocarbon administered to male rats and 1-chloro-2-propanol which was not quantified (EU RAR, 2008a).

Via the inhalation route, no studies are available. Via the dermal route, TCPP is extensively absorbed according to two *in vitro* percutaneous absorption studies using human skin membranes (EU RAR, 2008a).

In conclusion, exposure to TCPP via oral route results in extensive absorption, distribution, metabolism and excretion. After oral exposure, the average terminal plasma half-life (t½) was 48.7 hours. The longest tissue t½ was recorded in adipose tissue (up to 103.4 hours) but concentrations in tissues were low and bioaccumulation potential is therefore considered to be low. Urinary and faecal excretion is dose and administration route-dependent (oral and intravenous) and occurred quite rapidly. The observed biliary/faecal excretion ratio is indicative of enterohepatic recirculation (EU RAR, 2008a).

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated in this substance evaluation.

7.9.3. Sensitisation

Not evaluated in this substance evaluation.

7.9.4. Repeated dose toxicity

Not evaluated in this substance evaluation. However, some repeated dose toxicity (RDT) studies with TCPP have recently been performed. The results from these studies, which could be relevant for evaluation of carcinogenicity are described in section 7.9.6, whereas the results of the repeated dose toxicity studies relevant for the assessment for reproductive toxicity and endocrine disrupting properties of TCPP are presented in sections 7.9.7 and 7.10.2, respectively.

7.9.5. Mutagenicity

Several *in vitro* mutagenicity studies have been conducted with TCPP, the vast majority with a negative result. An *in vivo* rat bone marrow cytogenetics assay using a single intraperitoneal (i.p.) injection with 350 mg/kg bw yielded a negative result but this study did not demonstrate exposure of the target tissue (incidence of micronucleated polychromatic erythrocytes or normochromatic erythrocytes was not significantly increased in the treatment groups compared to the negative control). A Comet assay by with TCPP is referenced in the EU RAR (2008a). This Comet assay was conducted in liver with male rats and it yielded a negative result. Liver was chosen as the target organ because TCPP was shown to cause an increase in mutation frequency in the *in vitro* mouse lymphoma assay in the presence of S9 and also induced liver enlargement in repeated dose toxicity studies *in vivo*. According to the study summary, TCPP did not induce DNA damage in the

liver of male rats treated with either 750 or 1500 mg/kg bw in the Comet assay (EU RAR, 2008a). However, a slight increase in group mean tail moments and intensities compared with the concurrent control was observed after 3 hours at 1500 mg/kg bw. The eMSCA concludes that this finding could be treatment-related but that it is equivocal and with limited biological relevance. The EU RAR (2008a) assessed the data mentioned above and concluded that TCPP is not genotoxic *in vivo*.

As part of their bioassay program, the US NTP conducted a micronucleus assay in 2015 on male and female Sprague Dawley rats using TCPP. Peripheral blood was drawn from animals that were treated in the 90-day repeated dose toxicity studies. Animals were fed 0, 2500, 5000, 10000 or 20000 ppm TCPP in the diet, 5 days a week for 90 days. This study yielded a negative result in both male and female rats. A similar study was conducted in mice with doses of 0, 1250, 2500, 5000, 10000 or 20000 ppm TCPP and this study yielded a negative result in female mice and an equivocal result in male mice. Study summaries are available in the REACH registration dossier and raw data is available via the NTP website[4].

An assessment of the weight of evidence for mutagenicity supports that TCPP does not act directly with DNA.

7.9.6. Carcinogenicity

The carcinogenicity potential of TCPP was recently investigated by NTP in two two-year carcinogenicity studies in rats and mice (section 7.9.6.1). In addition, carcinogenicity studies have also been conducted with similar substances (TCEP and TCDP) as well as with the TCPP metabolite 1-chloro-2-propanol (section 7.9.6.2.2). Mechanistic studies with TCPP investigating the potential tumour mode of action (MoA) are also available (section 7.9.6.2.3).

7.9.6.1 Carcinogenicity studies with TCPP

NTP has conducted a 2-year carcinogenicity study in mice and rats (NTP, 2023). A perinatal study with rats was conducted prior to the 2-year study beginning *in utero* (gestation day (GD) 6 through lactation day (LD) 21) and through adulthood, which means that rats were exposed perinatally plus 2 years. Mice were exposed for 2 years. The studies were conducted from 2011 to 2013. The test article name represents the mixture of isomers. Its trade names are Amgard TMCP, Antiblaze 80, Antiblaze TMCP and Fyrol PCF. Two different lots were used for the studies in rats and mice. Chemical analysis of the test material was performed on both lots. The analysis identified four major isomeric components. They were identified as tris(1-chloro-2-propyl) phosphate (CASRN 13674-84-5) (rats: 64.77 %; mice: 68.06 %), bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CASRN 76025-08-6) (rats: 26.98 %; mice: 25.43 %), bis(2-chloropropyl) 2-chloroisopropyl phosphate (CASRN 76649-15-5) (3.99 %; mice: 3.55 %) and tris(2-chloropropyl) phosphate (CASRN 6145-73-9) (rats: 0.20 %; mice: 0.21 %).

Statistical analysis was performed using both pairwise comparison between the individual dose groups and the control group as well as an overall trend test. For the study in rats, Rao-Scott-adjusted Poly-3 tests were performed to account for litter effects while the study in mice used Poly-3 test without adjustment for potential litter effects.

7.9.6.1.1 Study in rats (with prenatal exposure)

In the carcinogenicity study in Sprague Dawley rats, 50 animals per sex were used in each

[4] https://cebs.niehs.nih.gov/cebs/data/publication/TR-602

dose group with exposure concentrations shown in Table 9.

Table 9: Exposure dose levels in male and female rats

TCPP in diet (ppm)	**0**	**2500**	**5000**	**10,000**	**20,000**
TCPP dose at week 102 (mg/kg bw/d) (males)	0	131.0	246.9	557.1	855.0
TCPP dose at week 102 (mg/kg bw/d) (females)	0	156.4	346.6	742.0	1109.2

Concentrations of the main isomer TCIPP were measured in the plasma after 3, 6, 12 and 18 months. A method was validated by NTP to quantitate TCIPP in female Sprague Dawley rat and B6C3F1 mouse plasma (Collins et al., 2019). The validated method has lower limits of quantitation and detection of ~5 and 0.9 ng/mL, respectively, in female rat plasma and can be used on samples as small as 50 µL.

Table 10: Plasmatic concentration of TCIPP in male and female rats

	TCPP in diet (ppm)	**0**	**2500**	**5000**	**10,000**	**20,000**
Sex	**Time (months)**					
Males	**3**	3.66 ± 1.17 ng/ml (10)	17.6 ± 4.34 ng/ml (10)	13.6 ± 1.72 ng/ml (10)	38.0 ± 6.27 ng/ml (9)	74.5 ± 7.65 ng/ml (10)
	6	Below detection	3.34 ± 1.53 ng/ml (10)	18.7 ± 2.4 ng/ml (10)	70.9 ± 18.40 ng/ml (9)	41.2 ± 4.40 ng/ml (10)
	12	3.77 ± 0.382 ng/ml (9)	5.48 ± 0.248 ng/ml (10)	28.2 ± 16.7 ng/ml (10)	31.7± 9.4 ng/ml (9)	31.9 ± 5.95 ng/ml (9)
	18	0.884 ± 0.207 ng/ml (8)	4.46 ± 0.417 ng/ml (9)	11.2 ± 1.75 ng/ml (9)	13.1 ± 1.02 ng/ml (9)	31.6 ± 7.49 ng/ml (8)
Females	**3**	8.60 ± 3.41 ng/ml (10)	39.3 ± 18.6 ng/ml (10)	30.2 ± 5.61 ng/ml (10)	50.0 ± 2.68 ng/ml (9)	78.4 ± 10.9 ng/ml (10)
	6	Below detection	8.31 ± 4.99 ng/ml (10)	6.84 ± 2.81 ng/ml (10)	19.5 ± 3.93 ng/ml (9)	7.21 ± 1.51 ng/ml (10)
	12	2.78 ± 0.437 ng/ml (9)	17.3 ± 4.79 ng/ml (9)	28.4 ± 7.04 ng/ml (10)	20.1± 3.79 ng/ml (9)	76.1 ± 35.1 ng/ml (10)

	18	1.40 ± 0.438 ng/ml (8)	7.75 ± 2.17 ng/ml (9)	15.9 ± 4.17 ng/ml (7)	23.0 ± 6.35 ng/ml (8)	17.4 ± 2.72 ng/ml (10)

As shown in the table, TCIPP was detected in plasma of the controls. According to the study report, sample preparation and analysis were attributed as the source of these low concentrations because of the ubiquitous presence of TCPP (NTP, 2023). TCIPP plasma concentrations in rats increased with increasing dose in general. However, this increase was not always in proportion with exposure concentrations. For instance, the concentration of TCIPP in female rats was higher in the 10,000 ppm dose group than in the 20,000 dose group after 18 months. TCIPP concentrations were also variable between time points for a given exposure concentration. No sex differences were observed as the TCIPP concentrations in males and females were within the same range. However, the generally varying TCIPP concentrations may have made possible differences difficult to reveal.

Histopathology:

Complete histopathology was performed on all control, 10,000 ppm and 20,000 ppm rats. In addition to gross lesions and tissue masses, tissues were examined according to OECD TG 451.

Overview of results in male rats:

Table 11: Survival and terminal body weight of male rats

TCPP dose	**0 ppm**	**2500 ppm**	**5000 ppm**	**10,000 ppm**	**20,000 ppm**
Survival (at 729 days)	50%	68%	68%	74%	62%
Mean life span (days)	651.9 ± 14.1	688.5 ± 9.9	699.0 ± 10.1	681.5 ± 16.1	696.5 ± 9.1
Terminal body weight in grams (% of controls)	574.4	570.7 (99.4%)	557.4 (97%)	544.6 (94.8%)	529.2 (92.1%)

Table 12: Incidences (%) of neoplasms in male rats

	0 ppm	**2500 ppm**	**5000 ppm**	**10,000 ppm**	**20,000 ppm**	**Historical control data from NTP 2-year feed studies (mean – range) (NTP, 2023)** **Comment**
Number of animals	**50**	**50**	**50**	**50**	**50**	
Hepatocellular adenomas (single + multiple)	2%	0%	0%	10%	6%	1/249 (0.4% ± 0.89%) Control significantly different from treated groups in Poly-3 trend test but not in Rao-Scott trend test

Hepatocellular carcinomas (single + multiple)	0%	0%	2%	6%	6%	2/249 (0.81% ± 1.1%) Control significantly different from treated groups in Poly-3 trend test but not in Rao-Scott trend test
Hepatocellular adenomas and carcinomas combined (single + multiple)	2%	0%	2%	16%	12%	3/249 (1.21% ± 1.1%) Control significantly different from treated groups in Rao-Scott trend test (p = 0.013)

Even though there was an increased incidence beyond the historical control data of both hepatocellular adenomas and carcinomas in treated male rats in the two highest dose groups, this difference was not statistically significantly different from controls with pairwise comparison. However, there was statistically significant increased incidence of combined hepatocellular adenomas and carcinomas in a trend test compared to controls (p = 0.013). Significant non-neoplastic lesions observed included an increase in eosinophilic, basophilic and mixed-cell foci (which can be precursor lesions of hepatocellular neoplasms) with a positive trend with increasing exposure concentration. Minimal hyperplasia was also observed in the bile duct.

According to the NTP report, the hepatocellular adenomas were well-circumscribed expansile masses comprising irregular plates of hepatocyte cords with eosinophilic to basophilic cytoplasm and occasionally vacuolated cytoplasm. Central veins and portal areas were occasionally entrapped in hepatocellular neoplasms. The hepatocellular carcinomas were described as large and invasive and composed of nodules of neoplastic hepatocytes. The NTP report notes that these neoplasms were also associated with increased cellular atypia accompanied by a few mitoses. Occasionally, there were areas of necrosis and hemorrhage within these masses.

No other biologically relevant significant differences in incidences of neoplastic or non-neoplastic lesions were observed in male rats. No adenomas were observed in male rats in the renal tubules of the kidneys in any of the groups. No carcinomas of the renal tubules were observed in male rats with the exception of 1 animal (2%) in the 5000 ppm group. It should be noted that these neoplasms are very rare in rats with <1% incidence in NTP historical control animals for all routes of exposure and 0% incidence in feed studies.

Overview of results in female rats:

Table 13: Survival and terminal body weight of female rats

TCPP dose	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm
Survival (at 729 days)	44%	62%	66%	68%	66%
Mean life span (days)	629.2 ± 16.3	675.6 ± 15.3	679.2 ± 12.8	683.3 ± 15.0	677.2 ± 12.1
Terminal body weight (% of controls)	371.4g	342.8g (92.8%)	353.7g (95.2%)	326.5g (87.9%)	309.1g (83.2%)

*: Statistically significant result (P<=0.05)

Table 14: Incidences (%) of neoplasms in female rats

	0 ppm	2500 ppm	5000 ppm	10,000 ppm	20,000 ppm	Historical control data from NTP 2-year feed studies, mean – range) (NTP, 2023) Comment
Number of animals	**50**	**50**	**50**	**50**	**50**	
Adrenal cortex (adenomas)	0%	0%	0%	4%	4%	Approx 2 % Control significantly different from treated groups in Poly-3 trend test but not in Rao-Scott trend test
Kidney, renal tubule (adenoma)	0%	0%	0%	0%	4%	<1% Control significantly different from treated groups in Poly-3 trend test but not in Rao-Scott trend test
Hepatocellular adenomas (single + multiple)	2%	6%	0%	6%	6%	4/249 (1.61% ± 1.68%)
Hepatocellular carcinoma (single + multiple)	0%	0%	0%	0%	0%	1/249 (0.4% ± 0.89%)
Ovary (benign granulosa cell tumor)	0%	0%	0%	0%	4%	<2% Control significantly different from treated groups in Poly-3 trend test but not in Rao-Scott trend test
Uterus (adenoma or adenocarcinoma)	6%	8%	12%	16%	18%	13/200 (6.5% ± 2.52%) Control significantly different from treated groups in Rao-Scott trend test (p = 0.043)
Uterus (stromal polyp)	10%	30%*	12%	14%	26%	37/200 (18.5% ± 9.43%);

*: Statistically significant result (P<=0.05)

According to the NTP study data, there were renal tubule adenomas in the kidneys of two female rats (4%) in the 20,000 ppm group. However, the increased incidence was not statistically significant in comparison to the control group. It should be noted that these neoplasms are very rare in rats with <1% incidence in NTP historical control animals for all routes of exposure and 0% incidence in feed studies. No other adenomas or carcinomas

in the renal tubules were observed in any of the groups in female rats.

Benign (adenomas) and malignant (adenocarcinomas) neoplasms were observed in the uterus of female rats. An exposure-related tripling in incidences was observed when comparing the control group and the high dose group, but there was no statistical significance by pairwise comparison to the control group. However, a statistically significant trend for adenoma or adenocarcinoma (combined) was observed ($p = 0.043$). The combined incidences of uterine adenomas and adenocarcinomas significantly exceeds historical controls.
Pre-neoplastic lesions such as atypical endometrial hyperplasia was also observed. Higher incidences of stromal polyps (non-neoplasms) were also observed in exposed rats. This difference was statistically significant in the low dose group. Solitary incidences of squamous cell carcinoma in the 10,000 and 20,000 ppm groups were also observed.

A statistically significant trend for benign ovarian granulosa cell tumors was observed. According to the NTP study report, there were higher incidences of malignant granulosa cell neoplasms in the 2,500 ppm (one) and 5000 ppm (three) groups and benign granulosa cell neoplasms in the 20,000 ppm group (two). These neoplasms are relatively uncommon and have <2% incidence in NTP historical control animals from feed as well as from all routes of exposure.

The incidences of hepatocellular adenomas were not significantly increased in female rats. The incidence of hepatocellular adenomas in the lowest and two highest exposure groups are slightly higher than controls. It is noted that two females in the 20,000 ppm group had multiple adenomas. No hepatocellular carcinomas were observed in any of the groups in female rats.

There was a statistically significant increase in eosinophilic, basophilic and mixed-cell foci in the liver. Statistically significant bile duct cysts (1/50, 6/50, 12/50, 19/50, 21/50) and bile duct hyperplasia was observed (7/50, 21/50, 24/50, 29/50, 11/50).

7.9.6.1.2 Study in mice

The study was performed in male and female B6C3F1/N mice. The average age of the mice was 5-6 weeks when the study began. 50 animals per sex were used in each dose group. Exposure concentrations were 0, 1250 (males only), 2500, 5000 or 10,000 (females only) ppm in feed.

Table 15: Exposure concentrations in mice

TCPP in diet (ppm)	**0**	**1250**	**2500**	**5000**	**10,000**
TCPP dose at week 102 (mg/kg bw/d) (males)	0	147.6	317.7	663.1	-
TCPP dose at week 102 (mg/kg bw/d) (females)	0	-	267	572.9	1359.8

Concentrations of the main isomer TCIPP were measured in the plasma after 3, 6, 12 and 18 months using a validated method (Collins et al., 2019). The validated method has lower limits of quantitation and detection of ~5 and 0.9 ng/mL, respectively, in female rat plasma and can be used on samples as small as 50 µL.

Table 16: Plasmatic concentration of TCIPP in male and female mice

	TCPP in diet (ppm)	**0**	**1250**	**2500**	**5000**	**10,000**
Sex	**Time (months)**					
Males	**3**	6.92 ± 3.14 ng/ml (5)	53.5 ± 33.2 ng/ml (5)	101 ± 58.5 ng/ml (5)	1180 ± 461 ng/ml (5)	-
	6	1.58 ± 0.683 ng/ml (5)	7.14 ± 2.21 ng/ml (5)	6.52 ± 1.42 ng/ml (5)	30.9 ± 9.18 ng/ml (5)	-
	12	1.05 ± 0.465 ng/ml 5)	11.0 ± 2.55 ng/ml (5)	47.2 ± 17.1 ng/ml (5)	174± 43.8 ng/ml (5)	-
	18	1.20 ± 0.488 ng/ml (4)	37.4 ± 34.4 ng/ml (3)	13.6 ± 3.60 ng/ml (4)	99.2 ± 28.8 ng/ml (5)	-
Females	**3**	15.1 ± 3.34 ng/ml (5)	-	15.7 ± 3.51 ng/ml (5)	29.7 ± 7.00 ng/ml (5)	159 ± 77.7 ng/ml (5)
	6	8.29 ± 1.96 ng/ml (5)	-	6.6 ± 1.74 ng/ml (5)	13.0 ± 3.49 ng/ml (5)	31.7 ± 5.05 ng/ml (5)
	12	1.50 ± 0.678 ng/ml (5)	-	19.3 ± 12.4 ng/ml (5)	65.1 ± 32.3 ng/ml (5)	265± 93.3 ng/ml (4)
	18	0.750 ± 0.173 ng/ml (5)	-	19.2 ± 8.66 ng/ml (5)	47.7 ± 14.0 ng/ml (5)	106 ± 57.3 ng/ml (5)

TCIPP was detected in plasma of the controls. According to the study report, sample preparation and analysis were attributed as the source of these low concentrations because of the ubiquitous presence of TCPP. TCIPP concentrations in mice increased in general with increasing dose but there were exceptions. For instance, the concentration of TCIPP in males in the 1250 ppm dose group was more than twice as high as the TCIPP concentration in the 2500 ppm dose group after 18 months. Male mice had higher plasma concentrations than females in general. This difference may be attributed to differences in feed consumption. Males exposed to 5000 ppm had TCIPP concentrations as high as 1180 ng/ml after three months but after 6 months, the concentration was reduced to 30.9 ng/ml. A huge standard deviation was associated with the former measurement.

Histopathology:

Complete microscopic examinations were performed on all mice. In addition to gross lesions and tissue masses, tissues were examined according to OECD TG 451.

Overview of results in male mice:

Table 17: Survival and terminal body weight of male mice

	0 ppm	**1250 ppm**	**2500 ppm**	**5000 ppm**
Survival (at 731 days)	78.0%	88.0%	84.0%	88.0%
Mean life span (days)	700.8 ± 9.5	715.6 ± 8.0	712.2± 7.2	724.6 ± 3.3
Terminal body weight (% of controls)	47.6g n = 38	46.2g (97.0%) n=44	43.7g (91.8%) n=42	39.1g (82.1%) n=43

*: Statistically significant result (P<=0.05)

Table 18: Incidences (%) of adenomas and carcinomas in male mice

	0 ppm	**1250 ppm**	**2500 ppm**	**5000 ppm**	**Historical control data from NTP 2-year feed studies (mean – range) (NTP, 2023)**
Hepatocellular adenoma (single + multiple)	42%	46%	36%	44%	60/149 (40.29% ± 3.74%)
Hepatocellular carcinoma (single + multiple)	10%	28%*	34%*	28%*	23/149 (15.44% ± 5.06%)
Hepatocellular adenoma or carcinoma (single + multiple)	46%	62%	62%	56%	73/149 (49.02% ± 3.64%)
Hepatocellular adenoma, carcinoma or hepatoblastoma (single + multiple)	48%	62%	62%	58%	74/149 (49.69% ± 2.92%)

*: Statistically significant result (P<=0.05)

There was no increase in incidence of hepatocellular adenomas in treated male mice when compared to controls. However, the incidence of hepatocellular carcinomas was statistically significantly increased in all treated groups compared to controls. Some animals had

multiple tumors. There appeared to be an increasing trend in combined adenomas, carcinomas or hepatoblastomas. Even though this difference did not reach statistical significance in any of the treated groups, the incidences in all treated groups were higher than the historical control data.

Renal tubule adenomas were observed in 2% of the control group. No adenomas were observed in any of the treated groups. Cytoplasmic alterations were observed in the renal tubules (0/49, 28/50, 40/50, 48/50).

No biologically relevant significant differences in incidences of neoplastic or non-neoplastic lesions were observed in the thyroid gland in male mice.

Overview of results in female mice:

Table 19: Survival and terminal body weight of female mice

	0 ppm	**2500 ppm**	**5000 ppm**	**10,000 ppm**
Survival (at 729 days)	92.0%	86.0%	90.0%	92.0%
Mean life span (days)	725.7 ± 2.0	709.6 ± 9.6	722.5± 3.4	722.6 ± 3.2
Terminal body weight (% of controls)	52.1g n = 46	45.3g (87.1%) n=43	38.7g (74.2%) n=45	32.2g (61.8%) n=46

Table 20: Incidences (%) of neoplasms in female mice

	0 ppm	**2500 ppm**	**5000 ppm**	**10,000 ppm**	**Historical control data from NTP 2-year feed studies (mean – range) (NTP, 2023)** **Comment**
Hepatocellular adenoma (single + multiple)	22%	10%	26%	46%*	17/150 (11.33% ± 9.24%) Control significantly different from treated groups in poly-3 trend test (p < 0.001)
Hepatocellular carcinoma (single + multiple)	2%	4%	10%	20%*	3/150 (2% ± 0%) Control significantly different from treated groups in poly-3 trend test (p < 0.001)
Hepatocellular adenoma or carcinoma (single + multiple)	24%	14%	32%	58%*	20/150 (13.33% ± 9.24%) Control significantly different from treated groups in poly-3 trend test (p < 0.001)

*: Statistically significant result (P<=0.05):

The carcinogenicity study from NTP show clear carcinogenic effect of TCPP on female mice. Both incidences of hepatocellular adenomas and hepatocellular carcinomas were statistically significantly increased in the high dose female mice. In addition, the controls were significantly different from treated groups in a trend test. The data does not report any hepatoblastomas. Non-neoplastic effects observed in female mice included eosinophilic focus (1/50, 7/50, 13/50, 16/50) and hepatocellular, cytoplasmic alterations (0/50, 0/50, 2/50, 48/50).

Malignant lymphomas were observed in the kidneys of female mice: 0 ppm: 2%; 2500 ppm: 2%; 5000 ppm: 4%; 10,000 ppm: 6%.

7.9.6.2 Other supporting studies

7.9.6.2.1 Repeated dose toxicity studies with TCPP (including NTP)

NTP three-month studies in rats and mice:

In a NTP 90-day study (NTP, 2023), rats were exposed perinatally (from gestation day 6 through postnatal day 21) plus for 90 days. The dose groups were 2500 ppm, 5000 ppm, 10,000 ppm and 20,000 ppm. Male rats in the 20,000 ppm group did not survive to study termination. Seven males were euthanized moribund, and the remaining were removed on study day 5.

A 15% increase in absolute liver weight was observed in male rats exposed to 10,000 ppm TCPP. Relative liver weights were increased 11% compared to the control group. For male rats exposed to ≤5000 ppm TCPP, liver weights were not statistically significantly different from controls. In females, a 13% and 19% increase in absolute liver weights were observed in rats exposed to 10,000 ppm and 20,000 ppm TCPP, respectively. Relative liver weights in female rats were also increased in a significant treatment-related manner.

Minimal bile duct hyperplasia in male and female rats in the 10,000 (male and female) and 20,000 ppm (female only) groups was also observed. Liver-related clinical chemistry parameters in serum (alkaline phosphatase (ALP), alanine 22 aminotransferase (ALT) and cholesterol) were altered in a treatment-related manner for both male and female rats. ALP and ALT concentrations were both decreased and cholesterol was slightly increased.

Changes in kidney weight was observed in both male and female rats exposed to TCPP. In males, nephropathy was observed in the controls (8/10) and in the high dose group (5/10). In these two groups, pelvic dilatation was also observed as well as renal tubule calculus in the 10,000 ppm dose group only (2/10 (20%)). In females, nephropathy was also observed in the controls (2/10) and in the high dose group (2/10). Since nephropathy was observed in both controls and high dose groups of both males and females, this effect is probably not treatment-related.

Thyroid was not weighted in the 90-day study. No observations in thyroid glands were reported.

In another NTP 90-day study (NTP, 2023), mice were exposed to 1250 ppm, 2500 ppm, 5000 ppm, 10,000 ppm or 20,000 ppm. All male and female mice survived to the end of the study with no clinical signs of toxicity in the TCPP-exposed groups.

Hepatocyte hypertrophy was observed in the liver of both male and female mice. The increased incidence of this effect was statistically significant in all dose groups from 5000 ppm compared to concurrent controls in both males and females. This indicates that the liver is a clear target organ following exposure to TCPP.

In male mice, renal tubule cytoplasmic alterations were observed in all dose groups and this effect was statistically significant from 2500 ppm. In male mice, statistically significant

(P<0.001) cytoplasmic alterations in the renal tubules were observed in all tested groups except the lowest dose of 1250 ppm. The incidences observed were 80% in the 2500 ppm group and 100% in both the 10,000 ppm and 20,000 ppm groups. No incidences were observed in the control group. An absolute kidney weight decrease was observed with increasing exposure concentration in male and female mice with the greatest effect observed in the 20,000 ppm groups (22% significant decrease in males; 13% significant decrease in females). Relative kidney weights were generally higher in male and female mice exposed to TCPP.

In female mice, casts protein was observed at 10% in the highest dose group and not in any of the other groups. Cytoplasmic alteration in the renal tubules were not observed in any of the groups in female mice.

Thyroid was not weighted in the 90 day study. No observations in thyroid glands were reported.

Other repeated dose toxicity studies from the TCPP REACH registration dossier:

The liver have been identified as the main target organ affected by TCPP in a 90-day RDT study in rats. Effects observed included statistically significant increases in absolute and relative liver weights in males at all doses and females at the two highest doses and periportal hepatocyte swelling in high dose groups. In addition, mild thyroid follicular cell hyperplasia in males at all doses and females at the highest dose was observed indicating that the thyroid is also an organ affected by TCPP. A LOAEL of 52 mg/kg/day (male rats) is derived from this study (Stauffer Chemical Co., 1981a). In a 4-week study in rats, the liver was identified as the target organ with increased liver weight changes observed at 1000 mg/kg, accompanied by hepatocyte hypertrophy in all males of this group (and one 100 mg/kg male) and changes in ALT activity (referenced in EU RAR 2008a).

7.9.6.2.2 Carcinogenicity studies with TCEP, TCDP and 1-chloro-2-propanol:

TCEP:

A two-year carcinogenicity study in F344/N rats and B6C3Fl mice with TCEP has been conducted by NTP (1991). Doses were 0, 44 and 88 mg/kg (five days/week) and rats and 0, 175 or 350 (5 days/week) in mice. Renal tubule hyperplasia and renal tubule adenomas was observed in both male and female rats and it was concluded that there was clear evidence of carcinogenic activity. Renal tubule adenomas was also observed in male mice but at a lower incidence and the level of evidence in mice was therefore considered to be equivocal (NTP, 1991). An 18-months study with TCEP has also been conducted in Scl:ddY mice with estimated doses of 0, 12, 60, 300, and 1500 mg/kg bw/d. The kidney and the liver were identified as target organs with significantly increased incidences of adenomas and carcinomas observed in both organs. This was accompanied by hyperplasia and hypertrophy of the urinary tubule epithelium (Takada *et al*., 1989 cited in EU RAR, 2009).

TCDP:

A two-year cancer study in Sprague Dawley rats with doses of 0, 5, 20 and 80 mg/kg/day has been conducted in 1981 (Stauffer Chemical Co., 1981b cited in EU RAR, 2008b). No cancer studies in mice are available. Similar to TCEP, the kidney is also a target organ of cancer induced by TCDP. Hyperplasia in the renal tubule epithelium occurred in 28/48 animals vs. 2/45 of the controls. A statistically significant increase in renal cortical adenomas was observed in both males and females at the medium and higher dose. No carcinomas were observed in the kidney during this study. In addition, statistically increased hepatocellular adenomas were observed in males and females in the high dose group.

1-chloro-2-propanol:

A 2-year carcinogenicity study on the TCPP metabolite 1-chloro-2-propanol has been conducted in male and female F344/N rats and B6C3F1 mice with 50 animals/sex in each dose group (NTP, 1998).

Rats were administered 0, 150, 325 or 650 ppm 1-chloro-2-propanol in drinking water (equivalent to average daily doses of approx. 15, 30, or 65 mg/kg during the first several months of the study and 8, 17 or 34 mg/kg for the remainder of the 2-year study). Survival and mean body weight of all exposed groups were similar to that of the controls. No treatment-related neoplasms or non-neoplastic lesions were observed in the study.

Mice were administered 0, 250, 500 or 1000 ppm 1-chloro-2-propanol in drinking water (equivalent to average daily doses of approximately 45, 75 or 150 mg/kg to males and 60, 105 or 210 mg/kg to females during the first several months of the study and 25, 50, or 100 mg/kg for the remainder of the 2-year study). Survival and mean body weight of all exposed groups were similar to that of the controls. No treatment-related neoplasms or non-neoplastic lesions were observed in the study.

7.9.6.2.3 Other supporting and mechanistic studies (including NTP)

In vitro Studies of TCPP:

Two cell transformation assays (*in vitro* transformed foci in BALB/3T3 cells) are included in the registration dossier. According to the Registrant, one yielded a positive result without dose response (Unpublished study report, 1978) and the other one yielded a negative result (Unpublished study report, 1980). For both tests, the testing material was identified as EC 807-935-0. Both studies are non-guideline, non-GLP studies and they are assessed by the Registrant as Klimisch 3. The eMSCA has not had a chance to further assess the positive study. A positive cell transformation assay can indicate both a genotoxic and a non-genotoxic carcinogenic response.

Other relevant *in vivo* studies:

One other *in vivo* study was found in the literature search (see 'Literature search ED and reproduction concern' in 7.10.2 for details). In a 5-day *in vivo* rat transcriptomics study, Gwinn *et al*. (2020) exposed Sprague Dawley rats (males) to TCPP once daily for five consecutive days by oral gavage. The tested TCPP doses were 0, 18.75, 37.5, 75, 150, 300, 600, 1000 and 2000 mg/kg. Liver and kidney were collected 24 h after the final exposure and total RNA was assayed using high-throughput transcriptomics (HTT) to determine transcriptional gene set dose (BMD) values. Absolute liver weights and liver weight/terminal body weight ratios were significantly increased in rats exposed to TCPP. Rats exposed to TCPP also exhibited significant increases in absolute right and left kidney weights and kidney weight/terminal body weight ratios based on trend tests. TCPP had active gene sets in 2322 in liver and 2182 in kidney out of a total of 4504. TCPP upregulated 7 out of 11 CAR and PXR-related biomarker genes and 8 out of 14 PPARα biomarker genes. In addition, the lowest transcriptional BMD (liver or kidney) in male rats was compared with the overall lowest apical (non-neoplastic or neoplastic) BMD in all sex and species (male or female rats or mice). The transcriptional BMD was lower (more sensitive) than the apical BMD for TCPP suggesting that a short-term, *in vivo* assay is generally predictive of BMD values for apical (histopathological) effects in long-term chronic studies.

7.9.6.3 Discussion and conclusion on the carcinogenicity of TCPP:

Body weight and internal dose:

In the NTP carcinogenicity study (NTP, 2023), body weights in the 20,000 ppm group were approximately 75% of control group values for male and female rats at the beginning of the post-natal exposure phase. The eMSCA considers this difference in weight to be a

potential effect of the prenatal exposure of the treated groups. This gap decreased within the duration of the study. For male rats in the 20,000 ppm group, the terminal mean body weight was within 8% of the controls whereas for females, the terminal body weight for the 20,000 ppm group was 17% lower than controls. It is well known that reduced weight and consumption can have a confounding effect on carcinogenicity parameters (OECD, 2002), but because mean body weights remained within 10% of the control groups during the majority of time intervals, the eMSCA finds that the indications of carcinogenicity in this study are likely to be directly treatment related. For mice, the differences in body weight were larger and are discussed below.

TCIPP concentrations in mice were generally higher than in rats. The estimated intake of TCPP, based on feed consumption data, may partly explain this difference. The higher internal concentrations in mice may explain some of the species differences observed for carcinogenicity in this study.

Potential target organs for carcinogenic activity:

The main target organ identified in the NTP 2023 carcinogenicity studies was the liver. In addition, effects were observed in the kidney and the uterus.

Liver: Several statistically significant neoplastic (adenomas and carcinomas) and non-neoplastic effects were observed in male rats. There was an increased incidence of hepatocellular adenomas and carcinomas individually and combined in male rats in the two highest dose groups. This incidence was higher compared to the relevant historical control groups but it was not significant with pairwise comparison with the concurrent control group. However, the incidence of hepatocellular adenomas and carcinomas combined in treated rats was significantly increased compared to the concurrent controls when using a trend test. Treated male rats had low reductions in terminal body weights and this corroborates that the carcinogenic effects are treatment-related (NTP, 2023).

In female rats, a slightly increased incidence of hepatocellular adenomas was observed in the low dose group and in the two highest dose groups. This was not statistically significant when compared to the concurrent control group but is was outside the incidence rate observed in historical control data. Two females in the 20,000 ppm group had multiple adenomas, which is an indication of a non-spontaneous event. There was also a statistically significant increased incidence of non-neoplastic effects. No hepatocellular carcinomas were observed in female rats (NTP, 2023).

In male mice, the incidence of hepatocellular carcinomas was significantly increased in all treated groups when compared to control group. Some animals had multiple tumors. The incidences observed in the dosed groups were also well beyond the incidence rate observed in historical control data. There was no increased incidences of hepatocellular adenomas in any of the dosed groups (NTP, 2023).

In female mice, there was a statistically significant increased incidence of hepatocellular adenomas and hepatocellular carcinomas both individually and when combined in the highest dose group. The treated groups were also significantly different from the control group in a trend test. The incidence of hepatocellular carcinomas was also increased in the low and mid dose groups when compared to the concurrent controls and the corresponding historical control data. Incidences of pre-neoplastic changes such as liver foci was also significantly increased (NTP, 2023).

According to Auerbach *et al*. (2018), hepatocellular carcinoma is the most common primary liver tumor in B6C3F1/N mice and Hoenerhoff *et al*. (2011) reports that spontaneous hepatocellular carcinomas in male mice average approximately 31% (range 16–56%, all routes, all vehicles) and female mice average 10% (range 2–46%, all routes, all vehicles) (NTP Historical Controls Report, All Routes and Vehicles, March 2010). However, the incidences of neoplasms observed in the NTP study were statistically significant when compared to the concurrent controls. In addition, the incidences were also beyond the

range observed in historical control data further supporting that the hepatocellular effects observed are treatment-related. There is clear evidence of carcinogenic activity in female mice due to the increase in both adenomas and adenocarcinomas and non-neoplastic effects. However, since treated female mice had reduced terminal body weights (74.2% and 61.8% of controls for the 5000 ppm and 10,000 ppm groups respectively), a confounding effect cannot be excluded. Food intake and clinical parameters in mice were not affected. In addition, a statistically significant increased incidence of adenomas and carcinomas were observed for TCEP in the liver and a statistically significant increased incidence of adenomas were observed for TDCP in the liver.

Kidney: The kidney is a target organ for carcinogenicity caused by TCEP and TDCP. This was not observed to the same extent for TCPP. The kidney appears to be a target organ for non-neoplastic effects of TCPP in mice as cytoplastic alterations were observed. There was also an increased incidence of renal tubule adenoma in female rats in the highest dose group (4%). This finding was statistically significant in a trend test. In addition, the incidence rate observed in historical controls is <1% and therefore, this finding my very well be treatment-related (NTP, 2023).

Uterus: In female rats, adenomas and adenocarcinomas were observed in the uterus. An exposure-related tripling of incidences was observed when comparing the control group and the high dose group, but there was no statistical significance by pairwise comparison. However, a statistically significant trend for adenomas or adenocarcinomas (combined) was observed in a trend test. The combined incidences of uterine adenomas and adenocarcinomas also exceeded the range observed in historical controls. An increased incidence of stromal polyps was also observed in female rats when comparing the control group to the exposure groups. This finding was statistically significant in the 2500 ppm dose group and in a trend test (NTP, 2023).

Thyroid gland: No neoplastic or non-neoplastic changes were reported for either rats or mice in the 90-day or 2-year NTP studies (NTP, 2023). This is in contrast to the study by Stauffer Chemical Co. 1981a cited in EU RAR 2008a, which reported hyperplasia in the thyroid glands of male Sprague Dawley rats at 52 mg/kg bw/day (LOAEL). The hyperplasia in the thyroid gland was observed alongside of increases in total and relative liver weights. Similar changes in liver weights were observed in the NTP 90-day and 2-year study in both male and female rats.

Table 21: Organ specific indications of carcinogenicity for TCPP, TCEP and TCDP

		Kidney	Liver	Adrenal glands	Thyroid
TCPP 90-day studies	Rats	In males, relative kidney weights were stat. sign. increased at the two highest doses associated with very mild cortical tubular degenerative changes in kidneys (3). Very mild cortical tubular degenerative changes in kidneys of high	Stat. sign. increased absolute and relative liver weight in all male dose groups and in mid and high dose females. Hypertrophy in the high dose males and females (3). Stat. sign. increased liver weight in both	No reported effects (3) (4).	One study observed mild thyroid follicular cell hyperplasia in males of all dose groups and females at the highest dose (3). Another study reported no effects (4).

		dose females (3). Alterations in kidney weight also observed but not considered treatment-related by study authors (4)	males and females (4) Bile duct hyperplasia observed in high dose group for both males and female (4).		
	Mice	Stat. sign. decreased kidney weight in high dose males and females. In males, stat. sign. cytoplasmic alteration in the renal tubules were observed in all tested groups (4).	Stat. sign. increased relative liver weights in all dose groups of males and females associated with hypertrophy in both males and females (4).	No reported effects (4).	No reported effects (4).
TCPP Two-year carc. Study (5)	Rats	No adenomas were observed in male rats. 2% carcinoma in renal tubules and 2 % carcinoma in urothelium were observed in the 5000 ppm group males. Female rats had a stat. sign. increase in adenomas (trend test).	Increased incidence of adenomas and carcinomas in male rats in the highest dose groups. Slightly increased incidence of hepatocellular adenomas in the higher dose group females. No hepatocellular carcinomas were observed in females.	Slightly higher incidences of cortical adenomas in two females each in the 10,000 and 20,000 ppm groups. Not stat. sign. Controls are stat. diff. from treated groups in trend test.	No increase in adenomas or carcinomas was observed. No biologically relevant significant differences in incidences of neoplastic or non-neoplastic lesions were observed.

	Mice	One adenoma in controls of male mice, no other adenomas. No carcinomas in any groups. No adenomas in female mice. No stat. sign. increase in carcinomas in female mice.	There was a stat. sign. increase in carcinomas in male mice and in adenomas and carcinomas in female mice.	No reported effects in mice.	No biologically relevant significant differences in incidences of neoplastic or non-neoplastic lesions were observed.
TCEP	Rats	Clear evidence for carcinogenicity at higher dose levels (primary kidney adenomas; focal renal tubule hyperplasia) (2).	Increased liver weight in males (2)	No relevant effects reported (2).	Slightly increased incidence of thyroid gland follicular cell carcinomas in males and females (2).
	Mice	Lesions (hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei) in an 18-month oral carcinogenicity study in mice (1). Weak evidence in B6C3F mice (primary adenocarcinoma; adenomas; hyperplasia) (2).	Statistically significantly high incidences of tumors (adenomas and carcinomas) (1).	No relevant effects reported (2).	No relevant effects reported (2).
TDCP	Rats	Stat. sign. increase in renal cortical adenomas observed at 20 and 80 mg/kg bw/d in male and female rats at 24 months (6).	Hepatocellular adenomas were observed at 80 mg/kg bw/d in male and female rats at 24 months (6).	Adrenal cortical adenomas were observed in male and female rats after two years (6).	No relevant effects reported (6).
	Mice	No relevant studies available.	No relevant studies available.	No relevant studies available.	No relevant studies available.

1) Takada et al. (1989) (cited in EU RAR, 2009); 2) NTP (1991); 3) Stauffer Chemical Co. (1981a); 4) NTP (2023; 90-day study) 5) NTP, (2023; 2 year study) 6) Stauffer Chemical Company (1981b).

Carcinogenic Modes of Action:

It is likely that TCPP does not interact with DNA directly (section 7.9.5) and therefore acts via non-genotoxic MoAs (NTP, 2023). There are some indications that the MoAs for TCPP as a non-mutagenic carcinogen could be via modalities CAR, PXR and PPARα (Gwinn *et al*., 2020; see also discussion in section discussion in 7.10.3). A MoA solely via PPARα is not considered relevant to humans (IARC 1994) but other MoAs such as CAR and PXR are relevant for humans (Boobis *et al*., 2006; Meek *et al*., 2003).

Conclusion:

Clear evidence of carcinogenic effects were observed in rats and mice in two-year carcinogenicity studies with TCPP. Liver was identified as a target organ and increased incidences hepatocellular adenomas and hepatocellular carcinomas were observed in both rats and mice. In female rats, there was also an increased incidence of renal tubule adenoma in the highest dose group and increased incidence of uterus adenomas and adenocarcinomas combined. There was also some evidence of an increased incidence of stromal polyps in female rats. It is concluded by the eMSCA that TCPP is a non-genotoxic carcinogen.

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

A combined literature search was performed for identification of relevant studies investigating reproductive toxicity and endocrine disruption (method described in section 7.10.2). This literature search, together with review of the data available in the registration dossier, revealed 11 *in vivo* studies relevant for assessment of reproductive toxicity (8 studies relevant for assessment of fertility and 7 studies relevant for assessment of developmental effects).

7.9.7.1. Fertility and sexual function

Table 22

Study overview, rodent *in vivo* studies on TCPP relevant for evaluation of effect on fertility. Effects described are statistically significant unless otherwise indicated (non-significant = n.s.). Reliability have been assessed using Klimisch score.

Reference	Study design	Relevant results	Test material
Stauffer Chemical Co., 1981a Published: Freudenthal, R.; & Henrich R.; 1999 Klimisch: 2 Non-guideline Non-GLP	90-day study in rats, n=20/sex Oral dosing in feed 52-62; 160-171; 481-570; 1349-1745 mg/kg bw/d (800, 2500, 7500, 20000 ppm) At necropsy the following reproductive toxicity	There was no investigation of the uterus weight or of estrous cyclicity *Body weight:* Mean terminal body weight was significantly decreased in both males (8%) and females (12%) in the high dose group. *Reproductive organ weights and histopathology:* Testes and ovary weight were not significantly affected.	Name: Fyrol PCF Specifications: Test material consists primarily of -tris(2-chloroisopropyl) phosphate (about 70%) -2-chloropropanol phosphate (about 23%)

	relevant endpoints were examined: -weight of testes and ovaries -macroscopic observations of prostate, mammary gland, testes, ovary, pituitary, uterus -histopathology of mammary gland, pituitary, testes, epididymis, prostate, seminal vesicles, ovaries, uterus	No statistically significant effects were seen on histopathology. *Liver* Absolute and relative liver weights were statistically significantly increased in male animals from all treatment groups (absolute: 16%, 16%, 15%, 31%; relative: 16%, 18%, 19%, 42%) and in female rats exposed to 7500 ppm (absolute: 17%; relative: 25%) and 20,000 ppm (absolute: 17%; relative: 33%).	

Bayer, 1991
 	
Klimisch: 2
 	
GLP
Guideline
EU B.7
	28-day repeated dose toxicity study in rats
	
	n=6/sex
	
	Oral gavage
	10, 100, 1000 mg/kg bw/day
	
	At necropsy, the following reproductive toxicity endpoints were examined:
	Weight of testes, macropathology and histopathology of testes, epididymis, seminal vesicles, prostate, ovaries, uterus.
	
	(There was no investigation of weight of uterus or ovaries, or of estrous cyclicity)
		Weight and histopathology of reproductive organs:
		No statistically significant changes in weights or histopathology of any of the reproductive organs was seen.
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4
			
			Specifications:
			Tris chloroisopropyl phosphate
			Purity: 97.85% including all isomers

TNO, 2007b
 	
Klimisch: 1
 	
GLP
	One-generation study (range finding) in rats
	n=10/sex
	
	Oral dosing in feed: 1500, 5000, 15,000 ppm
	(corresponding to app. 100, 330, 1000 mg/kg bw/d)
	
	Exposure 5 weeks premating to PND21
	
	Parental body weights were assessed throughout
		Body weights:
		During premating, a decrease in body weight was seen in F0 high dose males (5-8%).
		No effect was seen on body weight in F0 females during premating.
		
		During gestation, maternal body weights were decreased in mid (day 0-14, 5-6%) and high (day 7-14, 5-6%) dose animals.
		
		Around birth, body weights of F0 females were not significantly affected.
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4

	the study. Organ weights and semen quality were assessed at study termination. Offspring were assessed for postnatal mortality and growth.	During lactation, a decrease in maternal body weight gain was seen in low (19%) and high (38%) dose dams, resulting in a (10%) decrease in body weight at the end of lactation in high dose. This corresponded well with the decreased maternal food consumption (11-33%) seen on days 4-21 of lactation in high dose. Pup birth weight was similar between groups. *Delivery and Litters:* There were no effects on fertility index or live pups/litter at birth. *EAS-relevant effects:* Decreased prostate weight in F0 males was observed in low (absolute: 15%, relative: 11% n.s.) and high (absolute: 19%, relative: 13% n.s.) dose groups. Decreased uterus weight was observed in all dose groups in F0 females (absolute: 45-49%, relative: 42-47%). Terminal body weight was decreased in high dose F0 males (7%) and females (10%) but not to an extend that fully explains the observed effects on prostate and uterus. No effect on semen quality in F0. Organ weights and semen quality were not investigated in offspring.	

TNO, 2007a
 	
Klimisch: 1
 	
GLP
 	
OECD TG 416
	Two-generation study (OECD TG 416) in rats, n=28/sex
	
	Oral dosing in feed. Males: 85, 293, 925 mg/kg bw/d; females: 99, 330, 988 mg/kg bw/d.
	
	Exposure 10 weeks premating to PND 56 in F2.
	
	Endpoints in parental animals and offspring were assessed according to TG 416.
		Body weights
		During premating, a decrease in body weight was seen in F0 mid (week 10-11, 5%) and high (week 3-11, 5-11%) dose males. No effect was seen on body weight in F0 females during premating.
		
		During gestation, maternal body weights were decreased in high dose (day 7-21, 5%) F0 females.
		
		During lactation, body weights of F0 females were not significantly affected.
		
		Birth weights of F0-generation pups were similar between groups.
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4

		During lactation, F0-generation pup body weight gain was decreased from day 14 in mid (13-14%) dose and from day 7 in high dose (10-26%), resulting in a decrease in pup body weight at day 21 in mid (8%) and at day 14 and 21 in high (8-15%) dose. Throughout premating, body weights of F1 males were decreased in mid dose (6-8%, n.s. in week 1 and 5) and in high dose (11-16%). Body weight of F1 females were also decreased during premating in mid dose in week 2 to 11 (5-7%, n.s. in week 4) and in high dose in week 0 (13%) and week 1-11 (6-8 %). During gestation, maternal body weights were decreased in mid (7-9%) and high dose (8-11%) F1 females. During lactation, maternal body weights were decreased in mid (5-8%, n.s. at day 4) and high (6-10%) dose F1 females. In the F2-generation, decreased mean body weights were observed at day 28, 35, 42 in high dose males and females. *Delivery and Litters:* There were no effects on fertility index. The number of pups delivered was decreased in F0-generation pups in high dose (8%) and in F1-generation pups in mid (14%) and high dose (18%). *Sexual maturation:* Delayed vaginal opening in high dose F2 animals (7 days delay) and mid dose F2 animals (3 days delay). On the days around the time of vaginal opening, the decrease in body weight in high dose F2 females compared to controls was 11% on day 35 and 9% on day 42. In mid dose, the difference in body weight was 0.5-1% compared to controls. In both mid- and high dose F2 females, only 80-83% of the females reached criteria for vaginal opening, whereas this number was 92% in control females.	

		Delayed preputial separation was observed in high dose F2 animals (47 vs 44 days) but it may be explained by app. 11% lower body weight in the high dose animals at day 42 compared to controls. *Estrous cyclicity and uterus weight:* Estrous cycle length was increased in all dose groups in F0 (4.4 days in control, 4.8, 5.1 and 5.6 days in low, mid and high dose) and in high dose F1 females (4.7, 4.9, 5.0 and 5.8 days in control, low, mid and high dose). An increased number of acyclic females was observed in high dose F0 females (1/28, 0/28, 0/28 and 6/28 in control, low, mid and high dose) and a decreased number of cycles per animal was seen in high dose F0 (3.9, 3.7, 3.6, 3.0 in control, low, mid and high dose) and F1 females (3.8, 3.6, 3.7, 3.1 in control, low, mid and high dose). Estrous cyclicity is usually not affected by body weight. Further, body weight was not affected in any of the dose groups in F0 females during premating. Absolute and relative uterus weights were markedly decreased at termination in all dose groups in F0 females (absolute: 18-32%, relative 18-31%) and in high dose F1 females (absolute: 35%, relative: 31%). The terminal body weight was not affected in F0 females in any dose groups. In F1 females, terminal body weight was decreased in mid (5%) and high (7%) dose. *Ovary weight:* Absolute ovary weight was decreased in high dose F0 females (11%) and in high dose F1 females (10%, n.s.). Relative ovary weight was also decreased in F0 females (8%, n.s.) and F1 females (3%, n.s.). The terminal body weight was not affected in F0 females and decreased in high dose F1 females (7%). *Pituitary weight:* In F0 females, absolute and relative pituitary weight was decreased in high dose	

		(absolute: 6%, relative: 8%). Terminal body weight was not affected. In F1 females, absolute pituitary weight was decreased in all dose groups (low (12%), mid (6%) and high dose (18%)) and relative pituitary weights were decreased in low (12%), mid (5%, n.s.) and high dose (9%). Terminal body weight was decreased in mid (5%) and high dose (7%) but not in low dose. In F0 males, pituitary weight was not affected. In F1 males, absolute pituitary weight was decreased in high dose (13%). Terminal body weight was also decreased in high dose (16%) and relative pituitary weight was not affected. *Male reproductive organs:* No clear substance related effects were observed on weight of testes, epididymis, prostate or seminal vesicles. *Semen quality:* No effect on F0 or F1 semen quality.	
NTP, 2023 (Study conducted in 2009) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	3-months with perinatal study in rats F0 females: N=20 (0, 2500, 10000, 40000 ppm) or N=8 (5000, 20000 ppm) F1 rats: N=10/sex Exposure: F0 females: GD6-LD21, F1: Perinatal + 3 months Doses: Females: 0, 2500, 5000, 10,000, 20,000 ppm in feed (and 40,000 ppm in F0 females only, euthanized during gestation due to excess toxicity), corresponding to app.: 0, 236, 458, 906, 1890 mg/kg bw/day. Males: 0, 2500, 5000, 10,000 ppm in feed (and 20,000	The only reproductive organs weighed were testes and epididymis (not e.g. uterus, ovaries, pituitary). Due to poor quality of samples, an assessment of estrous cyclicity could not be made for female rats. *Delivery and litters:* In the high dose group (20,000 ppm), maternal body weight gain was decreased GD6-9 (87%) and as a result maternal body weight was decreased at GD9 (5%). However, maternal body weight gain was increased during most of the rest of the gestational period, though only significantly in GD12-15 (50%), and high dose maternal body weight was not different from control at the end of gestation (GD21). There were no effects on live litter size at birth or on pup mortality during postnatal life. *Body weight:* The terminal body weight in F1 males was decreased in low dose (6%), mid (6% n.s.) and high	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 obtained from Albemarle) Four major isomers identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 64.77 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 26.98 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.99 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.2 %) Impurities: 4.2 %

	ppm, but this group did not survive to study termination), corresponding to app.: 0, 223, 431, 911 mg/kg bw/day. (Body and organ weights of F1 males was reported in low, mid and high dose, corresponding to 2500 ppm, 5000 ppm and 10,000 ppm, whereas the 20,000 ppm group seemed to have been taken out). Age at necropsy: 17 weeks (F1)	dose (3% n.s.), but not significantly in the two highest doses. *Reproductive organs:* There was no significant effect on the weight of testes in F1. In F1 males, absolute epididymis weight was decreased in low (11%), mid (10%) and high (7%) dose. Also cauda epididymis weight was decreased in low dose (15%), mid (8%, n.s.) and high dose (3%, n.s.) but not significant in two highest doses. *Semen quality:* No effects were observed on testicular sperm count or percent motile sperm in F1 males dosed up to 10,000 ppm. *Histopathology:* No differences between control and 20,000 ppm groups observed in histopathology of the genital system or brain in F1 animals.	
NTP, 2023 (conducted 2009) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	3-months study in mice N=10/sex Exposure: 3 months Dosing: 0, 1250, 2500, 5000, 10,000, and 20,000 ppm in feed Corresponding to app.: Males: 0, 225, 473, 1050, 2509, and 4446 mg/kg bw/day. Females: 0, 204, 442, 924, 1841, and 3645 mg/kg bw/day Age at necropsy: 19-20 weeks	The only reproductive organs weighed were testes and epididymis (not e.g. uterus, ovaries, pituitary). Due to poor quality of samples, an assessment of estrous cyclicity could not be made for female mice. *Body weight:* Terminal body weight was decreased in males in the 2500 (11%), 5000 (16%), 10,000 (24%) and 20,000 ppm (29%) groups and in females in the 1250 (12%), 2500 (7%), 5000 (3%), 10,000 (9%) and 20,000 ppm (15%) groups. *Reproductive organs:* Testes (absolute and relative weight of right testis determined in all dose groups, absolute weight of left testis only in control and high dose): Decreased absolute right testis weight (8%) and left testis weight (9%) in high dose (20,000 ppm). Increased relative right testis weight in 2500 (12%), 5000 (17%), 10,000 (28%) and 20,000 (29%) ppm groups.	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 obtained from Albemarle) Four major isomers identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 64.77 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 26.98 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.99 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.2 %) Impurities: 4.2 %

		The observed decreased absolute testis weights are evaluated to be a consequence of the decreased body weights. The increased relative testis weights are evaluated to be due to the fact that the body weights are more markedly decreased than the testis weights. Epididymis (absolute weight of left epididymis and left cauda epididymis only examined in control and high dose): Decreased absolute epididymis (15%) and cauda epididymis weight (14%) in high dose (20,000 ppm). *Histopathology:* No differences between groups observed in histopathology of thyroid, genital system or brain.	
NTP, 2023 (conducted 2011-13) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	2-years with perinatal study in rats Exposure: F0 females: GD6-LD28, F1: Perinatal + 2 years F0 female rats: N=38 F1 rats (2-year study): N=50/sex F1 rats (internal dosimetry): N=10/sex Dosing: 0, 2500, 5000, 10,000, or 20,000 ppm in feed, corresponding to app.: Males: 0, 141, 294, 626, and 1,155 mg/kg bw/day. Females: 0, 156, 323, 674, and 1,295 mg/kg bw/day. Age at necropsy: F1: 109-110 weeks	Organ weights not reported and no investigation of estrous cyclicity There were no effects on live litter size at birth or on pup mortality during postnatal life. Differences in incidences of non-neoplastic lesions in reproductive organs in exposed animals compared to controls: Females: Uterus, cysts (0/50, 0/50, 0/50, 0/49, 2/50 - statistically significant trend); Uterus, cervix inflammation (0/50, 0/50, 0/50, 0/49, 3/50 – statistically significant trend). Males: Prostate, epithelium hyperplasia (0/50, 0/50, 5/50, 0/50, 3/50 – significantly different in mid dose compared to control). Testis, polyarteritis nodosa (13/50, 13/50, 9/50, 9/50, 3/50 – statistically significant trend and difference between high dose and control) Testis, germinal epithelium degeneration (21/50, 15/50, 14/50, 13/50, 8/50 - statistically significant trend and difference between three highest dose groups and control). Differences in incidences of primary tumours in reproductive organs in exposed animals	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 mixed with lot 134 obtained from Albemarle to form lot M072911NP) Four major isomers identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 68.06 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 25.43 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.55 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.21 %) Impurities: 2.55 % Due to ageing, control animals had high incidences of non-neoplastic lesions in numerous organs, including endocrine system, thyroid, genital system, mammary gland - challenging the

		compared to controls: Females: Uterus, adenoma or adenocarcinoma (combined) (3/50, 4/50, 6/50, 8/49, 9/50 – statistically significant trend and between high dose and control). Uterus, polyp stroma (5/50, 15/50, 6/50, 7/49, 13/50 – statistically significant difference between low dose and control as well as high dose and control). Ovary, granulosa cell tumour benign (0/50, 0/50, 0/50, 0/49, 2/50 – statistically significant trend). No biologically relevant significant differences in incidences of neoplastic or non-neoplastic lesions in other reproductive organs including epididymis, seminal vesicles, pituitary gland, preputial gland, penis, thyroid, mammary gland, oviduct and vagina. Neoplastic effects observed in other organs are described in section 7.9.6.	detection of non-neoplastic effects induced by TCPP exposure.
NTP, 2023 (conducted in 2011-13) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	2-years study in mice Exposure: 2 years Mice (2-year study): 50/sex Mice (internal dosimetry): 20/sex Dosing: Males: 0, 1250, 2500, 5000 ppm in feed, corresponding to app. 0, 160, 330, 711 mg/kg bw/day. Females: 0, 2500, 5000, 10000 ppm in feed, corresponding to app. 0, 329, 673, 1,491 mg/kg bw/day Age at necropsy: F109-111 weeks	Organ weights not reported and no investigation of estrous cyclicity. Differences in incidences of non-neoplastic lesions in reproductive and/or endocrine organs in exposed animals compared to controls: Females: Pituitary gland, pars distalis hyperplasia (17/50, 5/50, 4/50, 4/49 - statistically significant trend and all dose groups are different from control). Uterus, dilation (13/50, 18/50, 10/50, 8/50 – statistically significant trend). Differences in incidences of primary tumors in reproductive organs in exposed animals compared to controls: In females: Pituitary, pars distalis or unspecific site adenoma (3/50, 2/50, 1/50, 0/49 – non-statistically significant trend). No biologically relevant significant differences in	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 mixed with lot 134 obtained from Albemarle to form lot M072911NP) Four major isomers identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 68.06 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 25.43 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.55 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.21 %) Impurities: 2.55 %

		incidences of neoplastic or non-neoplastic lesions in other reproductive organs, including testes, epididymis, seminal vesicles, prostate, preputial gland, ovaries and vagina). Neoplastic effects observed in other organs are described in section 7.9.6.	Due to ageing, control animals had high incidences of non-neoplastic lesions in numerous organs, including endocrine system, thyroid, genital system, mammary gland - challenging the detection of non-neoplastic effects induced by TCPP exposure.

Conclusion of effects on fertility and sexual function

In a two-generation reproductive toxicity study (TNO, 2007a), TCPP exposure did not clearly affect fertility parameters. The mean number of pups delivered was lower in the F0 and F1 generations in the 333 and 1,000 mg/kg/day groups compared to the control group. However, TCPP caused clear delays on sexual maturation in both females (vaginal opening) and males (preputial separation) as well changes in reproductive function and organs that have endocrine-related modalities (see Sections 7.10.2 and 7.10.3). The reported LOAELs for F0 females was 99 mg/kg/day due to a significant decrease in uterus weights and effects on the estrous cycle (in both generations). Effects such as decreased body and absolute seminal vesicle weights were observed in F0 males exposed to 293 mg/kg/day. F1 males and females had LOAELs of 85 and 99 mg/kg/day, respectively. This assignment was based on a significant decrease in kidney weights in males and pituitary weights in females (EU RAR, 2008a; TNO, 2007a).

In repeated dose toxicity studies with or without perinatal exposure in rats and mice (NTP, 2023), there were no effects on live litter size at birth or on pup mortality during postnatal life. Due to limited sampling and/or quality of the samples, it is not possible to have a complete assessment of reproductive organs. In males, some effects were observed on accessory sex organs but testicular sperm count or percent motile sperm in F1 males were not affected. In mice, the observed decreased absolute testis weights were regarded to be a consequence of the decreased body weights. There was a decreased absolute epididymis and cauda epididymis weight in high dose but no differences between groups were observed in histopathology.

In the NTP 2-years carcinogenicity studies in rats and mice (NTP, 2023), an increased incidence of non-neoplastic lesions and tumours in uterus was observed, confirming that TCPP disrupts endocrine pathways via EAS modalities.

Taken together, although some endpoints are missing, the identified information shows that there is a concern for effects of TCPP on fertility and sexual function. Adverse effect on female reproduction and sexual maturation (uterine, ovary and pituitary weight, estrous cyclicity and vaginal opening) were seen following TCPP exposure. These effects are mainly discussed in the context of endocrine disruption in section 7.10.2.

7.9.7.2. Development

Table 23

Study overview, rodent *in vivo* studies on TCPP investigating effects on development. Effects described are statistically significant unless otherwise indicated (non-significant = n.s.). Reliability have been assessed using Klimisch score.

Reference	Study design	Relevant results	Test Material

Kawasaki *et al.,* 1982 Summarised in public ECHA dossier Klimisch: 2 Non-guideline Non-GLP	Developmental toxicity in rats, N=5 Oral gavage 5,7; 57; 571 mg/kg bw/d Exposure GD 0-20	From public ECHA dossier: No effects identified in the dams. No effects on foetal mortality, implantation number, resorption or foetal weight. *Skeletal malformations:* No statistically significant increases in the incidences of skeletal abnormalities. However, dose related increases in the incidences of cervical ribs and missing 13th ribs: In the low, mid and high dose groups, 77, 73 and 64 foetuses were examined and 1, 1, and 3 of them showed cervical ribs, respectively. No control foetuses demonstrated missing 13th rib, while 1, 2 and 5 foetuses in the low, mid and high dose group showed missing 13th ribs.	No info on test material composition in public ECHA dossier

TNO, 2007b
 	
Klimisch 1
 	
GLP
	One-generation study (range finding study for OECD TG 416) in rats
	n=10/sex/dose
	
	Oral dosing in feed: 1500, 5000, 15000 ppm (corresponding to app. 100, 330 and 1000 mg/kg bw/d)
	
	Exposure 5 weeks premating to PND21
	
	Parental body weights were assessed throughout the study. Organ weights and semen quality were assessed at study termination.
	Offspring were assessed for postnatal mortality and growth.
		Body weights:
		During premating, a decrease in body weight was seen in F0 high dose males (5-8%).
		No effect was seen on body weight in F0 females during premating.
		
		During gestation, maternal body weights were decreased in mid (day 0-14, 5-6%) and high (day 7-14, 5-6%) dose animals.
		
		Around birth, body weights of F0 females were not significantly affected.
		
		During lactation, a decrease in maternal body weight gain was seen in low (19%) and high (38%) dose dams, resulting in a (10%) decrease in body weight at the end of lactation in high dose. This corresponded well with the decreased maternal food consumption (11-33%) seen on days 4-21 of lactation in high dose.
		
		Pup birth weight was similar between groups.
		
		During lactation, pup body weight gain was decreased from day 14 in low (12-13%) and mid (26-27%) dose and from day 7 in high dose (24-46%), resulting in a decreased pup body weight at day 21 in mid dose (12-14%) and at day 14 and 21 in high dose (18-31%).
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4

		Delivery and Litters: There were no effects on fertility index or live pups/litter at birth. Increased pup mortality was seen in the high dose group (mainly caused by 1 litter with 8 pups dying at day 5-7). There was an increased number of runts in all dose groups at day 21 and in high dose also at day 7 and 14. Organ weights and semen quality were not investigated in offspring.	

TNO, 2007a
 	
Klimisch 1
 	
OECD TG 416
 	
GLP
	Two-generation study (TG 416) in rats, n=28/sex
	
	Oral dosing in feed: Males: 85, 293, 925 mg/kg bw/d; females: 99, 330, 988 mg/kg bw/d.
	Exposure 10 weeks premating to PND 56 in F2.
	
	Endpoints in parental animals and offspring were assessed according to OECD TG 416.
		Body weights:
		During premating, a decrease in body weight was seen in F0 mid (week 10-11, 5%) and high (week 3-11, 5-11%) dose males. No effect was seen on body weight in F0 females during premating.
		
		During gestation, maternal body weights were decreased in high dose (day 7-21, 5%) F0 females.
		
		During lactation, body weights of F0 females were not significantly affected.
		
		Birth weights of F0-generation pups were similar between groups.
		
		During lactation, F0-generation pup body weight gain was decreased from day 14 in mid (13-14%) dose and from day 7 in high dose (10-26%), resulting in a decrease in pup body weight at day 21 in mid (8%) and at day 14 and 21 in high (8-15%) dose.
		
		Throughout premating, body weights of F1 males were decreased in mid dose (6-8%, n.s. in week 1 and 5) and in high dose (11-16%).
		Body weight of F1 females were also decreased during premating in mid dose in week 2 to 11 (5-7%, n.s. in week 4) and in high dose in week 0 (13%) and week 1-11 (6-8 %).
		
		During gestation, maternal body weights were decreased in mid (7-9%) and high dose (8-11%) F1 females.
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4

		Birth weights of F1-generation pups were similar between groups. During lactation, maternal body weights were decreased in mid (5-8%, n.s. at day 4) and high (6-10%) dose F1 females. During lactation, F1-generation pup weight was decreased at day 21 in mid dose and at day 7, 14 and 21 in high dose. In the F2-generation (F1-generation pups after PND21/23), decreased mean body weights were observed at day 28, 35, 42 in high dose males and females. *Delivery and Litters:* There were no effects on fertility index. The number of pups delivered was decreased in F0-generation pups in high dose (8%) and in F1-generation pups in mid (14%) and high (18%) dose. The number of pups lost on day 1-4 was increased in F0-generation pups in high dose and in F1-generation pups in low and high dose. The number of runts was increased in the F0-generation pups from day 1-7 in low dose and day 1-21 in mid and high dose. In the F1-generation pups, the number of runts was also increased but only from day 14. At day 14 in high dose and at day 21 in all dose groups. *Anogenital Distance (AGD):* No observed effect on AGD in F1-generation pups (F2) (not investigated in F0-generation pups (F1)). *Sexual maturation:* Delayed vaginal opening in high dose F2 animals (7days delay) and mid dose F2 animals (3 days delay). On the days around the time of vaginal opening, the decrease in body weight in high dose F2 females compared to controls was about 10% (11% on day 35 and 9% on day 42). In mid dose, the difference in body	

		weight was 0.5-1% compared to controls. In both mid- and high dose F2 females, only 80-83% of the females reached criteria for vaginal opening, whereas this number was 92% in control females. Delayed preputial separation in high dose F2 animals (at 47 vs 44 days), but it may be explained by app.11% lower body weight in the high dose animals at day 42 compared to controls. *Estrous cyclicity and uterus weight:* Estrous cycle length was increased in all dose groups in F0 (4.4 days in control, 4.8, 5.1 and 5.6 days in low, mid and high dose) and in high dose F1 females (4.7, 4.9, 5.0 and 5.8 days in control, low, mid and high dose). An increased number of acyclic females was observed in high dose F0 females (1/28, 0/28, 0/28 and 6/28 in control, low, mid and high dose) and a decreased number of cycles per animal was seen in high dose F0 (3.9, 3.7, 3.6, 3.0 in control, low, mid and high dose) and F1 females (3.8, 3.6, 3.7, 3.1 in control, low, mid and high dose). Estrous cyclicity is usually not affected by body weight. Further, body weight was not affected in any of the dose groups in F0 females during premating. Absolute and relative uterus weights were markedly decreased at termination in all dose groups in F0 females (absolute: 18-32%, relative 18-31%) and in high dose F1 females (absolute: 35%, relative: 31%). The terminal body weight was not affected in F0 females in any dose groups. In F1 females, terminal body weight was decreased in mid (5%) and high (7%) dose. *Ovary weight:* Absolute ovary weight was decreased in high dose F0 females (11%) and in high dose F1 females (10%, n.s.). Relative ovary weight was also decreased in F0 females (8%, n.s.) and F1 females (3%, n.s.). The terminal	

		body weight was not affected in F0 females and decreased in high dose F1 (7%) females. *Pituitary weight:* In F0 females, absolute and relative pituitary weight was decreased in high dose (absolute: 6%, relative: 8%). Terminal body weight was not affected. In F1 females, absolute pituitary weight was decreased in all dose groups (low (12%), mid (6%) and high dose (18%)) and relative pituitary weights were decreased in low (12%), mid (5%, n.s.) and high dose (9%). Terminal body weight was decreased in mid (5%) and high dose (7%) but not in low dose. In F0 males, pituitary weight was not affected. In F1 males, absolute pituitary weight was decreased in high dose (13%). Terminal body weight was also decreased in high dose (16%) and relative pituitary weight was not affected. *Male reproductive organs:* No clear substance related effects were observed on weight of testes, epididymis, prostate or seminal vesicles. *Semen quality:* No effect on F0 or F1 semen quality.	

Unpublished study report, 2018
 	
Klimisch 1
OECD TG 414
GLP
	Developmental toxicity (TG 414) in rabbits,
	
	N=22
	
	Oral gavage
	75, 200, 500 mg/kg bw/day
	
	Exposure GD6-28
		Body weights:
		Maternal body weight gain was transiently decreased in mid and high dose. The decrease was significant in mid dose at day 9, 15, 21 and 24 and in high dose at day 9, 12, 15, 18 and 21.
		Maternal food consumption was transiently decreased in mid and high dose. The decrease was significant in mid dose at day 12-15 and 18-21 and in high dose at day 6-9, 9-12, 12-15, 15-18 and 18-21.
		Maternal terminal body weight was not significantly decreased in any dose group.
		
		Liver:
		Maternal relative liver weight was increased (14%) in high dose compared to control.
		Foetal weight was not significantly different between groups.
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4
			
			Details:
			Tris(1-chloroprop-2-yl) phosphate, isomer mixture (CAS 13674-84-5): 78.0 %
			
			Bis(1-chloropro-2-yl) 2-chloropropyl phosphate, isomer

		Skeletal malformations: An increased incidence of 13th rudimentary ribs were observed in high dose (10.6% per litter) compared to controls (3.8% per litter). An increased incidence of litters with soft tissue variations were observed in high dose (13.4% per litter) compared to controls (4.6% per litter).	mixture (CAS 76025-08-6): 19.6 % 1-Chloroprop-2-yl bis(2-chloropropyl) phosphate, isomer mixture (CAS 76649-15-5): 1.8 % Tris(2-chloropropyl) phosphate, isomer mixture (CAS 6145-73-9): 0.06 %
NTP, 2020 Klimisch 1 OECD TG 414 GLP	Developmental toxicity (OECD TG 414) in rats, N=20 Oral gavage 162.5, 325, or 650 mg/kg bw/day Exposure GD6-20	*Body weights:* Maternal body weight gain was not affected by treatment. Maternal food consumption was transiently affected in mid and high dose. In mid dose, it was significantly decreased at day 6-9 but increased at day 18-21. In high dose, it was decreased at day 6-9 and 9-12 and increased at day 19-21. Maternal terminal body weight was unaffected by treatment. *Liver:* Maternal absolute and relative liver weight was increased. This was significant as trends and for all doses compared to control (absolute: 9%, 16% and 20% in low, mid and high dose and relative: 8%, 14% and 27% in low, mid and high dose). Foetal body weight was unaffected by treatment. *Skeletal malformations:* Increased percentage of foetuses with lumbar rudimentary ribs in low (22%), mid (23% animals) and high dose (22%) compared to controls (14%).	Name: TCPP isomers: Specifications: - app. 68% tris(1-chloro-2-propyl) phosphate, CAS 13674-84-5, -app. 25% bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate Tris(Chloropropyl) Phosphate (CAS 76025-08-6) - app. 4% bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5); -app. 0,2% tris(2-chloropropyl) phosphate (CAS 6145-73-9).
NTP, 2023 (Study conducted in 2009) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance	3-months with perinatal study in rats F0 females: N=20 (0, 2500, 10,000, 40,000 ppm) or N=8 (5000, 20,000 ppm) F1 rats: N=10/sex Exposure: F0 females: GD6-LD21,	The only reproductive organs weighed were testes and epididymis (not e.g. uterus, ovaries, pituitary) *Delivery and litters:* In the high dose group (20,000 ppm), maternal body weight gain was decreased GD6-9 (87%) and as a result maternal body weight was decreased at GD9 (5%). However, maternal body weight gain was increased during most of the rest of the gestational	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 obtained from Albemarle) Four major isomeric components were identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 64.77 %)

with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	F1: Perinatal + 3 months Doses: Females: 0, 2500, 5000, 10,000, 20,000 ppm in feed (and 40,000 ppm in F0 females only, euthanized during gestation due to excess toxicity), corresponding to app.: 0, 236, 458, 906, 1890 mg/kg bw/day. Males: 0, 2500, 5000, 10,000 ppm in feed (and 20,000 ppm, but this group did not survive to study termination), corresponding to app.: 0, 223, 431, 911 mg/kg bw/day. (Body and organ weights of F1 males was reported in low, mid and high dose, corresponding to 2500 ppm, 5000 ppm and 10,000 ppm, whereas the 20,000 ppm group seemed to have been taken out). Age at necropsy: F1: 17 weeks	period, though only significantly in GD12-15 (50%), and high dose maternal body weight was not different from control at the end of gestation (GD21). There were no effects on live litter size at birth or on pup mortality during postnatal life. *Body weight:* The terminal body weight in F1 males was decreased in low (6%), mid (6% n.s.) and high dose (3% n.s.). *EAS-relevant investigations:* There was no significant effect on the weight of testes in F1. In F1 males, absolute epididymis weight was decreased in low (11%), mid (10%) and high (7%) dose. Also cauda epididymis weight was decreased in low (15%), mid (8%, n.s.) and high dose (3%, n.s.). No effects were observed on testicular sperm count or percent motile sperm in F1 males dosed up to 10,000 ppm. *Histopathology:* No differences between control and 20,000 ppm groups observed in histopathology of thyroid, genital system or brain in F1 animals.	bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 26.98 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.99 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.2 %) Impurities: 4.2 %
NTP, 2023 (conducted 2011-13) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory	2-years with perinatal study in rats Exposure: F0 females: GD6-LD28, F1: Perinatal + 2 years F0 female rats: N=38 F1 rats (2-year study): N=50/sex F1 rats (internal dosimetry): N=10/sex Dosing: 0, 2500, 5000, 10,000, or 20,000 ppm in feed, corresponding to	Organ weights not reported and no investigation of estrous cyclicity There were no effects on live litter size at birth or on pup mortality during postnatal life. Differences in incidences of non-neoplastic lesions in ED-relevant organs in exposed animals compared to controls: Females: Uterus, cysts (0/50, 0/50, 0/50, 0/49, 2/50 - statistically significant trend); Uterus, cervix inflammation (0/50, 0/50, 0/50, 0/49, 3/50 – statistically significant trend) Males:	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 mixed with lot 134 obtained from Albemarle to form lot M072911NP) Four major isomeric components were identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 68.06 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 25.43 %)

Practice Regulations	app.: Males: 0, 141, 294, 626, and 1155 mg/kg bw/day. Females: 0, 156, 323, 674, and 1295 mg/kg bw/day. Age at necropsy: F1: 109-110 weeks	Prostate, epithelium hyperplasia (0/50, 0/50, 5/50, 0/50, 3/50 – significantly different in mid dose compared to control) Testis, polyarteritis nodosa (13/50, 13/50, 9/50, 9/50, 3/50 – statistically significant trend and difference between high dose and control) Testis, germinal epithelium degeneration (21/50, 15/50, 14/50, 13/50, 8/50 - statistically significant trend and difference between three highest dose groups and control) Differences in incidences of primary tumours in ED-relevant organs in exposed animals compared to controls: Females: Uterus, adenoma or adenocarcinoma (combined) (3/50, 4/50, 6/50, 8/49, 9/50 – statistically significant trend and between high dose and control). Uterus, Polyp Stroma (5/50, 15/50, 6/50, 7/49, 13/50 – statistically significant difference between low dose and control as well as high dose and control). Ovary, granulosa cell tumour benign (0/50, 0/50, 0/50, 0/49, 2/50 – statistically significant trend) No biologically relevant significant differences in incidences of neoplastic or non-neoplastic lesions in other reproductive organs, including epididymis, seminal vesicles, pituitary gland, preputial gland, penis, thyroid, mammary gland, oviduct, vagina. Neoplastic effects observed in other organs are described in section 7.9.6	bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.55 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.21 %) Impurities: 2.55 % Due to ageing, control animals had high incidences of non-neoplastic lesions in numerous organs, including endocrine system, thyroid, genital system, mammary gland - challenging the detection of non-neoplastic effects induced by TCPP exposure.

Conclusion of effects on development

In both of the TG 414 studies in rats and in the prenatal developmental toxicity study in rabbits, there were no signs of foetal mortality, implantation loss or effects on foetal weight. However, indication of effects on skeletal malformations were observed in all three studies (Kawasaki *et al*., 1982; Unpublished study report, 2018 and NTP, 2020).

In the 1- and 2-generation reproductive toxicity studies that employed both pre- and postnatal exposure (TNO 2007a, 2007b), there were indications of increased pup mortality and delayed development in TCPP-exposed offspring.

In the 1-generation study used for setting doses in the two-generation study (TNO, 2007b), an increased number of runts was observed at day 4, 7, 14 and 21 in high dose (~1000 mg/kg bw/day) and at day 21 in low and mid dose (~100 & 330 mg/kg bw/day). In this study, mean maternal body weight was affected during gestation and lactation in the mid and high dose group but not in the low dose group. The observed effect on development of the pups could be partly explained by maternal toxicity in the two highest dose groups but it does not explain the effect on the pups in the low dose group.

In the two-generation study (TNO, 2007a), an increased number of runts was observed in all dose groups (~100, 330 & 1000 mg/kg bw/day) and a decrease in the mean number of pups delivered was observed in the mid dose group of F1 and the high dose groups of both generations. A decrease in pup weight was also noted during the lactation period. Pup mortality (PND1-4) was increased in the low and high dose groups of F0 and in the high dose group of F1 (although the latter was mainly due to the loss of one litter of a single dam on PND4).

A decrease in maternal body weight was also seen in the high dose F1 females and the effects observed in the offspring could be due to maternal toxicity. However, no significant effects were observed on mean maternal body weight during lactation in F0 so maternal toxicity seem not to be able to explain the effects on the developing pups completely. Based on the above, it is possible that TCPP has an effect on development of the pups.

Taken together, the identified information shows that TCPP is a developmental toxicant. Effects on anogenital distance (AGD), sexual maturation and reproductive organ development in offspring exposed to TCPP both pre- and postnatally are discussed in relation to endocrine disrupting effects in section 7.10.2.

7.9.8. Hazard assessment of physico-chemical properties

Not considered relevant by the eMSCA in this substance evaluation.

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

Not considered relevant by the eMSCA in this substance evaluation.

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

The available information is considered sufficient for the classification and labelling of TCPP. Carcinogenic effects were observed in the liver of both rats and mice and in the uterus of rats in NTP studies. It is concluded by the eMSCA that it is likely that TCPP is a non-genotoxic carcinogen. The observed effects on development and fertility could also warrant a classification for reproductive toxicity. However, no classification proposal is made in this conclusion document in view of the complexity of the information.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Endocrine disruption with relevance for the environment has not been evaluated in this substance evaluation.

7.10.2. Endocrine disruption - Human health

7.10.2.1. Literature search – reproductive toxicity and endocrine disruption

To ensure inclusion of all relevant literature, a single concept search strategy was used as suggested in ECHA/EFSA guidance document (ECHA/EFSA, 2018). The search strategy was used in PubMed and Web of Science. Since TCPP is a mixture of isomers, the search was performed on CAS number for TCPP as well as the CAS numbers for the individual isomers. Date of search, search string and number of hits are listed in the able below.

Table 24:

LITERATURE SEARCH				
Date of search	**Database**	**Search string**	**No. of articles**	**Comment**
12-05-2022	PubMed	**((((((((((((1-chloropropan-2-yl bis(2-chloropropyl) phosphate) OR (bis(1-chloropropan-2-yl) 2-chloropropyl phosphate)) OR (bis(1-chloropropan-2-yl) 2-chloropropyl phosphate)) OR (tris(2-chloropropyl) phosphate)) OR ("1244733-77-4")) OR ("13674-84-5")) OR ("76025-08-6")) OR ("76649-15-5")) OR ("6145-73-9")) OR (Tris(2-chloroisopropyl)phosphate)) OR (organophosphate ester flame retardant*)) OR (organophosphorus flame retardant*)**	1084	
12-05-2022	Web of Science	**((((((((((ALL=(1-chloropropan-2-yl bis(2-chloropropyl) phosphate)) OR ALL=(bis(1-chloropropan-2-yl) 2-chloropropyl phosphate)) OR ALL=(bis(1-chloropropan-2-yl) 2-chloropropyl phosphate)) OR ALL=(tris(2-chloropropyl) phosphate)) OR ALL=("1244733-77-4")) OR ALL=("13674-84-5")) OR ALL=("76025-08-6")) OR ALL=("76649-15-5")) OR ALL=("6145-73-9")) OR ALL=(Tris(2-chloroisopropyl)phosphate)) OR ALL=(organophosphate ester flame retardant*)) OR ALL=(organophosphorus flame retardant*)**	1646	No. of unique articles compared to PubMed: 824

After removal of duplicates, the total number of articles was 1906. These articles were screened in three refinement steps based on title, abstract and full text according to the procedure described in ECHA/EFSA guidance document (ECHA/EFSA, 2018). Articles were considered relevant when they contained primary data (i.e. not reviews) related to endocrine disruption or reproductive toxicity. Examples of articles not considered relevant included human exposure data without health parameters, methodological articles concerning detection and measurements of chemicals in samples (e.g. HPLC-MS methods), articles not including studies on TCPP or constituents of TCPP and articles not in English.

1st screen based on title: 1 article considered relevant, 116 articles possibly relevant, and 1789 articles considered irrelevant.

2nd screen based on abstract: 9 article considered relevant and 33 articles possibly relevant. These were categorized into 5 *in vivo* studies, 17 *in vitro* studies, 22 epidemiological studies, and 1 in silico. Seventy-five articles were considered irrelevant.

3rd screen based on full text: 2 *in vivo* and 12 *in vitro* studies.

The 3rd screen resulted in the final material.

Additionally, the *in vivo* studies included as part of the registration dossier were evaluated. Finally, a search in ToxCast (https:// https://comptox.epa.gov/dashboard/) was conducted on 14 June 2022 using the search term "13674-84-5" and "1244733-77-4". No results were obtained for the latter CAS number, which is the CAS number given in the registration dossier.

7.10.2.2. Endocrine disruption – Human health - *In vitro* studies

Table 25.

Study overview, *in vitro* studies on TCPP incl. ToxCast. Effects described are statistically significant unless otherwise indicated. Reliability have been assessed using Klimisch score.

Reference	Study design	Quick overview results	Results description	Study quality and assessment
Cao *et al.*, 2018	hERRγ binding affinity by fluorescence competitive binding assay, 2 minutes, 0.01-10 µM ERRγ-mediated luciferase reporter gene assay, HeLa cells transient transfection, 24 h exposure, 0.1-100 µM ≥98% Non cytotoxic concentrations as determined by WST-1 assay in HeLa cells	LOEC = 10 µM ERRγ binding affinity in dose dependent manner with maximum change in response of approximately 40% ERRγ activity inhibited at 10 µM with maximum change in response of approximately 40%	In this study the binding affinity and receptor activity was examined for the orphan human nuclear receptor estrogen-related receptor γ (hERRγ). TCPP displaced the fluorescent probe from the hERRγ LBD, but calculation of IC50 value was not done, as the response did exceed 50% decrease. TCPP showed inhibitory effect on the ERRγ transcriptional activity suggesting an inverse agonist according to the authors with a LOEC of 10 µM and maximum inhibition of approximately 40 %. All effects were observed at non-cytotoxic concentrations as determined by WST-1 assay in HeLa cells.	Klimisch 2. Positive controls in ERRγ binding affinity assay and reporter gene assay appear appropriate and show results in accordance with that previously reported according to the authors. The reported DMSO concentrations appear appropriate for the two assays, 1% and 0.1% for the ERRγ binding affinity assay and ERRγ reporter gene assay, respectively. Shortcomings: All experiments repeated three times. It is unclear if this describes technical replicates within an independent experiment or whether it describes three independent experiments. No CAS given, but chemical structure

				given for constituent with CAS number 13674-84-5 in the registration dossier
Dishaw *et al.,* 2011	Neurotoxicity, PC-12 cells, 50 µM, cell number, cell growth, neurite outgrowth and enzyme activities, 6 days exposure Cytotoxicity measured by trypan blue and visual scoring 95% purity		In this study, neurotoxicity was examined in PC-12 cells measuring effects of 50 µM TCPP on cell number, cell growth, neurite outgrowth, and enzyme activities of choline acetyltransferase and tyrosine hydroxylase. TCPP led to decreased cell number, no effect on cell growth, and promoted cholinergic phenotype	Klimisch 2 Cytotoxicity scored by two independent observers DMSO concentration 0.1% Experiments were performed on 4-5 separate cultures Shortcomings: No CAS number given for test substance, but chemical structure for TCPP shown Only one concentration tested
Föllman n *et al.,* 2006	ERα recombinant yeast reporter gene assay measuring β-galactosidase activity, 72-84 h exposure, 0.000001-100 µM. Agonism and antagonism Estrogenic effect measured as activity of alkaline phosphatase, Ishikawa cells, 72 h, 0.01-10 µM. Only agonism	No reported estrogenic effect	In this study estrogenicity of TCPP was examined in two model systems, a recombinant yeast ERα reporter gene assay and in Ishikawa cells by measuring estrogen dependent activity of alkaline phosphatase. Agonism and antagonism was measured in the former and only agonism in the latter assay. TCPP did not exhibit estrogenic nor anti-estrogenic activity in the two in vitro model systems.	Klimisch 3 Same effect measure in two different assays Shortcomings: No indication of number of experimental repeats Only one E2 concentration tested in Ishikawa cells. Full concentration range in Yeast assay. The Yeast assay does not appear very reproducible as the response for the positive control varies greatly in potency and concentration-response relationship between two experimental runs.

				No cytotoxicity reported No substance purity reported
Klose *et al.*, 2022	Developmental neurotoxicity *in vitro* battery: Neurosphere assay, 3D human primary neural progenitor (NCP) cell-based neurospheres, measure increase of sphere size, proliferation, migration and differentiation, up to 72 h cMINC assay, hiPSC-derived neural crest cells (NCC), measure cell migration, 24 h NeuriTox assay, Lung human mesencephalic cells (LUHMES), measure neutrite area, 24 h PeriTox assay, differentiated sensory neurons, measure neurite area and viability, 24 h Cell viability and cytotoxicity examined by different	Neurosphere assay: TCPP was negative in pre-screening (<20 µM) and was not tested further. cMINC assay: TCPP was negative in pre-screening (<20 µM) and was not tested further. NeuriTox assay: TCPP was negative in pre-screening (<20 µM) and was not tested further PeriTox assay: TCPP was negative in a pre-screening (<20 µM) and was not tested further	In this study the developmental neurotoxicity was examined in a battery of *in vitro* assays. TCPP did not lead to effects in any of the tested endpoints.	Klimisch 2 Vehicle (DMSO) 0.1% in experiments Authors report that the DNT battery perform with a specificity and sensitivity of 88 and 100%, respectively when testing known positives and negatives for human DNT with referral to other publication. Concentration-response tested, in 5 replicate reaction. Cell viability and cytotoxicity tested Shortcomings: Not clear if independent experiments performed

	means dependent on the assay No purity reported			
Kojima *et al.,* 2013	hERα, hERβ, hAR, hGR, $hTR\alpha_1$, $hTR\beta_1$, hRARα, and hRXRα transactivation assays, CHO-K1, 24 h, 0.1-30 µM PXR, hPPARα, and hPPARγ transactivation assay, COS-7 cells, 24 h, 0.1-30 µM All transient transfections All assays performed in agonist and antagonist mode, except hRARα, hRXRα, PXR, hPPARα, and hPPARγ, which were performed in agonist mode only. A β-galactosidase activity assay was used for cytotoxicity in both cell-lines >98% purity reported	REC20 = 4.9 µM In PXR reporter gene assay the reported REC20 was 4.9 µM ERα, ERβ, AR, GR, $TR\alpha_1$, $TR\beta_1$, PPARα and PPARγ no change in activity reported TCPP not cytotoxic based on β-galactosidase activity in both cell lines	In this study the agonistic and antagonistic activity of TCPP on eleven different receptors (ERα, ERβ, AR, GR, TRα1, TRβ1, RARα, RXRα, PXR, PPARα, and PPARγ) was examined. TCPP induced PXR activity with reported REC20 value of 4.9 µM. TCPP did not affect ERα, ERβ, AR, GR, $TR\alpha_1$ and $TR\beta_1$ activity in neither agonist or antagonist mode, nor RARa and RXRa agonistic activity. None of the reported data was at cytotoxic concentrations of TCPP	Klimisch 2 Three independent experiments in triplicates Effects reported at non-cytotoxic concentrations Shortcomings: Maximum tested concentration fairly low. Risk to miss activities at higher concentrations Classification as positives for agonism, only if more than 20% of positive control. The efficacy of the positive controls greatly influence these positive calls. Statistical analysis only performed for AR and GR activity data. Generally, materials and methods are described in other manuscripts.
Krivoshiev *et al.,* 2018	RNA seq, HepG2 cells, 72 h, 2.5 and 25 µM		In this study the toxicogenomic profile of TCIPP and TCEP was compared to identify common traits of effect.	Klimisch 2 Vehicle (DMSO) below 0.1%

	Cytotoxicity examined by resazurin assay		Affect genes involved in immune responses, steroid hormone biosynthesis, and xenobiotic metabolism pathways	Experiments performed in three independent experiments Treatment at non-cytotoxic concentrations
Liu *et al.,* 2012	H295R steroidogenesis assay, sex hormone levels, gene expression levels, adenocarcinoma cells, 48 h, 0.001-100 mg/L converted to 0.003 – 305 µM Estrogenic activity, MVLN cells, 72 h, 0.001-10 mg/L converted to 0.003-30.5 µM. Both agonism and antagonism tested in MVLN cells. MTT used for cell viability examination in H295R cells. No reporting for cell viability in MVLN cells.	LOEC=1 mg/L Significant increase in T and E2 at 1 and 100 mg/L, respectively. Effects on steroidogenic genes were observed No effects on estrogenic activity in MVLN cells	In this study the effects of TCPP on steroidogenesis at hormone and gene expression levels, as well as ER activity was examined. A significant increase in E2 and T was observed with TCPP exposure at 100 mg/L and 1 mg/L, respectively, in the H295R steroidogenesis assay. CYP11A1, CYP11B2 and HSD3β2 gene expression was significantly induced by TCPP, whereas CYP19A1 was not affected. SULT1E1 and SULT2A1 gene expression was significantly reduced with exposure. TCPP did not induce or inhibit estrogenic activity in the MVLN cells.	Klimisch 2 Vehicle control below 0.1% Cell viability measured in H295R and MVLN cells Reported effects only at non-cytotoxic concentrations. Positive controls appear appropriate Shortcomings: It is not transparent how many times the experiments have been repeated Purity of test substance not reported
Reers *et al.,* 2016	ER and AhR mediated gene expression, ECC-1 cells, 24 h, 0.01-20 µM AR mediated gene expression, LNCaP cells, 24 h, 0.01-20 µM	No effects reported on ER, AhR, and AR mediated gene expression.	In this study, the activities of TCPP on the AR, ER and AhR was examined in human prostate and endometrial cancer cells by measuring gene expression of receptor inducible genes. No effect on activity of ER, AR, and AhR by TCPP	Klimisch 3 Appropriate controls (E2, R1881, and TCDD) Shortcomings: Low maximum concentrations tested. Risk to miss substances with activity above the

	Agonism and antagonism both cell lines			tested concentration. No information on cytotoxicity measure No reporting of vehicle concentration No reporting on number of independent experiments Substance purity not reported
Rosenmai *et al.,* 2021	AR-EcoScreen, luciferase reporter gene assay, CHO-K1 cells, 24 h, 0.4-100 µM H295R steroidogenesis assay, adrenocarcinoma cells, 48 h, 0.8-50 µM ANSA-TTR displacement assay, 2 h, 0.1-500 µM AhR reporter gene assay, H4IIE cells, 24 h, 0.4-100 µM	EC_{50}: 3 µM for AhR agonism IC_{50}: 48 µM for AR antagonism IC_{50}: 224 µM for TTR displacement T levels H295: no effect E levels H295: no effect	In this study TCPP was examined in four assays, namely an AR and AhR reporter gene assay, TTR-ANSA displacement assay, and H295R steroidogenesis assay. TCPP led most potent effects on AhR activity with EC_{50} of 3 µM and a maximum change in response compared to vehicle control of 200%. In the AR reporter gene assay an antagonistic effect of receptor activity was observed with IC_{50} of 48 µM and a maximum change in response compared to vehicle control of 90%. The effect on ANSA displacement from TTR was less potent with IC_{50} of 224 µM and a maximum change in response of 40%. No effect was observed in the H295R steroidogenesis assay. All effects reported at non-cytotoxic concentrations.	Klimisch 2 Relevant controls included for all endpoints Vehicle concentration was kept constant and at relevant level for all endpoints Cytotoxicity was monitored for all cell-based assays All reported effect where at non-cytotoxic concentrations. At least three independent experiments performed for all assays Shortcomings: Substance ID not completely transparent. CAS number not identical to substance under evaluation but a mix of isomers with similar structures as TCPP. Substance purity not reported

Wang *et al.,* 2022	KGN human granulosa cells, 48 h exposure, 0.001-100 µM Ma-10 mouse Leydig cells, 48 h exposure, 0.001-100 µM C18-4 mouse spermatogonial cells, 48 h exposure, 0.001-100µM Purity 90%	LOEC=5 µM TCPP exposure from 5 µM led to an increase in the number of lysosomes in C18-4 cells, but not MA-10 and KGN cells. At 20 µM, a significant increase in cell viability and lysosome number was observed in some cell lines with TCPP exposure From 50 µM, TCPP increased number of lipid droplets in some cell lines No cytotoxicity of TCPP and no effect on mitochondrial parameters	In this study the effect of TCPP on three in vitro models related to reproductive function was examined. The cell types used was human granulose cells, mouse Leydig cells and spermatogonial cells. Cell survival, mitochondrial dynamics, oxidative stress, lysosomes and lipid droplets were analysed. TCPP was not cytotoxic in any of the cell lines. TCPP increased cell viability in MA-10 cells from 20 µM, but did not affect this parameter in KGN and C18-4 cells. No effect on mitochondrial parameter with TCPP exposure TCPP led to increased number of lysosomes from 20 µM exposure in C18-4 cells, but not MA-10 and KGN cells TCPP led to increased total area of lipid droplets from 50 µM in MA-10 and C18-4 cells, but no effect was seen in KGN cells.	Klimisch 2 Cell viability assessed and concentrations causing this were excluded before further analysis. Appropriate number of replicates (6-8) Shortcomings: Relatively high DMSO concentration of 0.5% in all wells
Zhang *et al.,* 2014	Rat ERα luciferase reporter gene assay, CHO-K1 cells, 24 h, exposure concentrations not stated by most likely 0.0001 - 10 µM. Ago and anta ERα activity in yeast two hybrid assay by measuring β-galactosidase, 4 hrs, exposure concentrations not stated by most likely 0.0001 - 10 µM. Ago and anta.		In this study, three methods were applied to examine the ability of TCPP to induce/inhibit estrogenicity, a rERα reporter gene assay, an ERα yeast hybrid assay, and a MCF7 proliferation assay. No anti-estrogenic or estrogenic activity was observed in any of the applied assays.	Klimisch 2 DMSO concentration was kept below 0.1% All assays performed in at least three independent experiments Three different assays used to show lack of estrogenicity of TCPP. Appropriate positive control, E2. Shortcomings: Cytotoxicity only examined in CHO-K1 cells and not MCF7 cells. Maximum concentration tested was most likely 10 µM. Risk of missing

	E-screen assay measured as proliferation, MCF-7 cells, 5 days, exposure concentrations not stated by most likely 0.0001 - 10 µM. Only ago Cytotoxicity was examined in CHO-K1 cells by MTS assay Purity 96%			low potent substances.
Zhang *et al.*, 2016	TRβ luciferase reporter gene assay, CHO-K1 cells, 24 h, 0.001-10 µM T-Screen assay measured as proliferation, GH3 cells, 96 h, 1 µM Agonism and antagonism tested in both models Effects at non-cytotoxic concentrations according to authors 96% purity	RIC_{20} of 1.2 µM Antagonistic effect on TRβ activity with RIC20 of 1.2 µM, No effect was observed in the T-screen assay nor TRβ-agonism.	In this study, a TRβ reporter gene assay and the T-screen assay were used to investigate the potential thyroid hormone disrupting capacity of TCPP. No TRβ agonistic activity was seen in the reporter gene assay. However, TCPP showed antagonistic activity with a RIC_{20} of 1.2 µM. No TR agonistic nor antagonistic effect was seen in the T-screen assay.	Klimisch 2 Positive controls appear appropriate DMSO concentration below 0.1% Three independent experiments Shortcomings: Low concentrations tested with risk of missing effects at higher concentrations Unclear how cytotoxicity was examined but authors state TCPP not cytotoxic at tested concentrations
CompTox Chemicals Dashboard 2022	AR activity		Six *in vitro* assays related to AR binding and activity was reported under the EDSP. Some were performed in both agonist and antagonist mode. TCPP did not lead to "active" hit calls for any of the applied assays.	
CompTox	ER activity		Six *in vitro* assays related to ER binding and activity was reported	

Chemicals Dashboard 2022			under the EDSP. Some was performed in both agonist and antagonist mode. TCPP did not lead to "active" hit calls for any of the applied assays.	
CompTox Chemicals Dashboard 2022	Aromatase inhibition		Under the EDSP program one assay for CYP19 inhibition was reported. TCPP did not lead to "active" hit call for this assay.	
CompTox Chemicals Dashboard 2022	Thyroid hormone receptor activity		Fine assays related to the TH system was performed under the SDSP. TCPP did not lead to "active" hit calls for any of the applied assays.	

Discussion - Endocrine disruption - in vitro effects

EATS

The literature search led to identification of 12 *in vitro* publications of potential relevance for endocrine disruptive potential for TCPP. In Table 25, a study summary of the identified publications are shown including reliability scores according to the Klimisch scoring system. Of the identified publications, six examined estrogenic effects, three examined androgenic or antiandrogenic effects, three examined effects on the thyroid hormone system, three investigated effects related to steroid hormone production, two investigated neurotoxicity and one publication investigated reproductive parameters.

Of the six studies examining estrogenicity, five did not find any estrogenic effect or specific effects on receptor activity for ERα/β (Follmann *et al*., 2006; Kojima *et al*., 2016; Liu *et al*., 2012; Reers *et al*., 2016, and Zhang *et al*., 2014). One study reported ability of TCPP to bind and activate the EERϒ (Cao *et al*., 2018). The study by Follmann *et al*., 2006 and Reers *et al*., 2016 were given a Klimisch score of 3, whereas the remaining studies were scored 2. Of the three studies examining effects on the AR, two reported no effects (Kojima *et al*., 2016 and Reers *et al*., 2016), whereas Rosenmai *et al*. (2021) reported inhibition of AR activity with an IC50 value of 48 µM. Of the two studies reporting no effect, one was given a Klimisch score of 3 (Reers *et al*., 2016). Further, these two studies both tested fairly low maximum concentrations (<30µM) of TCPP compared to Rosenmai *et al*. (2021) (100 µM), which could explain the discrepancies. Of the three studies dealing with interferences with the sex hormone synthesis, one study evaluated effects on sequenced RNA from HepG2 cells (Krivoshiev *et al.* 2018) and identified that the affected genes where, amongst other effects, involved in steroid hormone biosynthesis. The two other studies both used the H295R steroidogenesis assay. However, one study found increased levels of E2 and T with TCPP exposure (Liu *et al*., 2012), whereas the other study found no effect (Rosenmai *et al.,* 2021). Again, the maximum tested concentration may explain the differences in results as Liu et al. (2012) tested approximately 300 µM as the maximum concentration, whereas Rosenmai *et al*. (2021) tested 100 µM as the top concentration. Three studies included tests relevant for thyroid hormone system disruption. One study found that TCPP could displace ANSA from a TH transporter, TTR. Two studies examined effects on the TR activity with one study showing inhibition of the TRβ activity. Finally, two studies examined endpoints relevant for neurotoxicity and they only reported effects on cell number. One study examined effects in cell types (human granulosa, mouse Leydig cell and spermatogonial cells) relevant for reproductive toxicity (Wang *et al*., 2022).

In conclusion, the *in vitro* studies suggest that there is no evidence for estrogenic effects of TCPP on the ERα/β but that TCPP could affect ERRγ. There are some indications that TCPP may inhibit the activity of the AR, interfere with steroid hormone synthesis and affect parameter of the thyroid hormone system though the volume of studies performed to examine these latter endpoints are sparse.

Other modalities

NTP has collated some available information from Tox21 *in vitro* assays, which indicate that TCPP may affect other ED modalities than EATS. TCPP had activity in 10 assays of which 7 were related to xenobiotic homeostasis, including activation of the constitutive androstane receptor (CAR) pathway and the pregnane X receptor (PXR) signaling pathway, as well as inhibition of CYP 1A2, 2C19, 2C9, 3A4 and 2D6 (NTP, 2023). Further activities were observed in two assays for cytotoxicity and one for activation of the progesterone receptor (PR) signaling pathway.

NTP has also conducted a 5-day repeat dose *in vivo* mechanistic genomic study in male rats, focusing on transcriptomics in liver and kidney. The study showed that the androgen receptor was upregulated in the liver together with 7 out of 11 CAR and PXR-related biomarker genes and 8 out of 14 PPARα-related biomarker genes (NTP, 2023).

Further scrutiny of these findings and their implications for the understanding of effects of TCPP on the endocrine system observed *in vivo* are not included in the present evaluation, but may be relevant in further assessment of TCPP.

7.10.2.3. Endocrine disruption – *in vivo* effects in rodents

Table 26

Study overview, rodent *in vivo* studies on TCPP, relevant for assessment of endocrine disruption. Effects described are statistically significant unless otherwise indicated (non-significant = n.s.). Reliability have been assessed using Klimisch score. Some of the effects reported in this table are also reported elsewhere in this report but they are included here for completeness.

Reference	Study design	ED relevant results	Test material
Stauffer Chemical Co., 1981a Published: Freudenthal and Henrich, 1999 Klimisch: 2 Non-guideline Non-GLP	90-day study in rats, n=20/sex Oral dosing in feed 52-62; 160-171; 481-570; 1349-1745 mg/kg bw/d (800, 2500, 7500, 20,000 ppm) At necropsy, the following ED-relevant endpoints were examined: -weight of testes and ovaries -macroscopic observations of prostate, mammary gland, testes, ovary, pituitary, uterus -histopathology of mammary gland, pituitary, testes, epididymis, prostate,	No statistically significant changes in EAS relevant organs were seen. *Body weight:* Mean terminal body weight was significantly decreased in both males (8%) and females (12%) in the high dose group. *Reproductive organ weights:* Testes and ovary weight were not affected. *Thyroid histopathology:* Males: follicular hyperplasia: 0/20; 2/20, 2/20, 5/20, 8/20 Females: follicular hyperplasia: 0/20, 0/19, 0/10, 0/20, 5/20 There was no investigation of weight of thyroid, uterus or estrous cyclicity	Name: Fyrol PCF Specifications: consists primarily of -tris(2-chloroisopropyl) phosphate (about 70%) -2-chloropropanol phosphate (about 23%)

	seminal vesicles, ovaries, uterus		

Bayer, 1991
 	
Klimisch: 2
GLP
	28-day study in rats
	
	n=6/sex
	
	Oral gavage
	10, 100, 1000 mg/kg bw/day
	
	At necropsy, the following reproductive toxicity and ED-relevant endpoints were examined:
	-weight of testes
	-macropathology and histopathology of testes, epididymis, seminal vesicles, prostate, ovaries, uterus.
		No statistically significant changes in weights or histopathology of any of the EAS-relevant organs was seen.
		
		There was no investigation of weight of uterus, ovaries, estrous cyclicity or thyroid weight or histology
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4
			
			Specifications:
			Tris chloroisopropyl phosphate
			Purity: 97.85% including all isomers

TNO, 2007b
 	
Klimisch 1
GLP
	One-generation study (range finding) in rats
	n=10/sex
	
	Oral dosing in feed: 1500, 5000, 15,000 ppm
	(corresponding to app. 100; 330; 1000 mg/kg bw/d)
	
	Exposure 5 weeks premating to PND21
	
	Parental body weights were assessed throughout the study. Organ weights and semen quality were assessed at study termination.
	Offspring was assessed for postnatal mortality and growth.
		Body weights:
		During premating, a decrease in body weight was seen in F0 high dose males (5-8%).
		No effect was seen on body weight in F0 females during premating.
		
		During gestation, maternal body weights were decreased in mid (day 0-14, 5-6%) and high (day 7-14, 5-6%) dose animals.
		
		Around birth, body weights of F0 females were not significantly affected.
		
		During lactation, a decrease in body weight gain was seen in low (19%) and high (38%) dose dams, resulting in a (10%) decrease in body weight at the end of lactation in high dose.
		This corresponded well with the decreased maternal food consumption (11-33%) seen on days 4-21 of lactation in high dose.
		
		Pup birth weight was similar between groups.
		
		During lactation, pup body weight gain was decreased from day 14 in low (12-13%) and mid (26-27%) dose and from day 7 in high dose (24-46%), resulting in a decreased pup body weight at day 21 in mid dose (12-14%) and
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4

		at day 14 and 21 in high dose (18-31%). *Delivery and Litters:* There were no effects on fertility index or live pups/litter at birth. Increased pup mortality was seen in the high dose group (mainly caused by 1 litter with 8 pups dying at day 5-7). There was an increased number of runts in all dose groups at day 21 and in high dose also at day 7 and 14. *EAS-relevant effects:* Decreased prostate weight was observed in low (absolute: 15%, relative: 11%, n.s.) and high (absolute: 19%, relative: 13%, n.s.) dose F0 males. Decreased uterus weight was observed in all dose groups in F0 females (absolute: 45-49%, relative: 42-47%). Terminal body weight was decreased in high dose F0 males (7%) and females (10%) but not to an extent that fully explains the observed effects on prostate and uterus. No effect on semen quality in F0. Organ weights and semen quality were not investigated in offspring.	

TNO, 2007a
 	
Klimisch 1
GLP
	Two-generation study (TG 416) in rats, n=28/sex
	
	Oral dosing in feed: Males: 85, 293, 925 mg/kg bw/d; females: 99, 330, 988 mg/kg bw/d.
	Exposure 10 weeks premating to PND 56 in F2.
	
	Endpoints in parental animals and offspring were assessed according to TG 416.
		Body weights
		During premating, a decrease in body weight was seen in F0 mid (week 10-11, 5%) and high (week 3-11, 5-11%) dose males. No effect was seen on body weight in F0 females during premating.
		
		During gestation, maternal body weights were decreased in high dose (day 7-21, 5%) F0 females.
		
		During lactation, body weights of F0 females were not significantly affected.
		
		Birth weights of F0-generation pups were similar between groups.
		
		During lactation, F0-generation pup body weight gain was
			Name:
			1-chloropropan-2-yl bis(2-chloropropyl) phosphate;
			bis(1-chloropropan-2-yl) 2-chloropropyl phosphate;
			tris(1-chloropropan-2-yl) phosphate;
			tris(2-chloropropyl) phosphate
			
			EC 807-935-0;
			CAS 1244733-77-4

		decreased from day 14 in mid (13-14%) dose and from day 7 in high dose (10-26%), resulting in a decrease in pup body weight at day 21 in mid (8%) and at day 14 and 21 in high (8-15%) dose. Throughout premating, body weights of F1 males were decreased in mid dose (6-8%, n.s. in week 1 and 5) and in high dose (11-16%). Body weight of F1 females were also decreased during premating in mid dose in week 2 to 11 (5-7%, n.s. in week 4) and in high dose in week 0 (13%) and week 1-11 (6-8 %). During gestation, maternal body weights were decreased in mid (7-9%) and high dose (8-11%) F1 females. Birth weights of F1-generation pups were similar between groups. During lactation, maternal body weights were decreased in mid (5-8%, n.s. at day 4) and high (6-10%) dose F1 females. During lactation, F1-generation pup weight was decreased at day 21 in mid dose and at day 7, 14 and 21 in high dose. In the F2-generation (F1-generation pups after PND21/23), decreased mean body weights were observed at day 28, 35, 42 in high dose males and females. *Delivery and Litters:* There were no effects on fertility index. The number of pups delivered was decreased in F0-generation pups in high dose (8%) and in F1-generation pups in mid (14%) and high (18%) dose. The number of pups lost on day 1-4 was increased in F0-generation pups in high dose and in F1-generation pups in low and high dose. The number of runts was increased in the F0-generation pups from day 1-7 in low dose and day 1-21 in mid and high	

		dose. In the F1-generation pups, the number of runts was also increased, but only from day 14: At day 14 in high dose and at day 21 in all dose groups. *EAS-relevant effects* No observed effect on AGD in F1-generation pups (F2) (not investigated in F0-generation pups (F1)). Delayed vaginal opening in high dose F2 animals (7 days delay) and mid dose F2 animals (3 days delay). On the days around the time of vaginal opening, the decrease in body weight in high dose F2 females compared to controls was about 10% (11% on day 35 and 9% on day 42). In mid dose, the difference in body weight was 0.5-1% compared to controls. In both mid and high dose F2 females, only 80-83% of the females reached criteria for vaginal opening, whereas this number was 92% in control females. Delayed preputial separation in high dose F2 animals (at 47 vs 44 days) but it may be explained by app. 11% lower body weight in the high dose animals at day 42 compared to controls. *Estrous cyclicity and uterus weight:* Estrous cycle length was increased in all dose groups in F0 (4.4 days in control, 4.8, 5.1 and 5.6 days in low, mid and high dose) and in high dose F1 females (4.7, 4.9, 5.0 and 5.8 days in control, low, mid and high dose). An increased number of acyclic females was observed in high dose F0 females (1/28, 0/28, 0/28 and 6/28 in control, low, mid and high dose) and a decreased number of cycles per animal was seen in high dose F0 (3.9, 3.7, 3.6, 3.0 in control, low, mid and high dose) and F1 females (3.8, 3.6, 3.7, 3.1 in control, low, mid and high dose). Estrous cyclicity is usually not affected by body weight. Further, body weight was not affected in any of the dose groups in F0 females during premating.	

		Absolute and relative uterus weights were markedly decreased at termination in all dose groups in F0 females (absolute: 18-32%, relative 18-31%) and in high dose F1 females (absolute: 35%, relative: 31%). The terminal body weight was not affected in F0 females in any dose groups. In F1 females, terminal body weight was decreased in mid (5%) and high (7%) dose. *Ovary weight:* Absolute ovary weight was decreased in high dose F0 females (11%) and in high dose F1 females (10%, n.s.). Relative ovary weight was also decreased in F0 females (8%, n.s.) and F1 females (3%, n.s.). The terminal body weight was not affected in F0 females and decreased in high dose F1 (7%) females. *Pituitary weight:* In F0 females, absolute and relative pituitary weight was decreased in high dose (absolute: 6%, relative: 8%). Terminal body weight was not affected. In F1 females, absolute pituitary weight was decreased in all dose groups (low (12%), mid (6%) and high (18%) dose) and relative pituitary weights were decreased in low (12%), mid (5%, n.s.) and high (9%) dose. Terminal body weight was decreased in mid (5%) and high (7%) dose but not in low dose. In F0 males, pituitary weight was not affected. In F1 males, absolute pituitary weight was decreased in high dose (13%). Terminal body weight was also decreased in high dose (16%) and relative pituitary weight was not affected. *Male reproductive organs:* No clear substance related effects were observed on weight of testes, epididymis, prostate or seminal vesicles. *Semen quality:* No effect on F0 or F1 semen quality. *T-relevant effects:*	

		In F0 and F1 males, the absolute thyroid weight was not different from controls. The relative thyroid weight was increased in high dose F0 (15%) and F1 (26%) males and the terminal body weight was decreased in high dose F0 (10%) and F1 (16%) males. In F0 and F1 females, absolute thyroid weight was not different from controls. The relative thyroid weight was increased in high dose F0 (10%, n.s.) and F1 (9%, n.s.) females and the terminal body weight was decreased in high dose F0 (3%, n.s.) females and in high dose F1 (7%) females. *There was no investigation of thyroid histology*	
NTP, 2023 (Study conducted in 2009) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	3-months with perinatal study in rats F0 females: N=20 (0, 2500, 10,000, 40,000 ppm) or N=8 (5000, 20,000 ppm) F1 rats: N=10/sex Exposure: F0 females: GD6-LD21, F1: Perinatal + 3 months Doses: Females: 0, 2500, 5000, 10,000, 20,000 ppm in feed (and 40,000 ppm in F0 females only, euthanized during gestation due to excess toxicity), corresponding to app.: 0, 236, 458, 906, 1890 mg/kg bw/day. Males: 0, 2500, 5000, 10000 ppm in feed (and 20,000 ppm, but this group did not survive to study termination), corresponding to app.: 0, 223, 431, 911 mg/kg/day. (Body and organ weights of F1 males	The only EATS relevant organs weighed were testes and epididymis (not e.g. thyroid, uterus, ovaries, pituitary) Due to poor quality of samples, an assessment of estrous cyclicity could not be made for female rats or mice *Delivery and litters:* In the high dose group (20,000 ppm), maternal body weight gain was decreased GD6-9 (87%) and as a result maternal body weight was decreased at GD9 (5%). However, maternal body weight gain was increased during most of the rest of the gestational period, though only significantly in GD12-15 (50%) and high dose maternal body weight was not different from control at the end of gestation (GD21). There were no effects on live litter size at birth or on pup mortality during postnatal life. *Body weight:* The terminal body weight in F1 males was decreased in low dose (6%), mid (6%, n.s.) and high dose (3%, n.s.). *Epididymis weight:* In F1 males, absolute epididymis weight was decreased in low (11%), mid (10%) and high (7%) dose. Also cauda epididymis weight was decreased in low dose	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 obtained from Albemarle) Four major isomeric components were identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 64.77 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 26.98 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.99 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.2 %) Impurities: 4.2 %

	was reported in low, mid and high dose, corresponding to 2500 ppm, 5000 ppm and 10000 ppm, whereas the 20,000 ppm group seemed to have been taken out). Age at necropsy: F1: 17 weeks	(15%), mid (8%, n.s.) and high dose (3%, n.s.). *Testes and semen quality:* There was no significant effect on the weight of testes in F1. No effects were observed on testicular sperm count or percent motile sperm in F1 males dosed up to 10,000 ppm. *Histopathology:* No differences between control and 20,000 ppm groups observed in histopathology of thyroid, genital system or brain in F1 animals.	
NTP, 2023 (conducted 2009) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	3-months study in mice N=10/sex Exposure: 3 months Dosing: 0, 1250, 2500, 5000, 10,000, and 20,000 ppm in feed Corresponding to app.: Males: 0, 225, 473, 1050, 2509, and 4446 mg/kg/day. Females: 0, 204, 442, 924, 1841, and 3645 mg/kg/day Age at necropsy: 19-20 weeks	The only EATS relevant organs weighed were testes and epididymis (not e.g. thyroid, uterus, ovaries, pituitary). Due to poor quality of samples, an assessment of estrous cyclicity could not be made for female rats or mice. *Body weight:* Terminal body weight was decreased in males in the 2500 (11%), 5000 (16%), 10,000 (24%) and 20,000 (29%) ppm groups and in females in the 1250 (12%), 2500 (7%), 5000 (3%), 10,000 (9%), 20,000 (15%) ppm groups. *EAS relevant investigations:* Testes (absolute and relative weight of right testis determined in all dose groups, absolute weight of left testis only in control and high dose): Decreased absolute right testis weight (8%) and left testis weight (9%) in high dose (20000 ppm). Increased relative right testis weight in 2500 (12%), 5000 (17%), 10,000 (28%) and 20,000 (29%) ppm groups. The observed decreased absolute testis weights could be a consequence of the decreased body weights. The increased relative testis weights could be due to the fact that the body weights are more markedly decreased than the testis weights. Epididymis (absolute weight of left epididymis and left cauda	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 obtained from Albemarle) Four major isomeric components were identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 64.77 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 26.98 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.99 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.2 %) Impurities: 4.2 %

		epididymis only examined in control and high dose): Decreased absolute epididymis (15%) and cauda epididymis weight (14%) in high dose (20,000 ppm). No differences between groups observed in histopathology of thyroid, genital system or brain in F1.	
NTP, 2023 (conducted 2011-13) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	2-years with perinatal study in rats Exposure: F0 females: GD6-LD28, F1: Perinatal + 2 years F0 female rats: N=38 F1 rats (2-year study): N=50/sex F1 rats (internal dosimetry): N=10/sex Dosing: 0, 2500, 5000, 10,000, or 20,000 ppm in feed, corresponding to app.: Males: 0, 141, 294, 626, and 1,155 mg/kg bw/day. Females: 0, 156, 323, 674, and 1,295 mg/kg bw/day. Age at necropsy: 109-110 weeks (F1)	Organ weights not reported and no investigation of estrous cyclicity. There were no effects on live litter size at birth or on pup mortality during postnatal life. Differences in incidences of non-neoplastic lesions in ED-relevant organs in exposed animals compared to controls: Females: Uterus, cysts (0/50, 0/50, 0/50, 0/49, 2/50 - statistically significant trend); Uterus, cervix inflammation (0/50, 0/50, 0/50, 0/49, 3/50 – statistically significant trend). Males: Prostate, epithelium hyperplasia (0/50, 0/50, 5/50, 0/50, 3/50 – significantly different in mid dose compared to control). Testis, polyarteritis nodosa (13/50, 13/50, 9/50, 9/50, 3/50 – statistically significant trend and difference between high dose and control). Testis, germinal epithelium degeneration (21/50, 15/50, 14/50, 13/50, 8/50 - statistically significant trend and difference between three highest dose groups and control). Differences in incidences of primary tumours in ED-relevant organs in exposed animals compared to controls: Females: Uterus, adenoma or adenocarcinoma (combined) (3/50, 4/50, 6/50, 8/49, 9/50 – statistically significant trend and between high dose and control). Uterus, Polyp Stroma (5/50, 15/50, 6/50, 7/49, 13/50 – statistically significant difference	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 mixed with lot 134 obtained from Albemarle to form lot M072911NP) Four major isomeric components were identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 68.06 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 25.43 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.55 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.21 %) Impurities: 2.55 % Due to ageing, control animals had high incidences of non-neoplastic lesions in numerous organs, including endocrine system, thyroid, genital system, mammary gland - challenging the detection of non-neoplastic effects induced by TCPP exposure.

		between low dose and control as well as high dose and control). Ovary, Granulosa Cell Tumour Benign (0/50, 0/50, 0/50, 0/49, 2/50 – statistically significant trend). *No biologically relevant significant differences in incidences of neoplastic or non-neoplastic lesions in other EAST-relevant organs, including epididymis, seminal vesicles, pituitary gland, preputial gland, penis, thyroid, mammary gland, oviduct, vagina.* Neoplastic effects observed in other organs are described in section 7.9.6.	
NTP, 2023 (conducted in 2011-13) Klimisch: 2 No mentioning of OECD test guideline Conducted in compliance with NTP laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations	2-years study in mice Exposure: 2 years Mice (2-year study): 50/sex Mice (internal dosimetry): 20/sex Dosing: Males: 0, 1250, 2500, 5000 ppm in feed, corresponding to app. 0, 160, 330, 711 mg/kg bw/day. Females: 0, 2500, 5000, 10,000 ppm in feed, corresponding to app. 0, 329, 673, 1,491 mg/kg bw/day Age at necropsy: F109-111 weeks	Organ weights not reported. There was no investigation of estrous cyclicity. Differences in incidences of non-neoplastic lesions in ED-relevant organs in exposed animals compared to controls: Females: Pituitary gland, pars distalis hyperplasia (17/50, 5/50, 4/50, 4/49 - statistically significant trend and all dose groups are different from control) uterus, dilation (13/50, 18/50, 10/50, 8/50 – statistically significant trend). Differences in incidences of primary tumours in ED-relevant organs in exposed animals compared to controls: Females: Pituitary, pars distalis or unspecific site adenoma (3/50, 2/50, 1/50, 0/49 – non-statistically significant trend). No biologically relevant significant differences in incidences of neoplastic or non-neoplastic lesions in other EAST-relevant organs, including testes, epididymis, seminal vesicles, prostate, preputial gland, thyroid, ovaries, vagina). Neoplastic effects observed in other organs are described in section 7.9.6.	Isomeric mixture of tris(chloropropyl) phosphate (TCPP; lot 101 mixed with lot 134 obtained from Albemarle to form lot M072911NP) Four major isomeric components were identified as tris(1-chloro-2-propyl) phosphate (CAS 13674-84-5; 68.06 %) bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (CAS 76025-08-6; 25.43 %) bis(2-chloropropyl) 2-chloroisopropyl phosphate (CAS 76649-15-5; 3.55 %) tris(2-chloropropyl) phosphate (CAS 6145-73-9; 0.21 %) Impurities: 2.55 % Due to ageing, control animals had high incidences of non-neoplastic lesions in some organs, including adrenals, uterus, pituitary, kidney, liver, spleen, thymus - challenging the detection of non-neoplastic effects

			induced by TCPP exposure.

Discussion - Endocrine disruption – *in vivo* effects in rodents

The observed effects on estrous cyclicity, pituitary-, uterine- and ovary weight and the delayed vaginal opening show a pattern of effects on the female reproductive system.

Estrous cyclicity

A clear substance-related and dose-dependent effect on estrous cyclicity was observed in the OECD TG 416 study from 2007. Estrous cycle length was dose-dependently increased in all dose groups in F0 (4.4 days in control vs. 4.8, 5.1 and 5.6 days in low, mid and high dose, respectively) and was also significantly increased in high dose F1 females (4.7 in controls vs. 5.8 days high dose females). This corresponded with an increased number of acyclic females in high dose F0 females (1/28, 0/28, 0/28 and 6/28 in control, low, mid and high dose, respectively) and a statistically significant decreased number of cycles per animal in high dose F0 (3.9, 3.7, 3.6, 3.0 in control, low, mid and high dose) and F1 females (3.8, 3.6, 3.7, 3.1 in control, low, mid and high dose). Estrous cyclicity was not investigated in any of the other available *in vivo* studies.

Uterus

A marked substance-related effect on uterus weight was observed in the OECD TG 416 study from 2007 and it´s range-finding study. In F0 females from the two-generation study, absolute and relative uterus weights were markedly decreased at study termination in all dose groups (absolute: 18-32%; relative: 18-31%). A statistically significant decrease was also seen in high dose females from the F1 generation (absolute: 35%; relative: 31%). The terminal body weight was not affected in F0 females in any dose groups. In F1 females, terminal body weight was decreased by 7% in the high dose females. Hence, body weight decreases could not explain the marked effects on uterus weight.
In the one-generation study, even larger decreases in uterus weights were seen. In the F0 females, decreases in uterine weights were seen in all dose groups (absolute: 45-49%, relative: 42-47%). In this study, only high dose females showed a decrease in terminal body weight (10%) and therefore, systemic toxicity cannot explain the observed effects on the uterine weights.
Uterus weight was not investigated in any of the other available *in vivo* studies.

The decreased uterus weights might be linked to the observed effect on estrous cyclicity since the weight of uterus changes with stage of estrous but the very marked effects could also reflect a direct substance-related adverse effect on uterus.

In the NTP 2-years carcinogenicity studies in rats and mice, an increased incidence of non-neoplastic lesions and tumors in uterus was observed. The effects were not marked, but often reached statistical significance in trend tests. In the carcinogenicity studies in rats, the non-neoplastic lesions (cysts and cervix inflammation) showed a dose-related increase and were statistically significant as a trend. In the NTP 2-years carcinogenicity study in mice, a decreased incidence of dilation of uterus was observed. This effect also seemed to be dose related and was statistically significant as a trend. In the rat study, the increased tumor incidences in the uterus (adenoma or adenocarcinoma (combined) as well as polyp stroma) also seemed to be dose-related and were statistically significant as a trend. The combined incidence of uterus adenoma and adenocarcinoma was also statistically significant in the high dose group, whereas for the polyp stroma, the low dose was significantly different from controls. Higher incidences of polyp stroma was also observed in the higher dose groups without reaching statistical significance.

Histology of uterus was also investigated in some of the other evaluated *in vivo* studies but here, no clear substance-related findings were seen. It is possible that chronic exposure is needed before adverse histopathological effects on the uterus are seen, whereas changes in uterine weight seems to appear after much shorter exposure duration.

Pituitary

Clear substance-related effects on pituitary weight (6-18% decrease) was observed in the OECD TG 416 study from 2007. The effect was seen in all dose groups in F1 females and in high dose F0 females. No effect on the pituitary was observed in the males. Pituitary weight was not investigated in any of the other available *in vivo* studies.

In the NTP 2-year carcinogenicity study in mice, a decrease in pars distalis hyperplasia was observed in females. The effect was significant in all dose groups. In the same study, a non-significant decreasing trend in pituitary pars distalis or unspecific site adenoma was also observed in the females.

Histology of pituitary was investigated in some of the other evaluated *in vivo* studies, with no clear substance-related findings. As with the effects on the uterus, it is possible that chronic exposure is needed before adverse histopathological effects are seen, whereas changes in pituitary weight may appear after much shorter exposure durations.

Ovaries

Decreased ovary weight was observed in high dose F0 females in the OECD TG 416 study from 2007. A non-significant decrease was also observed in F1 females. Ovary weight was not investigated in any of the other available *in vivo* studies. In the 90-day study from 1981, luteal cysts were observed in 3/20 high dose females compared to 0/20 in the control group.

In the NTP 2-year carcinogenicity study in rats, a statistically significant increasing trend was seen in benign granulosa cell tumors in the ovaries.

Histology of the ovaries was investigated in a number of the other evaluated *in vivo* studies with no clear substance-related findings. None of the studies performed a quantitative evaluation of primordial and small growing follicles.

Vaginal opening

In the 2-generation study from 2007, vaginal opening was delayed by 7 days in high dose compared to controls. In the mid dose, the delay was 3 days compared to controls. In some cases, delays in sexual maturation can be fully explained by decreased body weights but this is not evaluated to be the case here. On the days around the time of vaginal opening, the decrease in body weight in high dose females compared to controls was only about 10% (11% on day 35 and 9% on day 42). This, in view of the eMSCA, does not explain the long delay of 7 days in mean age to reach vaginal opening criteria. In mid dose, the difference in body weight was only 0.5-1%, which certainly is not expected to affect the time for sexual maturation. Furthermore, only 80-83% of the females reached criteria in the mid and high dose groups, whereas this number was 92% in control females.

Thyroid:

In the 90-day study from 1981, a significant increase in animals with mild thyroid follicular cell hypertrophy was observed. The effect was significant in males from all dose groups and in high dose females. In the two-generation study (TNO 2007a), an increase in relative thyroid weight was observed in high dose F0 and F1 males. There was no investigation of thyroid histology or measurement of thyroid hormone levels in this study. No effects on thyroid histology were observed in the 90-day or 2-year NTP studies in rats and mice (NTP, 2023).

Other observations

Some effects, which could be induced through disruption of the endocrine system, were also observed in males, e.g.:

- Decreased prostate weight in low and high dose F0 males in the one-generation dose range finding study from 2007;

- Decreased epididymis weight in all dose groups in F1 males in the NTP 3-months perinatal study in rats;

- Decreased epididymis weight in the high dose group in the NTP 3-months study in mice;

- Increased incidences of epithelium hyperplasia in prostate in the NTP 2-years study in rats (significantly different in mid dose compared to control);

- Decreased incidences of polyarteritis nodosa and germinal epithelium degeneration in testes in the NTP 2-years study in rats (significant as a trend and as differences between dosed groups and control).

The effects were not fully consistent between generations and/or studies but supports the evidence that TCPP is an endocrine disruptor.

Summary

The observed effects on estrous cyclicity, pituitary-, uterine- and ovary weight and the delayed vaginal opening (VO) show a pattern of effects on the female reproductive system, which are evaluated to be induced through disruption of the endocrine system.

According to the ECHA-EFSA guidance for the identification of endocrine disruptors, changes in age at VO, estrous cyclicity and uterine weights are defined as EAS-mediated. This means that they are considered indicative of an EAS MoA and thus, in the absence of other severe systemic toxicity, also imply underlying *in vivo* mechanistic information (ECHA/EFSA, 2018). In this case, there is no other possible explanation than endocrine disruption leading to the observed changes in age at VO, estrous cyclicity and uterus weight in the females in the 2-generation study. The lowest observed adverse effect level for the effects on estrous cyclicity and uterus weight was the lowest tested dose level (85-99 mg/kg bw/d) at which level the female body weights were not affected and no other signs of e.g. starvation or other severe effects that in theory could affect the endocrine-related endpoints were observed.

In the performed two-generation study (TNO, 2007a), a decrease in the mean number of pups delivered was observed in the mid dose group of F1 and the high dose groups of both generations. This decrease could be a sign of decreased fertility, which may be related to the endocrine disruptive effect of TCPP on estrous cyclicity. It could also be a result of developmental toxicity independent of the endocrine disrupting effects of TCPP exposure (as discussed in section 7.9.7).

Changes in weight and/or histopathology in EATS-relevant organs were observed in some studies but not in others (e.g. testes, ovaries and thyroid). One possible explanation for this discrepancy between some of the study results may be the composition of the test material used. In the test material used in the 90-day study from 1981, there was approximately 23% tris(2-chloropropyl) phosphate (CAS 6145-73-9 according to ChemIDPlus) whereas this isomer only constitutes <1 % of the composition of the test substance in newer studies.

If the isomer 2-chloropropanol-phosphate was responsible for the increased incidence of thyroid follicular hyperplasia in male animals observed in the 90-days study, it leaves a

concern for thyroid disruption induced by 2-chloropropanol-phosphate but not for TCPP as the commercial mixture, which today contains only up to 1% of this isomer.

7.10.3. Conclusion on endocrine disrupting properties for human health

Based on the available evidence, TCPP can be identified as an endocrine disruptor on the sex hormonal system (EAS).

In vitro, there is no clear effects on ER or AR modalities, weak evidence for disruption of steroid synthesis and some indication of effects sensitive to, but not diagnostic of, ED modalities like CAR, PXR, PR and PPARα.

In vivo, exposure to the substance leads to increased estrous cycle length, decreased uterus and pituitary weight, decreased ovary weight and delayed vaginal opening.

According to the ECHA-EFSA guidance for the identification of endocrine disruptors (ECHA/EFSA, 2008), changes in age at vaginal opening as well as changes in estrous cyclicity and uterine weight are considered diagnostic of an EAS-mediated MoA and thus, in the absence of other severe systemic toxicity, also imply underlying *in vivo* mechanistic information (ECHA/EFSA 2018). These effects can therefore be used for the identification of TCPP as an endocrine disruptor without a need for further mechanistic information.

In conclusion, TCPP is an endocrine disruptor relevant for human health.

The available information is considered sufficient for the classification and labelling of TCPP. However, no classification proposal for endocrine disruption is made in this conclusion document in view of the complexity of the information and the recent introduction of the new CLP criteria for ED.

7.11. PBT and VPVB assessment

Not evaluated by the eMSCA in this substance evaluation.

7.12. Exposure assessment

Not evaluated by the eMSCA in this substance evaluation.

7.13. Risk characterisation

Not evaluated by the eMSCA in this substance evaluation.

7.14. References

Auerbach, S.; Xu, M.; Merrick, B.; Hoenerhoff, M.; Phadke, D.; Taxman, D.; Shah, R.; Hong, H.; Ton, T.; Kovi, R.; Sills, R. & Pandiri, A. (2018): "Exome Sequencing of Fresh-frozen or Formalin-fixed Paraffin-embedded B6C3F1/N Mouse Hepatocellular Carcinomas Arising Either Spontaneously or due to Chronic Chemical Exposure", *Toxicol Pathol*, vol. 2018, Aug;46(6), pp. 706-718. DOI: 10.1177/0192623318789398

Bayer 1991: Repeated Dose (28 Days) Toxicity (Oral); Unpublished study report

Boobis. A.; Cohen, S.; Dellarco, V.; McGregor, D.; Meek, M.; Vickers, C.; Willcocks, D. & Farland, W. (2006): "IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans", *Critical Reviews in Toxicology*, vol. 36:10, pp. 781-792.

Cao, L.; Ren, X.; Li, C. & Guo, L. (2018): "Organophosphate Esters Bind to and Inhibit Estrogen-Related Receptor γ in Cells", *Environmental Science & Technology Letters*, 2018, vol. 5 (2), pp. 68-73. DOI: https://doi.org/10.1021/acs.estlett.7b00558

Collins, B.; Slade, D.; Ryan, K.; Mathias, R.; Shan, A.; Algaier, J.; Aillon, K. & Waidyanatha, S. (2018): "Development and Validation of an Analytical Method to Quantitate Tris(chloroisopropyl)phosphate in Rat and Mouse Plasma using Gas Chromatography with Flame Photometric Detection", *Journal of Analytical Toxicology*, vol. 43(1), pp. 36-44. DOI: https://doi.org/10.1093/jat/bky048

Commission Delegated Regulation (EU) 2023/707): Commission Delegated Regulation (EU) 2023/707 of 19 December 2022 amending Regulation (EC) No 1272/2008 as regards hazard classes and criteria for the classification, labelling and packaging of substances and mixtures. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023R0707&from=EN

CompTox Chemical Dashboard (2022): https://comptox.epa.gov/dashboard/

Dishaw, L.; Powers, C.; Ride, I.; Roberts, S.; Seidler, F.; Slotkin, T. & Stapleton, H. (2011): "Is the PentaBDE replacement, tris (1,3-dichloropropyl) phosphate (TDCPP), a developmental neurotoxicant? Studies in PC12 cells", *Toxicology and Applied Pharmacology*, vol. 256 (2011) 281–289. DOI: https://doi.org/10.1016/j.taap.2011.01.005

ECHA (2018): "Screening Report – An assessment of whether the use of TCEP, TCPP and TDCP in articles should be restricted". Link: https://echa.europa.eu/documents/10162/17233/screening_report_tcep_tcpp_td-cp_en.pdf/e0960aa7-f703-499c-24ff-fba627060698?t=1523014289559

ECHA/EFSA (2018): "Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009". EFSA Journal 2018, 16(6):5311. DOI: https://doi.org/10.2903/j.efsa.2018.5311

ECHA (2022a): Assessment of regulatory needs. Group name: Chlorinated trialkyl phosphates. Link: https://echa.europa.eu/documents/10162/adb9db14-bd68-8b50-5e13-e987c1c04996

ECHA (2022b): Summary Report of the 23rd ED Expert Group Meeting, Link: https://echa.europa.eu/documents/10162/1459379/summary_report_edeg23_en.pdf/b03ea820-5fee-03fe-c7d7-09e8aed422a8?t=1667454685499

ECHA (2023): Regulatory strategy for flame retardants. DOI: 10.2823/854233

EU RAR (2008a): European Union Risk Assessment Report, Tris(2-chloro-1-methylethyl) phosphate, (TCPP), https://echa.europa.eu/documents/10162/17228/trd_rar_ireland_tccp_en.pdf

EU RAR (2008b): European Union Risk Assessment Report, Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP), https://echa.europa.eu/documents/10162/13630/trd_rar_ireland_tdcp_en.pdf/d5c05135-ed67-4c65-a090-a45ec72d9469

EU RAR (2009): European Union Risk Assessment Report, Tris (2-chloroethyl) phosphate, TCEP, https://echa.europa.eu/documents/10162/2663989d-1795-44a1-8f50-153a81133258

Föllmann, W. & Wober, J. (2006): "Investigation of cytotoxic, genotoxic, mutagenic, and estrogenic effects of the flame retardants tris-(2-chloroethyl)-phosphate (TCEP) and tris-(2-chloropropyl)-phosphate (TCPP) in vitro", *Toxicology Letters*, vol. 161 (2006), pp. 124–134. DOI: https://doi.org/10.1016/j.toxlet.2005.08.008

Freudenthal, R. & Henrich, R. (1999): "A Subchronic Toxicity Study of Fyrol PCF in Sprague-Dawley Rats", *International Journal of Toxicology*, vol. 18 (1999), pp. 173-176. DOI: https://doi.org/10.1080/109158199225468

Freudenthal, R. & Henrich, R. (2000): "Chronic Toxicity and Carcinogenic Potential of Tris-(1,3-Dichloro-2-propyl) Phosphate in Sprague-Dawley Rat", *International Journal of Toxicology* (2000) 19:2, 119-125. DOI: https://doi.org/10.1080/109158100224926

Gwinn, W.; Auerbach, S.; Parham, F.; Stout, M.; Waidyanatha, S.; Mutlu, E.; Collins, B.; Paules, R.; Merrick, B.; Ferguson, S.; Ramaiahgari, S.; Bucher, J.; Sparrow, B.; Toy, H.; Gorospe, J.; Machesky, N.; Shah, R.; Balik-Meisner, M.; Mav, D.; Phadke, D.; Roberts, G. & DeVito, M. (2020): "Evaluation of 5-day In Vivo Rat Liver and Kidney With High-throughput Transcriptomics for Estimating Benchmark Doses of Apical Outcomes". *Toxicol Sci*, vol. Aug 1;176(2) (2020), pp. 343-354. DOI: 10.1093/toxsci/kfaa081.

Hoenerhoff, M.; Pandiri, A.; Lahousse, S.; Hong, H.; Ton, T.; Masinde, T.; Auerbach, S.; Gerrish, K.; Bushel, P.; Shockley, K.; Peddada, S. & Sills, R. (2011): "Global gene profiling of spontaneous hepatocellular carcinoma in B6C3F1 mice: similarities in the molecular landscape with human liver cancer", *Toxicol Pathol*, vol. 2011 Jun;39(4), pp. 678-699. DOI: 10.1177/0192623311407213

IARC (1994): "Peroxisome Proliferation and its role in Carcinogenesis". IARC Technical Report No. 24.

Kawasaki *et al*. (1982), as summarised in ECHA public dossier: https://echa.europa.eu/da/registration-dossier/-/registered-dossier/1355/4/9

Klimisch, H.; Andreae, M. & Tillmann, U. (1997): "A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data", *Regul Toxicol Pharmacol*, vol. Feb;25(1) (1997), pp. 1-5. DOI: https://doi.org/10.1006/rtph.1996.1076

Klose, J.; Pahl, M.; Bartmann, K.; Bendt, F.; Blum, J.; Dolde, X.; Förster, N.; Holzer, A.; Hübenthal, U.; Kessel, H.; Koch, K.; Masjosthusmann, S.; Schneider, S.; Stürzl, L.; Woeste, S.; Rossi, A.; Covaci, A.; Behl, M.; Leist, M.; Tigges, J. & Fritsche, E. (2022): "Neurodevelopmental toxicity assessment of flame retardants using a human DNT in vitro testing battery", *Cell Biol Toxicol*, vol. Oct;38(5), pp. 781–807. DOI: https://doi.org/10.1007/s10565-021-09603-2

Kojima, K.; Takeuchi, S.; Itoh, T.; Iida, M.; Kobeyashi, S. & Yoshida, T. (2013): "In vitro endocrine disruption potential of organophosphate flame retardants via human nuclear receptors", *Toxicology*, vol. 314(1), pp. 76-83. DOI: https://doi.org/10.1016/j.tox.2013.09.004

Krivoshiev, B.; Beemster, G.; Spranges, K.; Blust, R. & Husson, S. (2018): "A toxicogenomics approach to screen chlorinated flame retardants tris(2-chloroethyl) phosphate and tris(2-chloroisopropyl) phosphate for potential health effects", *J Appl Toxicol*, vol. 38 (2018), pp. 459–470. DOI: https://doi.org/10.1002/jat.3553

Liu, X.; Ji, K. & Choi, K. (2012): "Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish", *Aquatic Toxicology*, vol. 15;114–115, pp. 173-181. DOI: https://doi.org/10.1016/j.aquatox.2012.02.019

Meek, M.; Bucher, J.; Cohen, S.; Dellarco, V.; Hill, R.; Lehman-McKeeman, L.; Longfellow, D.; Pastoor, T.; Seed, J. & Patton, D. (2003): "A Framework for Human Relevance Analysis of Information on Carcinogenic Modes of Action", *Critical Reviews in Toxicology*, vol. 33:6, pp. 591-653, DOI: 10.1080/713608373

NTP (National Toxicology Program) (1991): "Toxicology and Carcinogenesis Studies of Tris(2-chloroethyl) phosphate in F344/N rats and B6C3F1 mice". NTP TR 391.

NTP (National Toxicology Program) (1998): "Toxicology and carcinogenesis studies of 1-chloro-2-propanol (technical grade) (CAS NO. 127-00-4) in F344/N rats and B6C3F1 mice", NTP TR 477, NIH Publication No. 98-3967, September 1998

NTP (National Toxicology Program) (2010): "NTP Historical Controls Report - All Routes and Vehicles, Rats", March 2010. https://ntp.niehs.nih.gov/sites/default/files/ntp/historical_controls/ntp2000_2010/2010-03-22-hist-ratsallroutes.pdf

NTP (National Toxicology Program) (2020): "NTP Developmental and Reproductive Toxicity, Technical Report on the Prenatal Development Studies of Tris(chloropropyl) Phosphate, (CASRN 13674-84-5) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats (Gavage Studies)", NTP DART 01, June 2020. DOI: https://doi.org/10.22427/NTP-DART-01

NTP (National Toxicology Program) (2021): "Historical controls". Research Triangle Park, NC: U.S. 17 Department of Health and Human Services, National Institute of Environmental Health Sciences, 18 National Toxicology Program; 2021. https://ntp.niehs.nih.gov/data/controls/index.html

NTP (National Toxicology Program) (2023): "NTP Technical Report on the Toxicology and Carcinogenesis Studies of an Isomeric Mixture of Tris(chloropropyl) Phosphate Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice", NTP TR 602, June 2023. Link: https://ntp.niehs.nih.gov/sites/default/files/2023-06/tr602_508.pdf, Raw data available on DOI: https://doi.org/10.22427/NTP-DATA-TR-602

OECD (2002) "Guidance notes for analysis and evaluation of chronic toxicity and carcinogenicity studies". ENV/JM/MONO(2002)19.

Reers, A.; Eng, M.; Williams, T.; Elliot, J.; Cox, M. & Beischlag, T. (2016): "The Flame-Retardant Tris(1,3-dichloro-2-propyl) Phosphate Represses Androgen Signaling in Human Prostate Cancer Cell Lines", *J Biocem Molecular Toxicology*, vol. 30(5) (2016), pp. 249-257. DOI: http://doi.org/10.1002/jbt.21786

Rosenmai, A.; Winge, S.; Möller, M.; Lundqvist, J.; Wedebye, E.; Georgiev, N.; Johansson, H. & Vinggaard, A. (2021): "Organophosphate ester flame retardants have antiandrogenic potential and affect other endocrine related endpoints in vitro and in silico", *Chemosphere*, vol. 263 (2021) 127703. DOI: https://doi.org/10.1016/j.chemosphere.2020.127703

Stauffer Chemical Co. (1981a): "Fyrol PCF 3-month dietary sub-chronic toxicity in rats". Unpublished study report. Cited in EU RAR 2008a. Results published as Freudenthal and Henrich (1999).

Stauffer Chemical Co. (1981b): "A two year oral toxicity/carcinogenicity study of Fyrol FR-2 in rats." Unpublished study report. Cited in EU RAR 2008a. Results published as Freudenthal and Henrich (2000).

Takada, K; Yasuhara, K; Nakaji, Y; Yoshimoto, H; Momma, J; Kurokawa, Y; Aida, Y; Tobe, M. (1989). "Carcinogenicity study of tris(2-chloroethyl) phosphate in ddY mice", *J Toxicol Pathol*, vol. 2(2), pp. 213-222. DOI:10.1293/tox.2.213

TNO (2007a): OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)". Unpublished study report

TNO (2007b): Preliminary study dose range-finding study used to define the doses of the subsequent 2 -generation study. Unpublished study report

Wang, X.; Luu, T.; Beal, M.; Barton-Maclaren, T.; Robaire, B. & Hales, B. (2022): "The Effects of Organophosphate Esters Used as Flame Retardants and Plasticizers on Granulosa, Leydig, and Spermatogonial Cells Analyzed Using High-Content Imaging", *Toxicological Sciences*, vol. 186(2) (2022), pp. 269–287. DOI: https://doi.org/10.1093/toxsci/kfac012

Zhang, Q.; Lu, M.; Dong, X.; Wang, C.; Zhang, C.; Liu, W. & Zhao, M. (2014): "Potential Estrogenic Effects of Phosphorus-Containing Flame Retardants", *Environ. Sci. Technol*, vol. 48 (2014), pp. 6995−7001. DOI: https://doi.org/10.1021/es5007862

Zhang, Q.; Ji, C.; Yin, X.; Yan, L.; Lu, M. & Zhao, M. (2016): "Thyroid hormone-disrupting activity and ecological risk assessment of phosphorus-containing flame retardants by in vitro, in vivo and in silico approaches", *Environmental Pollution*, vol. 210 (2016), pp. 27-33. DOI: https://doi.org/10.1016/j.envpol.2015.11.051

Unpublished study report (1978): In vitro transformed foci in BALB/3T3 cells. Cell transformation assay.

Unpublished study report (1980): In vitro transformed foci in BALB/3T3 cells. Cell transformation assay.

Unpublished study report (2018): OECD Guideline 414 (Prenatal Developmental Toxicity Study).

7.15. Abbreviations

AC	Article Category
AGD	Anogenital distance
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Androgen receptor
ARN	Assessment of Regulatory Need
BMD	Benchmark Dose
bw	Body weight
CAR	Constitutive androstane receptor
Carc	Carcinogenicity
CKO-K1	Cell line derived from ovary of Chinese hamster
CLP	Classification, Labelling and Packaging
CoRAP	Community Rolling Action Plan
COS-7	Cell line that express nuclear large T and all proteins necessary for replication of appropriate circular genomes

CYP	Cytokrom P450 enzyme
DMSO	Dimethylsulfoxide
DNT	Developmental neurotoxicity
E2	Estradiol
EAS	Estrogen, androgen, steroidogenic modality
EATS	Estrogen, androgen, thyroid, steroidogenic modality
ED	Endocrine disruption
eMSCA	Evaluating Member State Competent Authority
ER	Estrogen receptor
ERC	Environmental Release Category
ERR	Estrogen related receptor
EU RAR	European Union Risk Assessment Report
GD	Gestation day
GH3	Cells that produce growth hormone
GLP	Good Laboratory Practice
GMT	Group Management Team
GR	Glucocorticoid receptor
hAR	Human Androgen receptor
HeLa	Human cell line derived from cancer cells
HepG2	Human hepatic cell line with high proliferation rates
hER	Human estrogen receptor
hERR	Human estrogen-related receptor
hGR	Human glucocorticoid receptor
hPPAR	Human peroxisome proliferator-activated receptor
hPXR	Human pregnane X receptor
hRAR	Human Retinoic acid receptor
hTR	Human thyroid receptor
HTT	High-throughput transcriptomics
IC	Inhibitory concentration
I.p	Intraperitoneal
LD	Lactation day
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration

LUHMES	Lung human mesencephalic cells
MCF-7	Brest cancer cell line
MoA	Mode of action
MVLN	Bioluminescent cell line used to study estrogenic activity
NCC	Neural crest cells
NCP	Neural progenitor
NTP	National Toxicology Program
N.s.	Not significant
OECD	Organization for Economic Cooperation and Development
PC	Product Category
PC-12	Cell line derived from rat adrenal medulla
PN	Prenatal
PNDT	Prenatal developmental toxicity
PPAR	Peroxisome proliferator-activated receptor
Ppm	Parts per million
PR	Progesterone receptor
PROC	Process Category
PUR foam	Polyurethane foam
PXR	Pregnane X receptor
RDT	Repeated Dose Toxicity
REC	Relative effective concentration
Repro	Reproductive toxicity
RMOA	Regulatory management option analysis
SU	Sector of end use
SULT	Sulfotransferase
SVHC	Substance of Very High Concern
T	Testosterone
TBD	To be decided
TCEP	Tris(2-chloroethyl) phosphate
TCIPP	Tris(1-chloro-2-propyl) phosphate (TCIPP)
TCPP	Reaction products of phosphoryl trichloride and 2-methyloxirane (the Substance)
TDCP	Tris[2-chloro-1-(chloromethyl)ethyl] phosphate

TCDP	2,2-bis(chloromethyl)trimethylene bis(bis(2-chloroethyl)phosphate) (also known as TCDPP)
TG	Test Guideline
TR	Thyroid hormone receptor
TTR	Thyroid hormone transporter
US NTP	United States National Toxicology Program
UVCB	Unknown or Variable composition, Complex reaction products or of Biological materials
WST-1	Assay to measure cell proliferation, viability and cytotoxicity