Effects of Triclosan on the Reproductive System

Subjects: Endocrinology & Metabolism

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Triclosan (TCS), 5-chloro-2-(2,4-dichloro phenoxy) phenol, is an endocrine-disrupting chemical often used as an antiseptic, disinfectant, or preservative. Triclosan is one of the antimicrobial agents used in cosmetic products, toothpaste, and disinfectants. Exposure to these compounds can lead to alterations in one or more signalling pathways, that may induce negative effects on reproduction, growth, survival, behavioural, and obesity. These changes can be passed on to subsequent generations. Studies in animals and humans suggest the possibility of harmful health outcomes, particularly for the reproductive system.

triclosan antimicrobial endocrine disruptor reproductive system

1. Animals

TCS has been widely studied in animals at the level of the reproductive system. It is also known that in this system, the effects are mainly due to the modulation of androgenic and estrogenic receptors.

In a study conducted by Ishibashi et al., several concentrations of triclosan were tested on embryos and adults of the *Oryzias latipes* fish. At concentrations of 625, 1250 and 2500 µg/L, no embryos were born, and they found that at 313 µg/L the incubation time increased significantly; however, fertility was not affected during the study [1].

Liu et al. used zebrafish (*Danio rerio*) to test the toxic effects of three concentrations of TCS (0.08, 0.16 and 0.25 mg/L) on the liver. The growth of the zebrafish showed no significant changes; however, there was an increase of 11.67–19.16% in the zebrafish weights and of 21.53–47.42% in the liver weights at exposures of 0.16 and 0.25 mg/L. At these concentrations, the superoxide dismutase (SOD) activity decreased while the malondialdehyde (MAD) levels gradually increased with different TCS concentrations. Thus, the authors demonstrated that exposure to TCS causes oxidative stress in the liver depending on the exposure time and concentration. This exposure created several hepatocellular changes, an increased hepatic plaque gap, necrosis, and atrophy, with the last two being concentration dependent. The same authors also observed a TCS concentration-dependent apoptosis and they quantified the expression of Bcl-2 and Bax to better understand which pathway of apoptosis was promoted by TCS. The expression of Bcl-2 at concentrations of 0.16 and 0.25 mg/L was significantly decreased while Bax was increased at concentrations of 0.08 and 0.25 mg/L. This relationship between Bax and Bcl-2 led the authors to hypothesise that hepatocyte apoptosis is directly related to the exposure of zebrafish to TCS [2].

Recently, Qiao et al. also tested 2, 20 and 200 µg/L of TCS in zebrafish, observing no mortality or any abnormality in their behaviour during the experiment. Fertilisation was not affected at any of the concentrations; however, the births decreased. Furthermore, in males, the testosterone levels decreased with the higher TCS concentration while the oestradiol and vitellogenin (VTG) levels increased in the 20 and 200 µg/L of TCS groups. On the other hand, in females, there was only a decrease in the VTG levels at 20 and 200 µg/L of TCS. These results indicate that TCS has an estrogenic influence in zebrafish, with males being more sensitive than females. There was also a significant decrease in mature spermatozoa and destruction of the testis structure at the concentration of 200 µg/L of TCS, and in females, an increase in the number of immature oocytes was observed at the same concentration [3].

Another model widely used for in vivo studies are rats. Kumar et al. observed that the administration of 5 mg/kg/day to male Wistar rats did not induce a significant change in the testes weight, but higher doses of TCS induced a significant change in the testes and sexual tissues. The weight of the testes, epididymis, ventral prostate, vas deferens and seminal vesicles decreased by 20–50% with 10 mg/kg/day and 35–49% with 20 mg/kg/day. The authors also quantified several hormones and observed a decrease of 38.5% in the luteinizing hormone (LH), 17% in the follicle stimulating hormone (FSH), 35% in cholesterol, 31% in pregnenolone and 41% in testosterone after a dose of 20 mg/kg/day [4].

Regarding the study of gender differences in reproduction, in the following year, 2010, Stoker et al. performed a daily administration of TCS (9.375; 37.5; 75; 150 mg/kg) to female Wistar rats, between the Postnatal day (PND) 22 and the PND 42 (PND = 0, day of birth of the females used in the study), to examine the effects of TCS on female pubertal development. They analysed if the time until the opening of the vaginal canal was affected, as well as the histology of the uterus and ovaries, and some hormones including E2 and LH. The authors observed that this compound altered the reproductive development and response to exogenous oestrogens. They also observed a lower age for the opening of the vaginal canal with the administration of 150 mg/kg, which was an estrogenic response of the triclosan since stimulation through oestrogens is required for the canal to open. The serum oestradiol concentrations at PND 42 were decreased followed by administration of TCS at 37.5 mg/kg and 150 mg/kg, while the LH levels did not show significant changes. Thus, the study suggested that TCS increases oestrogen activity with a potential to alter the oestrogen-dependent functions. In summary, the authors concluded that TCS affects the reproductive development and uterine responses to exogenous oestrogens in developing females [5].

Two years later, in 2012, Jung et al. analysed the estrogenic activity of TCS using well-established models, both in vivo and in vitro. For the in vivo approach, female Sprague-Dawley rats were treated with 17α-ethinyl-oestradiol (1 mg/kg) and TCS (7.5, 37.5 and 187.5 mg/kg), between PND 19 and 21. The TCS increased the uterine weight and mRNA expression for complement 3, which is an oestrogen-sensitive gene in the uterus, in immature mice after a treatment with TCS at 37.5 mg/kg and 187.5 mg/kg. This shows that TCS has estrogenic activity. In vitro, the estrogenic activity upon TCS exposure was analysed on the expression of calbindin-D9k (CaBP-9k), which is a biomarker for the detection of endocrine disruptors in GH3 cells (i.e., Wistar rat epithelial cells). The expression of CaBP-9k was increased in the presence of TCS, which demonstrates the endocrine disrupting nature of TCS [6]. In

a similar study by Louis et al., also using female Wistar rats between PND 19 and 21, doses of ethinyl-oestradiol (0.125, 0.25, 0.5, 1, 2 and 3 µg/kg) or ethinyl-oestradiol combined with TCS (2.3, 4.69, 9.375 and 18.75 mg/kg) were administered orally. No changes in the body weight were observed; however the uterine weight was increased when ethinyl-oestradiol (1, 2 and 3 µg/kg) was combined with TCS (4.69, 9.375 and 18.75 mg/kg). The expression of CaBP-9K was also analysed, but they found no changes when treated with TCS alone; however, the coupling of ethinyl-oestradiol and TCS caused an increase in its expression, but not significantly higher than the expression increase with the ethinyl-oestradiol alone. The authors concluded that TCS can alter the uterine response in the presence of low concentrations of ethinyl-oestradiol [7].

Regarding the effects of TCS in *Mus musculus* rats' gestation, Crawford et al. compared several doses of TCS (0, 87, 262, 523 and 785 mg/kg) with BPA. Females receiving the TCS dose on gestational days 0 and 1 showed no significant differences in the implantation sites when compared with the control. On gestational day 2, significant differences were already observed for the 785 mg/kg triclosan dose and on day 3 for the 523 mg/kg triclosan dose. When exposed to 18 and 785 mg/kg of triclosan from gestational days 1–3, the number of implantation sites decreased significantly on gestational day 6. The number of implantation sites was also reduced after an injection of these two doses on gestational day 3 and only of the higher dose on gestational day 2 [8]. Thus, this study concluded that TCS may affect intrauterine implantation when administered in combination with BPA.

In a different study, 3-month-old rats were administered with 1, 10 and 100 mg TCS/kg/day from gestational day 6. The TCS levels were measured up to gestational days 11 and 16. An exposure to TCS demonstrated a dose-dependency in increasing foetal death. The incidence of spontaneous abortion at doses 10 and 100 reached 60% and 80%, respectively. The level of oestrogen sulphotransferase protein (EST) was decreased at gestational day 16 at the highest dose, as were its plasma and placental activities. These abortions were most likely caused by the inhibition of EST activity leading to placental thrombosis and degeneration [9]. Thus, the authors concluded that higher levels of TCS may lead to the occurrence of spontaneous abortions due to the inhibition of EST activity.

One year later, in 2016, Feng et al. recorded the total and uterine weights of female mice throughout gestation after an administration of TCS (0, 30, 100 and 300 mg/kg), and noted a lower weight gain in the groups where 300 and 600 mg/kg of TCS was administered when compared to the control; however, significant differences were only found regarding the uterine weight in the 600 mg/kg group. The tissues presenting the highest concentration of TCS were the placenta, liver, and kidneys with 12.83, 9.52 and 8.74 µg/g, respectively. In the placenta, the mean TCS levels following 0, 30, 100 and 300 mg/kg of TCS administrations were 0.033, 13.05, 28.23 and 55.83 µg/g, respectively. The LH and FSH levels were identical to the control group, whereas the levels of human chorionic gonadotropin (hCG), prolactin, progesterone, oestradiol, and testosterone were significantly reduced in all the exposure groups when compared to the control. Thus, the placenta may be a target of TCS in rats during the gestation period [10].

In the same way, a study performed by Montagnini et al. tested the effect of low doses of TCS (0.8, 2.4, and 8.0 mg/kg) over the course of three generations of Wistar rats named F0, F1 (the offspring of F0) and F2 (the offspring of F1). The TCS was orally administered to the F0 generation only between PND 49 and 120 in females, while in

males it continued until PND 140. Thus, weight changes, food consumption during the pre-mating period, sexual behaviour assessment, male organs of the F1 generation, plasma testosterone quantification, all sperm parameters, testicular histomorphometry, sexual and physical development of the F1 and F2 generations and neuronal behavioural tests were accounted for. They concluded that these concentrations of TCS had no significant effect other than a reduction in the viability and motility of spermatozoa from the F1 generation being administered with 2.4 mg/Kg at PND 140. Doses administered between PND 49 and PND 140 did not affect the sperm parameters; therefore, the authors concluded that TCS could have an impact on gametogenesis during the foetal period [11].

More recently, Raj et al. analysed the effects of accumulated TCS on the histopathology and secretory functions of the epididymis, seminal vesicle, and sperm indices in adult Swiss-strain rats. Four different doses of triclosan (40, 80, 160 and 320 mg/kg) were administered for 42 consecutive days. All the concentrations significantly decreased the epididymal and seminal vesicle weights and led to a decrease in the sperm percentage and viability, and sperm count; furthermore, the percentage of non-viable spermatozoa increased. The histology of the epididymis was marked with changes throughout its length with disorganization and the appearance of vacuoles. The final chromatographic study showed an accumulation of TCS in the epididymis and seminal vesicle. Thus, Raj et al. concluded that TCS caused alterations in the epididymis and seminal vesicle, as well as in the sperm indices, epididymal sialic acid levels and fructose levels of the seminal vesicle [12].

Concerning the effect of this compound on the placenta, in 2010, TCS was tested on the placental tissue from ewes with gestation periods ranging between 126–130 days. In this study it was demonstrated that the TCS was a potent inhibitor of placental EST activity. This inhibition was analysed, and the authors observed that it occurred mainly competitively; therefore, it can be assumed that TCS occupies the substrate binding sites. The role of placental EST is not yet fully known; however, alterations in its expression are thought to be associated with spontaneous abortions and foetal loss [13], as previously demonstrated in rats by Wang et al. [9].

In summary, TCS has a variety of reproductive effects in fish, rats and even sheep. The decreased fertility beyond the induction of abortion was the most observed consequence in any of the models. This may be due to the way the substance acts on the receptors or on oestradiol concentrations, as observed by Wang et al., where they demonstrated an inhibition of EST activity which caused a thrombosis in the placenta. Furthermore, TCS also appears to affect the serum concentration of several hormones such as LH, FSH, hCG, pregnenolone and cholesterol, which can lead to reproductive problems such as foetal loss and decreased fertility in subsequent generations.

2. Humans

In humans, TCS has not been widely studied yet, with most of the existing studies being related to the hormonal levels of EST, FSH and the effects at the foetal-placental level, namely, spontaneous abortions.

The first authors to demonstrate that TCS could be associated with adverse reproductive effects were Wang et al., finding an association between urinary TCS concentration levels and increased spontaneous abortions. These authors recruited a population of 452 women at 14 and 24 weeks of pregnancy with mean ages of 28 and 27 years, respectively. Their E2 levels at low-TCS concentrations were lower than the control; however, at high-TCS levels there were no changes. The plasma EST levels were reduced at both low- and high-TCS concentrations, while the EST activity at high-TCS concentrations was lower compared to the control and the low-TCS concentrations [14]. This increase in the number of abortions at higher TCS concentrations may be due to decreased EST activity caused by the TCS, as it was already demonstrated in animals.

Miscarriages may be linked to an inhibition of the placental 11β-HSD2 enzyme. In this sense, Zhang et al. observed that triclosan altered the expression of 11β-HSD2 in human syncytiotrophoblasts and examined the apoptotic mechanism induced by TCS. At concentrations of 0.1 μM and higher of TCS, the mRNA levels decreased significantly, and at the protein level there was a significant inhibition over a similar range of concentrations. There was also a decrease in the viability of syncytiotrophoblasts in a concentration dependent manner (0.001–10 μM), with statistical significance at concentrations above 0.1 μM. Moreover, the authors observed that triclosan induced the apoptosis of syncytiotrophoblasts and that the inhibition of 11β-HSD2 was explained by the induction of apoptosis [15].

In 2017, an epidemiological study also demonstrated the association between the urinary TCS levels of 401 pregnant women (at 16 weeks of gestation) and birth weight, length, head circumference and length of gestation. Urine was collected at 16 and 27 weeks of gestation, and TCS was detected in 91% and 83% of the samples, respectively, with a mean concentration of 16 ng/mL. This study showed a positive association between the urine triclosan concentration and a moderate reduction in birth weight, length, head circumference and gestational length [16].

Two years later, Jurewicz et al. analysed the association between triclosan concentration and ovarian reserve, which is a marker of female fertility. They counted the antral follicles and quantified the anti-müllerian hormone, follicle stimulating hormone and oestradiol. The authors recruited 511 menstruating women (25–39 years old) that provided blood and urine samples. The blood samples were taken at the beginning of the menstrual cycle, approximately between the second and fourth day, so that the hormones could be counted, while the antral follicle counts were performed in both ovaries during the beginning of the follicular phase of the menstrual cycle. Urine TCS concentrations (0.3–1677.68 ng/mL) were shown to be negatively associated with the antral follicle count, while the anti-müllerian hormone, follicle stimulating hormone and oestradiol did not show a statistic relevance. Thus, the authors concluded that TCS may be associated with decreased female fertility, although further studies are needed to demonstrate the mechanism involved in this effect [17].

The following year, Yuan et al. compared the levels of TCS present in urine with the sperm quality in a Chinese population, between 2018 and 2019. From the 406 subjects (aged between 21 and 56), 57.1% had never smoked and 83.5% had never consumed alcoholic beverages. The parameters analysed included the sperm volume, concentration, count, total, progressive and non-progressive motility, immobility, percentage of hyperactivated

sperm, mean velocity, curvilinear velocity, straight line velocity, lateral head deviation amplitude, linearity, sway, beat cross frequency and straightness. TCS was detected in 74.6% of the samples with a mean of 1.7 µg/L. None of the parameters studied were associated with TCS exposure, although a higher TCS exposure showed higher motility parameters indicating a potential non-linear influence of TCS on sperm quality [18].

In summary, the effects caused by TCS in humans are very similar to those observed in animal models. Spontaneous abortion caused by chronic exposure to TCS is the most observed consequence, although the specific pathway by which it occurs is not yet known. Furthermore, sperm motility is also disrupted by TCS exposure.

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