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Evaluation of reproductive toxicity in rats treated with triclosan

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ABSTRACT

Triclosan (TCS) is an antibacterial agent used in a variety of consumer products such as: soaps, deodorant, and toothpaste, among others. Some studies have reported the (anti)androgenic effects of TCS in the male reproductive system, raising concerns about its effects on the reproductive axis. In this study, the (anti)androgenicity of TCS was evaluated in the Hershberger assay in 52-day old male Wistar rats. Additionally, the sexual behavior, sperm motility, sperm viability, and testicular histomorphometry were evaluated in a second protocol to investigate the reproductive effects of TCS in 49-day old male Wistar rats. The dosages were administered based on the acceptable daily intake for TCS, in addition to 3 and 10-fold higher doses. Our results demonstrated that TCS, in the doses administered, did not act as an endocrine disrupter (ED), with no (anti)androgenic effect in the Hershberger assay and without interfering with the parameters evaluated in the reproductive toxicity study.

1. Introduction

Interest in monitoring environmental contaminants (such as pesticides, heavy metals, therapeutic drugs, phenolic derivatives, and/or other persistent xenobiotics) is mainly due to recognition of their negative effects relative to environmental endocrine disruption in wildlife and human health [1]. Endocrine disrupters (EDs) are environmental contaminants described by the International Programme on Chemical Safety as an exogenous substance or mixture that alters function(s) of the endocrine system with adverse health effects in an intact organism, its progeny, or (sub)populations [2]. In human populations, exposure to EDs points towards an association with reproductive disorders, such as early puberty [3,4], poor sperm quality and/or function [5,6], and prostate cancer [7].

Triclosan (TCS) (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is a phenolic compound with antibacterial properties applied to a variety of consumer products, such as personal care products (cosmetics, toothpaste, shampoo, hand soaps, deodorants, and body oils), textiles, and toys, among others [8–10]. Due to its widespread use over the past decades, TCS has become one of the most commonly detected contaminants in solid and water environmental compartments [11,12]. In rivers in the state of São Paulo (Brazil), the measurable concentrations of TCS in surface waters ranged from 2.2 to 66 ng/L [13], and as TCS is only partially removed in wastewater treatment plants [12], this compound has also been found in drinking water [14].

In humans, the major routes of exposure to TCS occur through topical absorption and the gastrointestinal tract [15–17], in addition, it has been demonstrated that frequent contact with TCS is associated with higher concentrations of this compound in the urine of individuals ≥6 years of age [18], in breast milk [19], and in human umbilical cord blood plasma [20]. Some experimental evidence has demonstrated the potential for TCS to act as an ED in the reproductive system. In Sprague-Dawley rats, TCS showed a tendency to accumulate in the epididymis after a single oral administration (50 mg/kg), and after 8 weeks of treatment starting on post-natal day (PND) 42, decreased daily sperm production was observed at doses of 50 and 200 mg/kg [21]. Moreover, the oral treatment (200 mg/kg) for 31 days decreased serum testosterone in immature male Wistar rats (23 days old) [22], and in 10 week old rats, there was a decrease in the synthesis of androgens followed by reduced daily sperm production, after 60 days of oral administrations (20 mg/kg) [23]. *In vitro*, TSC demonstrated antiandrogenic action by disrupting the activity of the adenylyl cyclase

Abbreviations: EDs, endocrine disrupters; TCS, triclosan; CTR, control; PND, postnatal day; TP, testosterone propionate; EPA, Environmental Protection Agency; LABC, levator ani bulbocavernosus; DSP, daily sperm production; LST, total length of the seminiferous tubules; TSV, tubule seminiferous volume; πR2, area of transverse section of seminiferous tubules.

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enzyme, decreasing biosynthesis of testosterone in rodent Leydig cells [24], and inhibiting transcriptional activation by testosterone in human embryonic kidney cells [25], while exhibiting androgenic activity by displacing testosterone of the androgen receptor binding domain in androgen-responsive breast cancer cells [26].

Therefore, considering the extensive use of TCS, its occurrence in the environment, its endocrine-disrupting potential in the male reproductive system and given the few data available in the literature regarding the reproductive toxicity of TCS, this study aimed to detect possible (anti)androgenic effects of TCS using the Hershberger assay, in addition to evaluating the sexual behavior, sperm motility, sperm viability, and testicular histomorphometry in a reproductive toxicity study.

2. Material and methods

2.1. Drugs

TCS was obtained from Vivimed Labs Limited (Habsiguda Hyderabad, India) (CAS no. 3380-34-5, 99.38% pure), and testosterone propionate (TP) (CAS no. 57-85-2, 99.41% pure) and flutamide (CAS no. 13311-84-7, 99.60% pure) were obtained from Fragon (São Paulo, Brazil). Except for TP, which was administered subcutaneously, all chemicals were dissolved in corn oil (vehicle) and administered orally, by gavage, in a volume of 2.5 ml/kg.

2.2. Animals

Male and female Wistar rats were obtained from the colony of the State University of Londrina and maintained in a controlled environment with a temperature of 21 ± 2 °C; a 12 h light/dark cycle (lights on at 6:00 AM); and free access to regular lab chow (Nuvilab™, Quimtia SA, Brazil) and tap water. Animals were housed in collective polypropylene cages (29 × 18 × 13 cm) with wood shavings as bedding, and were mated after 1 week of acclimatization. Litters with 8–10 pups were used and, if litters had more than 10 pups, culling was conducted. The day of birth was considered postnatal day (PND) 0 and male pups were weaned on PND 21. After weaning, male pups were distributed for the Hershberger assay or assessment after puberty. No littermates were used in the same experimental group. All animal procedures were approved by the State University of Londrina Ethics Committee for Animal Research (CEUA/UEL: 283.2015.27) and were elaborated and developed based on the principle of the three R's (Refine, Reduce, and Redesign).

2.3. Hershberger assay

The study design followed the guideline OPPTS 890.1400 from the U. S. Environmental Protection Agency (EPA) [27]. On PND 42, the rats were anesthetized with sodium pentobarbital (40 mg/kg, ip) and castrated by making an incision in the scrotum and removing both testes and epididymis with ligation of blood vessels and seminal ducts. On PND 52, the rats were randomly assigned to the experimental groups. Weight variation among the animals on the first day of treatment was 10%.

In order to evaluate the possible androgenicity of TCS, the rats were distributed into the following groups (n = 6/group):

- CTR (vehicle): rats were treated with corn oil (solvent of TCS);
- TP: rats were treated with 0.4 mg/kg of TP;
- TCS 0.8: rats were treated with 0.8 mg/kg of TCS;
- TCS 2.4: rats were treated with 2.4 mg/kg of TCS;
- TCS 8.0: rats were treated with 8.0 mg/kg of TCS;

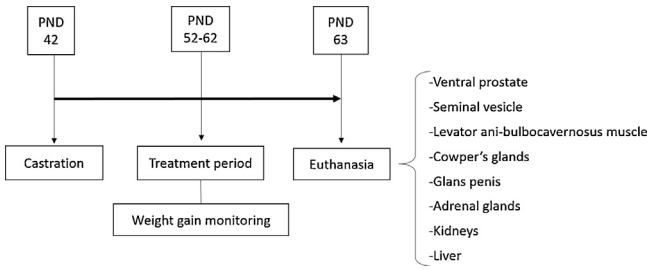

Fig. 1. Diagram of Hershberger assay design. PND: postnatal day.

For evaluation of the possible antiandrogenicity of TSC, the rats were distributed into the following groups (n = 6/group):

- TP: rats were treated with 0.4 mg/kg of TP.
- TP + flutamide: rats were treated with 0.4 mg/ kg of TP in addition to 3 mg/kg of flutamide.
- TCS 0.8 + TP: rats were treated with 0.8 mg/kg of TCS in addition to 0.4 mg/kg of TP.
- TCS 2.4 + TP: rats were treated with 2.4 mg/ kg of TCS in addition to 0.4 mg/kg of TP.
- TCS 8.0 + TP: rats were treated with 8.0 mg/ kg of TSC in addition to 0.4 mg/kg of TP.

All chemicals were administered for 10 consecutive days after a 3-h food restriction. Twenty-four hours after the final dose (i.e., PND 62), the animals were weighed and euthanized by decapitation. The ventral prostate, seminal vesicle (plus fluids and coagulating glands), levator ani-bulbocavernosus (LABC) muscles, paired Cowper's glands, glans penis, liver, paired kidneys and paired adrenals were removed, trimmed free of fat and weighed. In addition, during the 10-day treatment period, animals were observed daily for mortality, morbidity, and general signs of toxicity, such as changes in behavior (e.g., agitation, lethargy, and hyperactivity), neurological changes (e.g., convulsions, tremors, muscle rigidity, and hyperreflexia), and autonomic signs (e.g., lacrimation, piloerection, pupil size, and unusual respiratory patterns). The experimental protocol is diagrammed in Fig. 1.

2.4. Reproductive toxicity study

The study treatment period was undertaken following the principles of the OECD Guideline for Testing of Chemicals 416 [28] designed to provide general information concerning the effects of a xenobiotic on the reproductive system. On PND 49, the male rats were randomly distributed into four groups (n = 10/group):

- CTR (control): rats were treated with corn oil;
- TCS 0.8: rats were treated with 0.8 mg/kg of TCS;
- TCS 2.4: rats were treated with 2.4 mg/kg of TCS;
- TCS 8.0: rats were treated with 8.0 mg/kg of TCS.

The treatment was performed by gavage once a day, always following the same administration schedule routine (11:00 a.m.–1:00 p.m.) and was conducted until PND 140.

2.4.1. Dose justification

According to the U. S. EPA [29], the acceptable daily intake of TCS is up to 0.3 mg/kg, for humans. This value was corrected discounting the allocation factor adopted for TCS exposure in drinking water [30,31], which corresponds to 20% (i.e., 0.2) of the acceptable daily intake value. Subsequently, an approximate value of 0.2 mg/kg was generated, which was applied to the $BW^{3/4}$ scale [32] for dosimetric adjustment, where the weight of a human of 70 kg was consid-