



## NOTE

Toxicology

# Effects of tris(1,3-dichloro-2-propyl) phosphate on epididymal sperm parameters in adult male rats

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**ABSTRACT.** Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is widely used as a flame retardant and is known to exhibit anti-androgenic effects *in vitro* and *in vivo*. To assess the reproductive toxicity potency of TDCIPP, we investigated the effects of 7 days of TDCIPP oral administration on epididymal sperm motion and concentration in adult male Wistar–Imamichi rats. Thirty-five days after the final administration, sperm parameters were evaluated by computer-assisted sperm analysis. Results showed that sperm swimming progression and vigor and sperm concentration in TDCIPP-treated rats were unexpectedly higher than those in control rats. TDCIPP did not significantly affect the percentage of motile sperms or sperm swimming pattern. These results contribute to the understanding of the biological effects of TDCIPP.

**KEY WORDS:** anti-androgenic substance, computer-assisted sperm analysis, flame retardant, tris(1,3-dichloro-2-propyl) phosphate, sperm

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Organophosphorus flame retardants (OPFRs) are widely used in many commercial products. OPFRs are currently detectable in the environment [4, 9, 12, 16] and the human body [3, 17] as a consequence of this use. This presence of OPFRs raises a concern for risk to ecosystems and human health. Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is an OPFR, and its toxicity is reported [7, 19, 26]. We recently reported that TDCIPP causes hepatic and renal toxicity, along with a reduction in alkaline phosphatase, and anemia coinciding with a reduction in reticulocyte count [13].

TDCIPP exhibits anti-androgenic activity *in vitro* [14, 25] and *in vivo* [10]. Androgens are generally important for spermatogenesis in adulthood, and administration of anti-androgenic substances to adult animals negatively affects sperm parameters, such as sperm number, motility, and velocity [15, 18, 20, 22]. No study has addressed the effects of TDCIPP anti-androgenic activity on sperm parameters in adulthood. To assess the reproductive toxicity potency of TDCIPP, we investigated the effects of 7 days of TDCIPP oral administration on epididymal sperm parameters in adult male Wistar–Imamichi rats using computer-assisted sperm analysis (CASA).

We purchased 8-week-old male Wistar–Imamichi rats (n=18) from the Institute for Animal Reproduction (Ibaraki, Japan). Animals were housed (three animals per cage) in polycarbonate cages with free access to food (Oriental Yeast Co., Ltd., Tokyo, Japan) and water. They were kept in a controlled environment (temperature: 25°C ± 1°C, humidity 50% ± 10%) with 12-hr light/dark cycle (lights on from 8:00 to 20:00 hr) and acclimated for 7 days. Before the initial oral administration, body weights were checked to confirm no significant difference among treatment groups. Rats were orally administered sesame oil (5 ml/kg, Sigma-Aldrich, St. Louis, MO, USA, CAS No. 8008-74-0) as a control (n=6) or TDCIPP (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan, CAS No. 13674-87-8, Purity: >93.0%) dissolved in sesame oil (250 mg/kg/day group: n=6, 650 mg/kg/day group: n=6) for 7 days. The dose of 650 mg/kg/day caused overt hepatic and renal toxicity, but the dose of 250 mg/kg/day did not [13].

Spermatogenesis in rats occurs at approximately 7 weeks, and when adult male rats were administered flutamide, a general

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androgen antagonist, for 6 days, a decrease in spermatid number was confirmed 35 days after final administration [15]. Thus, we collected sperm 35 days after final administration. Rats were weighed before sperm collection and decapitated under isoflurane anesthesia (Pfizer Japan Inc., Tokyo, Japan); testes and epididymis were removed. Testes and right epididymis were weighed. The sperm was collected from the left cauda epididymis. Sperm parameters were measured using CASA as described previously [23], including the percentage of motile sperm (MOT), percentage of progressive motile sperm (PROG), average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat-cross frequency (BCF), straightness (STR), linearity (LIN), and concentration (CONC). In the present study, we considered MOT and PROG as indicators for the percentage of motile sperm; VAP and VSL for sperm swimming progression; VCL, ALH, and BCF for sperm swimming vigor; and STR and LIN for sperm swimming pattern, which was based on the classification used in a previous study [5]. Animal use protocols were reviewed and approved by the Animal Care and Use Committee of Meiji University (IACUC19-0005).

Effects of TDCIPP on the body weight, organ weight, and sperm parameters were evaluated using Dunnett's test. Statistical analyses were performed using Bell Curve Excel Statistics for Windows (SSRI, Tokyo, Japan). The threshold for statistical significance was  $P < 0.05$ .

Body weight, organ weight, and sperm parameters in each group 35 days after the end of TDCIPP oral administration are shown in Table 1. No significant effect of TDCIPP on body weight or testes and epididymis weights was observed. TDCIPP administration did not affect the indicators of the percentage of motile sperm or swimming pattern. TDCIPP-treated rats showed higher sperm swimming progression, with statistical significance observed in animals receiving 250 mg/kg/day. Sperm swimming vigor, VCL and ALH, was significantly higher in both treatment groups. TDCIPP-treated rats showed higher CONC, with statistical significance observed in animals receiving 650 mg/kg/day.

In the present study, the effect of 7 days of TDCIPP oral administration on the epididymal sperm parameters was investigated. The present study found that a dose of 250 mg/kg/day, which does not cause overt toxicities [13], affected some of the sperm parameters. Unexpectedly, although anti-androgenic substances typically decrease sperm parameters such as sperm number, motility, and velocity in adulthood [15, 18, 20, 22], TDCIPP induced an increase in some of these parameters.

A possible explanation for this finding might be, first, follicle-stimulating hormone (FSH) may be involved in enhancing some sperm parameters. FSH is secreted from the anterior pituitary and plays a role in maintaining spermatogenesis. Low sperm number, sperm swimming progression, and sperm swimming vigor were observed in FSH receptor knockout mice compared with those in wild-type mice, but the percentage of motile sperm and sperm swimming pattern were not changed [8]. These effects on sperm parameters are consistent with the sperm parameters observed in the present study, supporting the involvement of FSH. FSH secretion is regulated by negative feedback from testosterone. Therefore, the anti-androgenic activity of TDCIPP could stimulate FSH secretion, resulting in an increase in some sperm parameters. Second, although no report on the androgenic

**Table 1.** Body weight, organ weight, and sperm parameters in adult rats 35 days after the end of tris(1,3-dichloro-2-propyl) phosphate oral administration

	Control	250 mg/kg/day	650 mg/kg/day
Body and organ weight			
Body weight (g)	460.1 ± 14.3	441.1 ± 13.0	452.6 ± 10.5
Testes (g)	2.90 ± 0.07	3.04 ± 0.04	2.91 ± 0.04
Epididymis (g)	0.64 ± 0.02	0.69 ± 0.04	0.63 ± 0.02
Sperm parameter			
Percentage of motile sperm			
MOT (%)	64.8 ± 2.8	68.7 ± 2.3	67.7 ± 2.9
PROG (%)	35.2 ± 1.7	42.2 ± 2.4	41.3 ± 2.5
Sperm swimming progression			
VAP (µm/sec)	103.8 ± 4.2	118.7 ± 5.4*	116.8 ± 2.7
VSL (µm/sec)	81.2 ± 3.4	95.0 ± 4.8*	91.8 ± 2.3
Sperm swimming vigor			
VCL (µm/sec)	141.8 ± 4.7	162.9 ± 5.7*	163.6 ± 3.7*
ALH (µm)	5.8 ± 0.2	6.5 ± 0.1*	6.6 ± 0.1*
BCF (Hz)	8.5 ± 0.6	8.2 ± 0.4	8.2 ± 0.7
Sperm swimming pattern			
STR (%)	75.2 ± 0.5	76.3 ± 0.5	75.2 ± 0.5
LIN (%)	56.3 ± 1.0	56.8 ± 0.8	55.7 ± 0.9
Sperm concentration			
CONC (million/ml)	3.2 ± 0.3	4.6 ± 0.6	5.0 ± 0.5*

Data are expressed as mean ± SEM. MOT, percentage of motile sperm; PROG, percentage of progressive motile sperm; VAP, average path velocity; VSL, straight-line velocity; VCL, curvilinear velocity; ALH, amplitude of lateral head displacement; BCF, beat-cross frequency; STR, straightness; LIN, linearity; CONC, concentration. \* $P < 0.05$  vs. control.

activity of TDCIPP exists, flutamide, a general anti-androgenic chemical, sometimes acts as an androgenic substance [21]. Thus, TDCIPP might exhibit androgenic activity on spermatogenesis. Third, an *in vitro* study investigating the endocrine disrupting potency of OPFRs indicated that TDCIPP exhibits weak estrogenic activity [25]. Estrogen is involved in spermatogenesis, such that estrogen receptor  $\alpha$  knockout mice display low sperm numbers [6]. Therefore, results in the present study may be due to the estrogenic activity of TDCIPP. Fourth, the alteration of some sperm parameters might not be caused by hormonal activity. For example, oxidative stress detrimentally affects spermatogenesis resulting in infertility [2], and antioxidant administration increases sperm number and motion [1, 24]. Thus, TDCIPP may act via antioxidant activity in testis to increase some sperm parameters. Examination of testis histology to identify the stage of spermatogenesis affected by TDCIPP, and along with hormone measurements, is key to assess the aforementioned hypotheses.

In summary, the present study demonstrated that TDCIPP did not exhibit typical anti-androgenic activity on sperm parameters in adulthood. Our results, i.e., TDCIPP, an anti-androgenic chemical, enhances some epididymal sperm parameters, provide interesting information for both toxicology and physiology fields and contribute to the understanding of the biological effects of TDCIPP. As sperm parameters are related to fertility [11], TDCIPP may affect reproduction of animals. We administered TDCIPP to adult rats, but it is unclear if rats were administered TDCIPP during puberty when testicular maturation occurs. Moreover, it is also unclear whether TDCIPP affects sperm morphology, which is affected by anti-androgenic substances. Substantial study is still needed to assess the reproductive toxicity of TDCIPP further.

CONFLICT OF INTEREST. The authors declare no conflict of interests.

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