Tris(methylphenyl)phosphate (TMPP), CAS no. 1330-78-5

Synonyms: Tricresyl phosphate (TCP)

TMPP is a mixture of the 3 isomers ortho-, metha-, and para cresols (Figure 1) and is used in the manufacturing of e.g. plastics, organophosphate flame retardants and solvents. TMPP is registered under REACH with a tonnage band of 1 000 - 10 000 tonnes per annum and is classified as Rep. 2 or self-classification as Repr. 1B; H360: May damage fertility or the unborn child . It is used in a variety of products such as furniture, electronics, textiles etc., and has been detected in the environment and in humans. The ortho isomer of TMPP, known as tri-*ortho*-cresyl phosphate (TOCP), is the most toxic of the 3 isomers and have been identified to cause neurotoxicity in humans and susceptible animals.

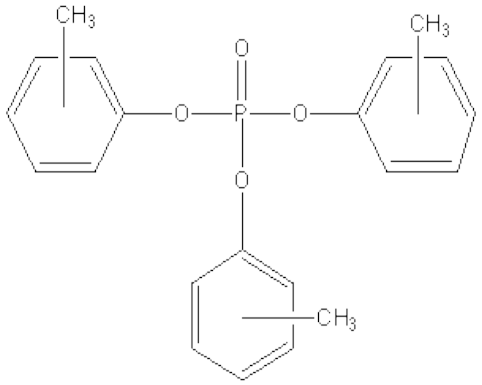

Figure 1. 2D structure from https://www.huidziekten.nl/allergie/stoffen/tricresyl-phosphate.htm

4. Human health hazard assessment

4.10.3 Endocrine disruption

4.10.3.1 General approach – human health

4.10.3.2 In vitro information indicative of endocrine activity

Reers et al. (2016)

Summary: In the present study, the effects of 6 organophosphate flame retardants **(**OPFRs), including TMPP (CAS no. 1330-78-5, purity not reported), on androgen receptor (AR), estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) agonism and antagonism *in vitro* were studied. AR-mediated gene expression was studied by exposing metastatic prostate cancer LNCaP cells to chemicals in doses between 0.01-20 µM and measuring mRNA and protein accumulation. Effects on ERα and AhR were studied in endometrial carcinoma cells (ECC-1 cells), at the same dose ranges. For both studies both the agonistic activity and the antagonistic activity (with positive controls: E2 for ER activity in ECC-1, TCDD (a dioxin) for AhR activity, and R1881 (synthetic androgen) for AR-activity in LNCaP cells). TMPP did not cause any effects on AR or ERα and AhR gene expression in the exposed LNCaP and ECC-1 cells, respectively, in both the agonistic and antagonistic mode.

Study quality and assessment: The material and methods section is not explicit with missing information on the measured mRNA and proteins in the two cell lines, purity of the tested chemicals, cytotoxicity information. The results from the first part of the study are also not explicitly presented. Overall, the quality of this study is assessed to be low. The evidence for lack of ERα, AR or AhR agonism or antagonism is weak due to the low quality of the study.

Schang et al. (2016)
Summary: In the present study, the effects of 7 OPFRs, including TMPP (CAS no. 1330-78-5, purity not reported), on MA-10 mouse Leydig tumor cells were compared to those of the brominated flame retardant BDE-47. First cell viability and cell count was assessed after exposing the MA-10 cells for 48h with 0, 1, 2, 5, 10, 20, 50 and 100 µM TMPP. Next, cells were treated with 0, 10.1, 1 and 10 µM TMPP for 48 h followed by measuring the superoxide production. Similarly the production of progesterone was assessed, both with cells only exposed to TMPP for 48 h, or TMPP and 2h post-treatment with dbcAMP (1 mM) or luteinizing hormone (LH) (100 ng/ml), and the progesterone production was normalized to cell number. Finally, the MA-10 cells were exposed to 10 µM TMPP for 48 h with or without 2 h post treatment with dbcAMP or LH, and the quantification of the following steroidogenesis related genes: *Star, Tspo, Hsd3b, Lhcgr, Cyp11a1, Adcy3*. Each test was run in 5-7 independent experiments. TMPP significantly decreased cell viability at concentrations of 20 µM or above and reduced the cell number at all tested concentrations except at 2 µM. Superoxide and progesterone production was increased in the 10 µM dose, but no significant effects were seen on the gene expression on steroidogenic genes.

Study quality and assessment: The study well-described and –designed and only information on the purity of TMPP is missing. Overall, the study is assessed to be of high quality. The study provides moderate evidence of a steroidogenic MoA due to increased progesterone production although the exact mechanisms underlying this effect were not identified.

Bradley et al. (2015)
Summary: The present study used chicken eggs to test TMPP (CAS no. 1330-78-5, ≥89.0% purity) for effects on chicken embryo neurodevelopment. On day 0 of incubation, eggs were injected into air cell with 50 µM TMPP in dimethyl sulfoxide (DMSO) in doses of 0 (n=15), 10, 100 or 1000 ng/g egg (n=20/dose group), or used as a non-injected control ((n=15). Incubation mortality, successful pipping and hatching were recorded. At day 7 through 9 post-hatching the chicks were tested in multiple behavioural tests such as open field, righting reflex, angled balance beam, gait analysis and wing flap reflex to assess their locomotor function, fear, desire for social reinstatement, motor reflexes, coordination, balance, and neuromuscular strength. At day 10 post-hatching, the chicks were euthanized and left cerebrum was used for cholinergic analyses, while cerebellum was used for histological evaluation of purkinje cell number.

The two control groups, non-incubated and DMSO incubated did not show any differences and were combined into a single control group. TMPP did not affect, survival o hatching, and no treatment effects were seen in the behavioural tests, except for a non-significant decreased righting reflex in the 10 ng TMPP/g egg. On histology, a non-significant increase in the number of cerebellar purkinje cells were observed at the highest 1000 ng/g egg group.

Study quality and assessment: Overall the study is well described and assessed to be of high quality. The study provides no evidence for ED MoAs and only very weak evidence for neurodevelopmental effects in chickens.

Crump et al. (2014)
Summary: The present study used chicken eggs to test TMPP (CAS no. 1330-78-5, 93.1% purity) for effects on chicken embryo development. In total 110 fertilised chicken embryos were used; 20 for dimethyl sulfoxide (DMSO) control, 10 for 7.8 ng/g, 20 for 780 ng/g, 8600 ng/g, 43,700 ng/g and 261,400 ng/g, respectively. Pipping success was calculated as the number of pippings by day 22 divided by the number of total fertile eggs per treatment group. Time to pip was also recorded. At day 22, embryos were euthanized and tarsus length, body mass and liver mass was assessed and liver somatic index (LSI = liver mass / body mass * 100) was calculated. Thyroid glands were harvested from the first 6-8 embryos and used for thyroxin (T4) measurement. The hepatic right lobe was removed and used for RNA extraction (n=8/treatment group) of CYP2H1, CYP3A37, deiodinase 1, 2 and 3, insulin-like growth factor 1 (IGF1), liver basic fatty acid-binding protein (LFABP), transthyretin (TTR) and uridine 5'-diphospho-glucuronosyltransferase 9 (UGT1A9). Whole blood was collected for free T4, bile acid and genetic sex determination. Pipping success in TMPP treatment groups were unaffected but an increase in deformities was observed in the embryos in the highest dose group, including reduced tarsus length and increased LSI with discoloured livers. TMPP up-regulated CYP2H1 and CYP3A37, UGT1A9 (not significant) and LFABP and downregulated TTR, as well as affected a number of other genes in the liver. T4 levels in plasma and thyroid glands were unaltered, but bile acid concentration in plasma was increased in the highest dose group.

Study quality and assessment: The study is well-described and assessed to be of high quality. The study provides only very weak evidence of ED MoAs due to effects on TTR gene levels as well as enzymes that could regulate hormone turnover.

Kojima et al. (2013)
Summary: In the present study, the effects of 11 OPFRs, including TMPP (here one of the TMPP isomers tricresyl phosphate (TCP) with CAS no. 78-30-8, >98% purity), on the agonistic and antagonistic activity on human nuclear receptors were studied. A transiently transfected CHO-K1 (Chinese hamster ovary cell line) cell-based transactivation assays were used for testing the agonistic and antagonistic activities of $1 \cdot 10^{-7}$-$3 \cdot 10^{-5}$ M TCP on estrogen receptor (ER)α, ERß, androgen receptor (AR), glucocorticoid receptor (GR), retinoic acid receptor (RAR)α, retinoic X receptor (RXR)α and thyroid hormone receptor (TR)α and ß. Likewise, the simian kidney COS-7 cell-based reporter gene assay was used to test TPC's activity against pregnane X receptor (PXR), peroxisome proliferator-activated receptor (PPAR) α and PPARγ. All assays were run in 3 independent experiments. TCP showed sight cytotoxicity in both the CHO-K1 and COS-7 cells at the highest concentration of $3 \cdot 10^{-5}$ M. In the CHO-K1 cells, TCP was a weak ERα agonist, an anti-androgen against 5α-dihydrotestosterone (DHT)-induced AR activity, and antagonized hydrocortisone (HC)-induced GR activity. No effects on ERß, RARα, RXRα, TRα and TRß activity were measured. I the COS-7 cells, TCP caused PXR agonism and slight PPARγ agonistic activity but no PPARα activity.

Study quality and assessment: The description of the material and method section could be more detailed and include more information on the agonism and antagonism modes of the assays. Also, the exact doses are not reported but can be read from the figures. Overall, the study quality is assessed to medium. The study provides moderate evidence for multiple ED MoAs of TCP, including oestrogenic and anti-androgenic activity, as well as GR antagonism and PXR agonism.

Liu et al. (2012)
Summary: The present study investigates the effects of 6 OPFRs, including TMPP (here TCP, CAS no. 1330-78-5, 90% purity), on steroidogenesis in H295R cells, estrogenicity or anti-estrogenicity in MVLN cells, and hormone levels and syntheses in zebrafish. Here only the experiments in the H295R and MVLN cells are presented. H295R cells were exposed to 0.0, 0.001, 0.01, 0.1,1, 10 and 100 mg/L TCP for 48 hrs. First cytotoxicity was assessed in the MTT bioassay, and the non-cytotoxic doses were further evaluated for effects on hormone production. Next, 0.0, 0.001, 0.01, 0.1, 1 and 10 mg/L TCP was tested for estrogen receptor (ER) binding as well as ER antagonism using 100 nM 17ß-estradiol (E2) in the MVLN luciferase assay. Here also only doses below cytotoxicity were evaluated. All experiments were run in 3 replicates.

TCP caused cytotoxicity at concentrations of ≥10 mg/L in both the H295R and MVLN cells. In the H295R cells, TCP increased E2 (≥0.01 mg/L) and testosterone (T, ≥0.1 mg/L) production, and resulted in an increased E2:T-ratio at 0.01 and 1 mg/L. TCP induced transcription of CYP11A1 (≥0.1 mg/L), CYP11B2, CYP19A1 and HSD3ß2 at 1 mg/L, and down-regulated gene transcription of SULT1E1 and SULT 2A1 at 1 mg/L. In the MVLN cells TCP did not bind to ER but significantly and in a concentration-dependent manner reduced the binding of E2 to ER.

Study quality and assessment: The study is well-described and thorough and assessed to be of high quality. It provides strong evidence of both a steroidogenic and anti-oestrogenic MoA of TCP in human cells lines.

4.10.3.3 In vivo effects with regard to an endocrine mode of action

Bolon et al. (1997)
Summary: This is an additional study using ovaries from mice tested previously in the NTP Reproductive Assessment by Continuous Breeding (RACB) bioassays for 15 different chemicals, including TMPP (here TCP, CAS no. and purity not reported). The testing and reproductive outcomes of TCP in the RACB assay has been described in Chapin et al (1988) and summarised in an NTP RACB report. In the present study, the ovaries from the exposed and control animals were sectioned and the number of follicles at 3 different stages were counted. Up to five experienced technicians performed the follicle counts, and the variation between pairs of technicians ranged from 0-30%. Then the results of the follicle counts were compared with the reproductive outcomes from the RACB study.

TCP has been shown to cause decreased fertility in both male and female mice (Chapin et *al.*1988), but no effects on the follicle counts at the tested TCP doses were seen in the present study.

Study quality and assessment: The study is well-described, although the CAS no. and purity of the chemical is not stated. Also it should be noted that the large interobserver variation of up to 30% might have affected the results. Overall, the study is assessed to be of medium quality. No ovarian toxicity of TMPP was observed in the present study and this effect can therefore not explain the reduction in female fertility in mice seen after TMPP exposure. No other potential MoAs underlying the reduced female fertility were explored and the present study therefore does not provide any evidence of an ED MoA.

Latendresse et al. (1995)
Summary: The study investigated the functional effects of TMPP (here TCP, CAS no. 1330-78-5, no purity reported) and butylated triphenyl phosphate (BTP) induced adrenal and ovarian tissue changes on hormone levels and oestrous cycle in intact, adult female rats. First, female rats were exposed to 0.0 or 0.4 g/kg TCP by a single oral dose for 20 (n=9), 40 (n=6) or 60 days (n=3). Vaginal cytology was taken daily to study the effects of TCP on oestrous cycle. Next, female rats were exposed to 0.0 or 0.4 g/kg TCP by a single oral dose for 20 days (n=12), and blood samples, ovaries and adrenal glands were used after killing the animals. Estradiol, androstenedione and corticosterone concentration in serum was analysed as was cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), alanine transaminase, albumin and total protein. The TCP exposed rats had normal oestrous cycle. Histopathological changes in the adrenal glands and ovaries were seen and it was hypothesized that these changes could alter steroid hormone production and metabolism. Estradiol levels were elevated but no other effects were seen on hormone levels

Study quality and assessment: Overall the study is thorough, however the purity of the tested compound is not clear and only a single dose of TMPP was tested. Based on this the quality of the study is assessed to be of medium quality. The study provides moderate evidence for effects on female estradiol production or metabolism but the exact MoA(s) behind the elevated estradiol levels is not elaborated. The evidence for female reproductive adverse effect are moderate due to the histopathological changes in the ovaries. It is hypothesized that these may result in altered steroid hormone production or metabolism.

Latendresse et al. (1994a)
Summary: The present study investigates the effect of TMPP (here TCP, CAS no. 1330-78-5, no purity reported) and butylated triphenyl phosphate (BTP) on rat testes, ovaries and adrenal glands. Sexually mature male and female rats were exposed to 0.0 or 0.4 g/kg TCP by a single oral dose for 20, 40 or 60 days (n=3/sex/dose group). After the exposure period, the rats were sacrificed and ovaries, testes, epididymides and adrenal glands were excised and weighed and then undergoing different histological examinations.

No macroscopic changes in the ovaries were seen but at the microscopic level the ratio of interstitial tissue to follicles and corpora lutea was increased and the ovarian interstitial cells from all TCP-exposed female rats were larger than the control group. The weight of testes from the 60 days TCP exposed rats was decreased, and the seminiferous tubules showed altered morphology in all TCP-exposed animals. The adrenal glands of both TCP-exposed female and male rats were enlarged and had morphological changes. Due to an editorial error in the manuscript in the form of a picture covering text, tables and figures the remainder of the results section cannot be evaluated here.

Study quality and assessment: Apart from the editorial error, the study is well-described. There is only 3 animals per group and only 1 dose of TMPP is tested, and the purity of TMPP is not reported. With these limitations the quality of the study is assessed to be medium. The study provides strong evidence of ovary and testicular toxicity as well as effects on the adrenal glands in rats, but it does not study the underlying MoAs and it is therefore not clear if the adverse effects are due to ED MoAs.

Latendresse et al. (1994b)
Summary: The present study investigates the effect of TMPP (here TCP, CAS no. 1330-78-5, no purity reported) and butylated triphenyl phosphate (BTP) on female and male rat reproductive performance using a modified NTP continuous breeding protocol. First, sexually mature male and female rats were paired and exposed to 0.0 (n=40 pairs) or 0.4 g/kg TCP (n=20 pairs) by a single oral dose for 7 days pre-breeding, 63 days during breeding and 28 days post breeding. Body weight, fertility, litters/pair, live pups/litter, gender and mean weights of pups were registered. Next, the rats were used in an 8 days cross-mating study with either: control males x control females, TCP males x control females or TCP females x control males (n=20/pairs/group). Vaginal plug and smears for sperm as well as oestrous stage was assessed daily. Then all F0 animals were sacrificed and ovaries, testes, epididymides, vagina and uterus, liver and adrenal glands were excised and weighed. The body weights of all exposed rats were significantly decreased as was the number of litters/pair and live pups/litter. In the cross-mating study only the exposed males showed a reduced fertility with no litters produced. No effects on reproductive performance of the exposed females were seen. Both sexes had increased adrenal gland and liver weights. The treated males had lower testicular and epididymal weights, while the ovaries in the treated females had increased weights.

Study quality and assessment: Overall the study is thorough, however the purity of the tested compound is not clear and only 1 dose of TMPP was tested. Based on this the quality of the study is assessed to be of medium quality. The study provides moderate evidence of male reproductive adverse effects of TMPP as well as testicular and ovarian toxicology but does not study the underlying MoAs and it is therefore not clear if the adverse effects are due to ED MoAs.

Chapin et al. (1988)
Summary: This study evaluates the effect of TMPP (here TCP, CAS no. 1330-78-5, purity 74.9%) on reproductive performance in mice and consists of 4 tasks: 1) a range finding 14-day study, 2) a 98-days continuous breeding study, 3) a cross-mating study with F0 animals from 2), and 4) an offspring assessment of F1 animals. In 1), adult mice were exposed to 0.0, 0.437, 0.875, 1.75, 3.5 or 7.0% TMPP via feed for 14 days (n= 8/sex/group), and based on signs of toxicity and mortality the doses for 2) were set. In 2), animals were put in to breeding pairs and exposed to 0.0 (40 breeding pairs), 0.05, 0.01 or 0.2% TMP (corresponding to approximately 62.5, 125, and 250 mg/kg/day, 20 pairs/dose group) via feed for 98 days. After the last litter was reared, the F0 animals were used in a cross-mating study, i.e. task 3, to determine the affected sex in the F0 animals. Three groups were formed: control males paired with control females, control males paired with 0.2% TMPP females, and control females paired with 0.2% TMPP males. Following this the F0 animals were necropsied. The last litters from the control, 0.5% and 0.1% TMPP groups in task 2) were used in task 4) to study the effects on the F1 animals. When the F1 animals were sexually mature they were paired with an opposite sex from the same dose group but another litter and kept until first delivery. In 1) Doses of 1.75% or above resulted in general toxicity and increased mortality. In 2), the highest dose of 0.2% TMPP resulted in hind limb weakness and reduced postpartum weights in the females, and increased number of dead pups, as well as reduced litters/pair, and pub BW. In 3) the fertility of both males and females was affected. The sperm motility and concentration were decreased, and the number of abnormal sperm was increased. Atrophy was seen in the seminiferous tubules. In 4) there was a trend toward lower mating and fertility indexes, and the number of live pups/litter was decreased in the 0.1% TMPP F1 pairs. Sperm motility was decreased and number of abnormal sperm increased in F1

males but the number of sperm s were unaffected. No histopathological changes in the reproductive organs were found.

Study quality and assessment: Overall the study is thorough, but it is not clear how many animals that were used in 3) and 4). Also the exact ages of the animals used in 2) and 3) is not clear from the text but can in most cases be read from the tables. Based on this the quality of the study is assessed to be of medium quality. The study provides moderate-strong evidence of male and female reproductive adverse effects of TMPP as well as effects on offspring reproductive performance but does not study the underlying MoAs and it is therefore not clear if the adverse effects are due to ED MoAs.

Carlton et al. (1987)

Summary: The present study evaluates the reproductive toxicity of TCP. Male Long Evans rats 4-6 weeks old were given 0, 100 or 200 mg/kg TCP (CAS no. and purity not reported) per day by oral gavage for 56 days prior to breeding and during the 10 days breeding period and were then killed and blood, the reproductive tract and sperm were examined (n=12/dose group). Female Long Evans rats age 4-6 weeks were treated with 0, 200, 400 mg/kg per day by oral gavage 14 days prior to breeding, during breeding, gestation and lactation until killed at postnatal day 21, where blood and the reproductive tract were examined (n=24/dose group). One male was mated with 2 females: 0 mg/kg males with 0 mg/kg females, 100 mg/kg males with 200 mg/kg females, and 200 mg/kg males with 400 mg/kg females. Blood and hormone analyses were made on the litters at PD 21. No clinical signs of toxicity were detected in the male and female F0 animals at all doses. The fertility rates of TCP exposed rats were remarkably reduced compared with the controls. The 200 mg/kg TCP exposed male rats showed adverse reproductive effects with reduced epididymis weights, necrosis and degeneration of seminiferous tubules, and reduced sperm concentration, motility and progressive movement, and increased abnormal morphology. Histopathological alterations were found in the ovaries of 400 mg/kg TCP exposed females.

Study quality and assessment: Overall the study is thorough and well-described, although no information on the CAS no. and purity of TCP is reported. The study is assessed to be of medium quality. The study provides strong evidence of male and female reproductive adverse effects of TCP but does not study the underlying MoAs and it is therefore not clear if the adverse effects are due to ED MoAs.

Somkuti et al. (1987a)

Summary: A 14 day dose-range study was conducted on tris-*o*-cresyl phosphate (TOCP, the most toxic isomer of TMPP, 99% purity, no CAS. no. reported) followed by a 63 day sub-chronic study of TOCP and tris-*p*-cresyl phosphate (TPCP, less toxic isomer of TMPP, 97% purity, no CAS. no. reported). In the 14 day study, adult male Fischer were exposed to 0, 100, 200, 400, 800 or 1600 mg/kg TOCP for 14 days (n=8/dose group), and 2 animals/group were prepared for histopathology, and the remaining 6 animals were used for other assays. Next, a 63 day dose-response study was performed by exposing rats to 0, 10, 25, 50, 75 or 100 mg/kg TOCP or 100 mg/kg TPCP daily (n=10/group). Five animals from each dose group were used for histopathology of testes and epididymis, and the remaining 5 animals were used for other assays: activity of nonspecific testes esterase (NSE) and neurotoxic esterase (NTE) was measured in testes, sperm motility, density and morphology were assessed, interstitial testis fluid testosterone levels was measured.

In the 14-day study dose-dependent increase in mortality and cholinergic toxicity were seen in animals exposed to ≥ 200 mg/kg TOCP, and a decrease in sperm density and testicular histopathology were observed in all animals treated with TOCP. In the 63-day study, animals treated with ≥50 mg/kg TOCP showed decrease in weight gain and testis:body weight ratio as well as dose-dependent decreases in sperm motility and density and increase in abnormal sperm morphology. Histopathology in testes and epididymis were seen at doses ≥ 25 mg/kg TOCP, and TOCP also resulted in decreased NTE (≥50 mg/kg) and NSE (≥10 mg/kg) activity. In the TPCP group, only lower sperm density was observed.

Study quality and assessment: Overall the study is thorough and well-described, however it is only the TOCP and TPCP isomers that are tested here. It is not always clear from the material and methods section, which analyses were performed in the 2 studies. Due to these shortcomings, the study is assessed to be of medium quality. The study provides strong evidence of testicular toxicity and male reproductive adverse effects of TOCP in rats but does not study the underlying MoAs and it is therefore not clear if the adverse effects are due to ED MoAs.

Somkuti et al. (1987b)

Summary: In the present study changes in rooster testis histopathology, biochemistry and sperm motility after tris-*o*-cresyl phosphate (TOCP, the most toxic isomer of TMPP, 99% purity, no CAS. no. reported) TOCP oral exposure was studied. Adult leghorn roosters were treated with corn oil (n=10), a single oral dose of 750 mg/kg TOCP and killed after 24 h or 18 days (n=3/group), or 100 mg/kg daily TOCP for 18 days (n=10). Brain, plasma, and left testis were removed quickly after the animals were dead and brain acetylcholinesterase (AChE), testis nonspecific esterase (NSE), testis neurotoxic esterase (NTE), and plasma butyrylcholinesterase (BuChE) were measured. Sperm motility and density were assessed. No clinical signs of toxicity was observed in the single dose 750 mg/kg 24h treatment group, but in the 750 mg/kg 18 days treatment group and the 100 mg/kg daily 18 days treatment group, signs of neurotoxicity was observed. Sperm motility and density were remarkably reduced in the 100 mg/kg daily treated group and in this group 50% of the 10 roosters has disorganized seminiferous epithelium. Inhibition of testis NSE activity was reduced in the 750 mg/kg 24 h treatment group, and the NTE activity was reduced in 750 mg/kg 24 h treatment group and the 100 mg/kg daily 18 days treatment group.

Study quality and assessment: Overall the study is thorough and well-described, however it is only the TOCP isomer that is tested here. The study is assessed to be of strong quality. The study provides moderate evidence of testicular toxicity and male reproductive adverse effects of TOCP in roosters but does not study the underlying MoAs and it is therefore not clear if the adverse effects are due to ED MoAs.

Somkuti et al. (1987c)

Summary: The aim of the present study was to characterize the time course of tris-*o*-cresyl phosphate (TOCP, the most toxic isomer of TMPP, 99% purity, no CAS. no. reported) induced testicular toxicity. Male Fisher 344 rats were exposed to 0 or 150 mg TOCP/kg for 3, 7, 10, 14 and 21 days daily. Body weights and clinical signs were noted during exposure. At the end of exposure, the animals were killed and trunk blood, brain and testes were sampled. A second group of 10 animals

were used in a recovery study and first treated for 21 days, 2 were killed, the rest were allowed 98 days of recovery before they were killed and examined.

No clinical signs of toxicity were observed in any animals, but a decrease in sperm number was seen after 10 days of exposure and sperm motility was reduced after 14 days exposure. The testis to body weight ratio was decreased in rats exposed for 21-days. The testis enzyme nonspecific esterase and neurotoxic esterase activities were reduced at all the time points. No effects on serum LH, FSH or testosterone levels were seen. In the recovery study, the sizes of testes in the TOCP treated groups were decreased and they were devoid of germinal cells indicating irreversible testicular toxicity of TOCP.

Study quality and assessment: Overall the study is thorough and well-described, however it is only the TOCP isomer that is tested here. The study is assessed to be of strong quality. The study provides strong evidence of irreversible testicular toxicity and male reproductive adverse effects of TOCP in rats but does not study the underlying MoAs and it is therefore not clear if the adverse effects are due to ED MoAs.

REACH dossier

Toxicity to reproduction*:*

All applicant-reported data are from published studies that have already been included here.

Developmental toxicity / teratogenicity:

001 Key | Experimental results

Summary: The applicant reported information originates from an unnamed study report from 2004 and is from a Prenatal developmental toxicity study (EPA OPPT 870.3700). Briefly, adult male and female rats were mated, and females evident of mating were selected for further studies. They were then dosed daily by oral gavage with TCP (CAS no. reported in the dossier, purity not stated) from gestation day 10 to 19 with 0, 20, 100, 400 or 750 mg/kg/day and sacrificed on GD 20. Clinical signs of toxicity, body weights, fertility and gravid uterus weight were some of the endpoints reported. The foetuses were weighted and examined for malformations macroscopically and by histology.

The pregnant dams showed salivation from 100 mg/kg, decreased body weight/ (gain) and lower gravid uterus weights from 400 mg/kg/day. The foetuses in all treatment groups had lower weight with a dose-related response. No malformations in relation to the doses were observed.

Study quality and assessment: The study was performed under GLP and followed the EPA OPPT 870.3700 guideline, but since this is only a summary of a study report an assessment of the quality is not possible. The study however does not provide any evidence for ED related MoAs or adverse effects.

4.10.3.4 Summary of the plausible link between adverse effects and endocrine mode of action

Some ED MoAs for TMPP have been reported in both *in vitro* and *in vitro* studies (Table 1), including effects on steroidogenesis *in vitro* with increased oestrogen, progesterone and testosterone levels and up-regulation of enzymes involved in their production as well as down-regulation of enzymes important for their turn-over. The *in vitro* effects on steroidogenesis and oestrogen production might explain the increased oestrogen level measured in exposed adult female rats (Table 2). PXR agonism, ERα agonism, anti-oestrogenicity and AR antagonism MoAs were found *in vitro*, however in a study from 2016 the ER and AR MoAs could not be confirmed. Overall, there is moderate evidence for ED MoA of TMPP, mainly with regards to effects on steroidogenesis.

A number of studies in rats, mice and roosters, found adverse effects (Table 2) on male fertility with histopathological changes in testes and epididymides and evidence of adversely affected sperm parameters and altered activity of testicular esterases. Reduced sperm motility and increased number of sperms with abnormal morphology were also observed in rat F1 males exposed *in utero* to TMPP in a continuous mating study. Thus, there is strong evidence for adverse reproductive toxicity effects in adult males. There is a biologically plausible link between these male reproductive effects and ED MoA(s) of TMPP, especially effects on steroidogenesis leading to increased oestrogen levels. In female rats, histopathological changes in ovaries have been found with no to weak effects on their fertility. A single study in mice report effects on female fertility after TMPP exposure, but in a retrospective study examining the ovaries from these mice no effects were found in follicle counts. For both sexes, histopathological effects on adrenal glands in rats after TMPP exposure were observed, and these changes might result in modulation of the endocrine system leading to e.g. altered fertility. Weak evidence for adverse effects on the developing foetus have been found with effects on tarsus length and liver mass and gene expression in exposed chicken embryos and inconsistent deformities in rat foetuses.

Overall, the evidence for ED MoA(s) is moderate and the evidence for adverse effects of TMPP is strong, and the link between the adverse effects on reproduction and gonads to ED MoAs is evaluated as moderate. clear

In conclusion, TMPP meet the WHO definition of an endocrine disruptor with adverse effects on male fertility linked to effects steroidogenesis leading to increased oestrogen levels.

Additional literature not included in the evaluation

NTP RACB summary (1997): This is a summary of the NTP Reproductive Assessment by Continuous Breeding testing of TMPP in mice that has been described in the paper by Chapin et al. (1988).

Health Canada (2016): This is a draft screening assessment report that among others assess reproductive toxicity of TMPP and it uses references already included here.

Table 1. Overview of *in vitro* and *in vivo* endocrine disrupting (ED) mode(s) of action (MoA(s)) of TMPP.

Reference	**MoA**		**Quality of study**	**Evidence for ED MoA**
	In vitro	*In vivo*		
Reers et al. (2016)	No ERα, AhR, or AR agonistic or antagonistic activity in two human cancer cells		Low	Weak
Shang et al. (2016)	Effects on basal progesterone production in mouse Leydig tumor cells		High	Moderate
Bradley et al. (2015)		Effects on chicken embryo neurodevelopment: no effects on chick behaviour or neurochemistry after *in ovo* exposure to TMPP. Non-significant increase in purkinje cell number in cerebellum in the highest exposure group.	High	None-weak
Crump et al. (2014)		Effects on chicken embryo development: the highest dose-level of TMPP resulted in deformities such as reduced tarsus length and increased liver mass, as well as altered liver gene expression with up-regulation of CYP2H1 and CYP3A37, UGT1A9 (not significant) and LFABP, and down-regulation of TTR	High	Weak
Kojima et al. (2013)	ERα and PXR agonism, AR antagonism and very weak PPARγ agonism. No effects on ERß, TRα, TRß, RARα, RXRα, and PPARα activities were measured		Medium	Moderate
Liu et al. (2012)	Effects on steroidogenesis in H295R cells: increased T and E2 production, induction of CYP11A1, CYP11B2, CYP19A1 and HSD3ß2 and downregulation of SULT1E1 and SULT2A1. Anti-oestrogenic in MVLN cells.		High	Strong

Androgen receptor (AR), estrogen receptor (ER) α and ß, aryl hydrocarbon receptor (AhR), glucocorticoid receptor (GR), retinoic acid receptor (RAR)α, retinoic X receptor (RXR)α, thyroid hormone receptor (TR)α and ß, liver basic fatty acid-binding protein (LFABP), transthyretin (TTR) and uridine 5'-diphospho-glucuronosyltransferase 9 (UGT1A9), pregnane X receptor (PXR), peroxisome proliferator-activated receptor (PPAR) α and PPARγ

Table 2. Overview of potential endocrine-related adverse effects of TMPP.

Reference	Species, n	Adverse effects	Quality of study	Evidence for adverse effects
Bolon et al (1997)	Mice, ovaries from the Chapin et al (1988) study	No effect on follicle counts was seen.	Medium	None
Latendresse et al (1995)	Rats, 3-12/group	Histopathological effects on ovaries and adrenal glands and elevated serum estradiol levels were found in exposed adult female rats.	Medium	Moderate
Latendresse et al (1994a)	Rats, 3/sex/dose group	Histopathological effects on ovaries and testes as well as adrenal glands from both sexes were found.	Medium	Moderate
Latendresse et al (1994b)	Rats, 20/pairs/group	Adverse effects on male fertility as well on ovarian and testes weights.	Medium	Strong
Chapin et al (1988)	Mice, 8-40 males and females/group	Reduced fertility in both sexes was seen in the highest dose group. On necropsy reduced sperm motility and increased number of abnormal sperms were seen in both F0 and F1 males, with only a reduced number of sperms in the F0 males.	Medium	Moderate-Strong
Carlton et al. (1987)	Rats, 12 males and 24 females/ dose group	Males (200 mg/kg): reduced sperm concentration, motility, progressive movement, and increased abnormal sperm morphology, reduced epididymis weight, and necrosis and degeneration of seminal vesicles. Females (400 mg/kg): reduced litter size, histopathological changes in the ovaries	Medium	Strong
Somkuti et al. (1987a)	Rats, 8 males (14-days) or 10 males	Reduced sperm motility and density and increased number of abnormal sperm in the 50, 75 and 100 mg/kg groups. Histopathology in testes and epididymides were seen	Medium	Strong

Reference	Species, n	Adverse effects	Quality of study	Evidence for adverse effects
	(63 days)/ dose group	in all treatment groups ≥ 25 mg/kg, and decreases in testicular esterases were also seen as a response to TOCP treatment		
Somkuti et al. (1987b)	Roosters, 10 (100 mg/kg/ day in 18 days) or 3 (750 mg/kg and killed after 1 or 18 days)	Reduced sperm density and motility, and disorganized seminiferous tubules in the 100 mg/kg group, inhibited activity of 2 testicular esterases in the 750 mg/kg 1 d group	High	Moderate
Somkuti et al. (1987c)	Rats, 8 males/time point and 10 males in the recovery group	Reduced sperm motility (≥14 days) and density (≥10 days), and reduced testis:BW ratio (≥21 days), inhibited activity of 2 testicular esterases (≥3 days), no effects of LH, FSH and testosterone in serum Recovery study: decreased testis size and absence of germinal cells after 98 days of recovery	High	Strong
REACH dossier	Rats, 25 females/dose group	Maternal toxicity and lower foetal weight was seen in the treatment groups. Inconsistent deformities in the foetuses were seen and these were most likely not related to the treatments	High	None

Luteinizing hormone (LH), Follicle stimulating hormone (FSH)

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