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The Effects of Triclosan on Puberty and Thyroid Hormones in Male Wistar Rats

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Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a potent antibacterial and antifungal compound that is widely used in personal care products, plastics, and fabrics. Recently triclosan has been shown to alter endocrine function in a variety of species. The purpose of this study was to determine effects of triclosan on pubertal development and thyroid hormone concentrations in the male rat. Weanling rats were exposed to 0, 3, 30, 100, 200, or 300 mg/kg of triclosan by oral gavage from postnatal day (PND) 23 to 53. Preputial separation (PPS) was examined beginning on PND 33. Rats were killed on PND 53, organ weights were recorded and serum was collected for subsequent analysis. Triclosan did not affect growth or the onset of PPS. Serum testosterone was significantly decreased at 200 mg/kg, however no effects were observed on androgen-dependent reproductive tissue weights. Triclosan significantly decreased total serum thyroxine (T4) in a dose-dependent manner at 30 mg/kg and higher (no observed effect level of 3 mg/kg). Triiodothyronine (T3) was significantly decreased only at 200 mg/kg, but thyroid stimulating hormone was not statistically different at any dose. Liver weights were significantly increased at 100 mg/kg triclosan and above suggesting that the induction of hepatic enzymes may have contributed to the altered T4 and T3 concentrations, but it does not appear to correlate with the T4 dose-response. This study demonstrates that triclosan exposure does not alter androgen-dependent tissue weights or onset of PPS; however, triclosan exposure significantly impacts thyroid hormone concentrations in the male juvenile rat.

Key Words: **triclosan; puberty; thyroid hormone.**

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Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a chlorinated phenolic antibacterial compound that is widely used in soaps, toothpastes, cosmetics, fabrics and plastics (McMurry *et al.*, 1998), typically at a concentration of 0.1–0.3% (Sabaliunas *et al.*, 2003). The structure of triclosan (Fig. 1) is similar to bisphenol A and dioxins (Cabana *et al.*, 2007), and it is degraded into various chlorinated dibenzo-*p*-dioxins by heat and ultraviolet irradiation (Kanetoshi *et al.*, 1987). This compound is a broad-spectrum antibacterial and antifungal agent (Bhargava and Leonard, 1996; Lyman and Furia, 1968) that blocks lipid synthesis by specifically inhibiting the enoyl-acyl carrier protein reductase (ENR or FabI) (Heath *et al.*, 1999; Newton *et al.*, 2005) in both prokaryotic and eukaryotic cells (Guillen *et al.*, 2004; Lygre *et al.*, 2003; Villalain *et al.*, 2001).

Triclosan is frequently detected in urban effluent waters (Kolpin *et al.*, 2002). Concentrations have been measured in effluents up to 37.8 μg/l (Hua *et al.*, 2005) and in surface water up to 431 ng/l (Morrall *et al.*, 2004). Triclosan is also detected in human blood plasma (Hovander *et al.*, 2002), breast milk (Dayan, 2007) and urine (Calafat *et al.*, 2008).

Triclosan has been shown to bioaccumulate and have endocrine effects in fish and amphibians (Adolfsson-Erici *et al.*, 2002; Foran *et al.*, 2000; Ishibashi *et al.*, 2004; Veldhoen *et al.*, 2006). In medaka, changes in fin length and trends in sex ratios suggested triclosan to be a weak androgen (Foran *et al.*, 2000). Other studies of medaka showed effects on hatchability of fertilized eggs (Ishibashi *et al.*, 2004). Plasma vitellogenin concentrations were reported to be significantly increased in male medaka at 20 and 100 μg/l, but not at 200 μg/l (Ishibashi *et al.*, 2004), indicating that triclosan may have estrogenic activity. However, *in vitro* studies report that triclosan showed anti-estrogenic activity in MCF7 human breast cancer cells, by inhibiting estrogen-responsive gene activity (10μM) and inhibiting 17β-estradiol–induced stimulation of growth of MCF7 cells (10μM, Gee *et al.*, 2008). Other *in vitro* studies report that triclosan inhibits transcriptional activity induced by

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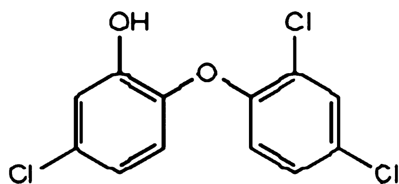

FIG. 1. Chemical structure of triclosan.

testosterone at 10μM (with no cytotoxic effects observed at this dose, Chen *et al.*, 2007) and decreases testosterone-induced CAT reporter gene activity in S115 + A mouse mammary tumor cells at 0.1μM (Gee *et al.*, 2008). However, we observed that triclosan decreased cell viability in the H295R cell line at concentrations above 3μM (Stoker and Cooper, unpublished observations).

In North American bullfrogs, triclosan exposure during the premetamorphic stage altered the rate of triiodothyronine (T3)–induced metamorphosis and thyroid hormone receptor mRNA expression (Veldhoen *et al.*, 2006). In a 4-day study using weanling female rats, a dose-dependent decrease in serum thyroxine (T4) was observed (100 mg/kg and higher), with a no observed effect level of 30 mg/kg/day (Crofton *et al.*, 2007).

If a decrease in serum T4 does indeed occur in rats, one potential mechanism of action could be an alteration in hepatic enzyme activity. P450 enzymes are important in metabolism of compounds such as toxicants, drugs and carcinogens (Zuber *et al.*, 2002), and induction of P450s in liver microsomes from rats and mice treated with triclosan has been demonstrated (Hanioka *et al.*, 1996, 1997; Jinno *et al.*, 1997; Kanetoshi *et al.*, 1992). Adult male Wistar rats dosed ip with triclosan for four days caused a dose-dependent increase in 7-benzyloxyresorufin-*O*-debenzylase (BROD) and 7-pentoxyresorufin-*O*-dealkylation (PROD) enzyme activities, as well as a CYP2B protein expression (Hanioka *et al.*, 1997), suggesting triclosan is a phenobarbital (PB)-type inducer. This hypothesis is supported by the finding that triclosan has been shown to induce hepatic PROD, BROD, and ethoxyresorufin-*O*-deethylase (EROD) activity as well as increase CYP450 2B1/2 protein expression *in vitro* (Jinno *et al.*, 1997). However, Hanioka *et al.* (1996) reported that acute exposure to triclosan caused a competitive inhibition of EROD, and noncompetitive PROD inhibition in triclosan-treated rat liver microsomes *in vitro*.

The effects of triclosan exposure during the period of pubertal development are not known and few studies have examined effects of this compound on the endocrine system in mammals. Due to the concerns about its known environmental contamination, the endocrine disrupting properties seen in various systems and the potential for triclosan to have adverse effects on human health, a male pubertal study was performed to evaluate the effects of triclosan on pubertal development and thyroid function in the male Wistar rat.

MATERIALS AND METHODS

Animals

Timed-pregnant female Wistar rats were purchased from Charles River Labs, (Raleigh, NC) and shipped to arrive on gestation day 13. Upon arrival the rats were housed one per cage in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility maintained at 22 ± 1°C, 45–52% humidity and on a 14:10-h light:dark cycle (on 0800 h, off 2200 h). Food (Purina laboratory rat chow 5001) and water were provided *ad libitum*. The day of parturition was designated postnatal day (PND) 0.

The protocol employed in this study is the Endocrine Disruptor Screening Program (EDSP) male pubertal protocol. An overview of this protocol is shown in Fig. 2 and a review of this assay can be found on the EDSP web site (http://www.epa.gov/scipoly/oscpendo/index.htm). On PND 3, the litters were randomly standardized to ten pups each to maximize uniformity in growth rates. On PND 21, all male pups were weaned and weighed to the nearest 0.1 g and weight ranked. The male pups from each litter were distributed across the treatment groups, so that each group received one male pup from each litter. Pups were also randomly assigned so that treatment groups had similar body weight means and variances. Males were housed two per cage. Two blocks of animals were treated with triclosan; block 1: control, 3, 30 and 300 ($n = 10$) mg/kg/day and block 2: control ($n = 15$), 100 and 200 (both $n = 8$) mg/kg/day.

Treatment

Triclosan was administered daily (0800–0900 h) by oral gavage at a volume of 0.5 ml per 100 g body weight beginning on PND 23 and continuing for 31 consecutive days until the day of necropsy. The body weight of each pup was recorded daily and the dose administered each day was adjusted for body weight. Triclosan (CAS#3380-34-5, Calbiochem, San Diego, CA) with 99.5% purity was used for this study. Doses were prepared in amber bottles in fresh corn oil and selected on the basis of the oral LD_{50} (5000 mg/kg) in rats. Control animals received corn oil only from PND 23 to 53 at the same time.

Pubertal Development

Preputial separation. The separation of the foreskin of the penis from the glans penis, preputial separation (PPS), is an early reliable marker of the progression of puberty in the male rat (Korenbrot *et al.*, 1977). In the present study, PPS was monitored beginning on PND 33, until all males showed separation. All males were examined once daily at approximately the same time each day. A partial separation with a thread of cartilage remaining was recorded as "partial," but only the day of complete separation was used in the data analyses.

Necropsy

The day before necropsy the rats were placed in a holding room adjacent to the necropsy room. This room was maintained under the same lighting conditions as the animal room but the holding room location allowed for each animal to be decapitated immediately (routinely less than 15 s) after removal from their home cage. On the day of necropsy (after 31-day exposure) the males were decapitated between 1000 and 1200 h and trunk blood collected. The time of necropsy within the 2-h period was balanced among dose groups to

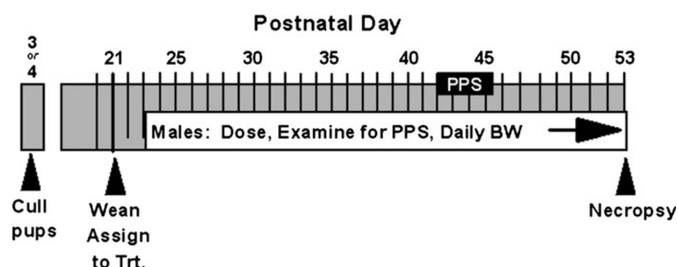

FIG. 2. The timeline for the male pubertal protocol currently used in the U. S. EPA EDSP Tier 1 Screening Battery.

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minimize stress-induced changes in hormones due to diurnal fluctuations. The anterior pituitary, liver, kidneys, adrenals, testes, ventral prostates, epididymides, levator ani-bulbocavernosus (LABC) muscle, seminal vesicles with coagulating gland (with fluid) were removed and weighed. Epididymides, left testis, and thyroid (with the bracketing trachea) were fixed in 10% neutral phosphate-buffered formalin and 24 h later transferred to 70% ethanol. Tissues were then processed in paraffin for histology (hematoxylin and eosin [H&E] stain) and pathology.

Radioimmunoassays

Blood was allowed to clot at room temperature for 1 h and then centrifuged at 1260 × g for 30 min, the serum collected and stored frozen at −80°C for subsequent hormone assays. The anterior pituitaries were frozen on dry ice and stored at −80°C for subsequent hormonal analyses.

Serum testosterone, androstenedione, luteinizing hormone (LH), T4, T3, thyroid stimulating hormone (TSH), and prolactin (PRL) radioimmunoassays were performed on the serum collected. Total T3, T4, testosterone, and androstenedione were measured using Coat-a-Count RIA kits obtained from Diagnostic Products Corporation (Los Angeles, CA). If samples were below the lowest standard a lowest detectable limit was assigned based on the sensitivity of the assay. For Androstenedione, the lowest detectable limit was 0.15 ng/ml. Several samples in the control (6/25) and 100 (5/8) mg/kg groups were assigned this value, whereas one animal in the 30 mg/kg group and all animals in the 200 (8/8) mg/kg group were assigned 0.15 ng/ml. The lowest limit of detection was 1.0 μg/dl for T4 and several samples in both the 200 (5/8) and 300 (7/10) mg/kg group were assigned this value. One animal in the 200 mg/kg group was assigned 0.2 ng/ml in the testosterone assay. All others were within detectable limits. Inter- and intra-assay coefficients of variation were testosterone, 3.5 and 2.3%, respectively; androstenedione, 1.16 and 2.6%, respectively, T4, 2.4 and 6.2%; T3, 2.4 and 1.3%, respectively.

The TSH, LH, and PRL radioimmunoaasays were performed using the following materials supplied by the National Hormone and Pituitary Agency: iodination preparation (I-9-TSH; I-10-LH; I-6-PRL); reference preparation (RP-3- all hormones); and antisera (S-6-TSH; S-11-LH; S-9-PRL). Iodination material was radiolabeled with ^{125}I (Perkin Elmer, Shelton, CT) by a modification of the chloramine-T method (Greenwood *et al.*, 1963). Labeled antigen was separated from unreacted iodide by gel filtration chromatography as described previously (Goldman *et al.*, 1986). Sample serum and pituitary homogenate were pipetted with appropriate dilutions to a final assay volume of 500 μl with 100mM phosphate buffer containing 1% bovine serum albumin (BSA). Standard reference preparations were serially diluted for the standard curves. Primary antisera (200 μl) in 100mM potassium phosphate, 76.8mM ethylenediaminetetraacetic acid, 1% BSA, and 3% normal rabbit serum (pH 7.4) were pipetted into each assay tube, vortexed, and incubated at 5°C for 24 h. One-hundred microliters of the iodinated hormone were then added to each tube, and the tube was vortexed and incubated for 24 h. A second antibody (Goat Anti-Rabbit Gamma Globulin, Calbiochem, at a dilution of 1 unit/100 μl) was then added, vortexed and incubated 24 h. The samples were centrifuged at 1260 × g for 30 min, the supernate aspirated, and the sample tube, with pellet, was counted on a gamma counter. Inter- and intra-assay coefficients of variation were TSH, 2.2 and 1.5%, respectively; LH, 11.6 and 0.12%, respectively; PRL, 8.4 and 1.0%, respectively.

Histology

Following paraffin embedding, central 4- to 6-μm sections of the testes and 4- to 6-μm longitudinal sections of epididymides were H&E stained for pathological evaluation, which was performed by Experimental Pathology Laboratories, Inc. (Research Triangle Park, NC). Testes (control and 100–300 mg/kg treated) were evaluated for gross lesions, retained spermatids, missing germ layers or types, multinucleated giant cells or sloughing of spermatogenic cells into the lumen. Epididymides from the same dose groups were also evaluated for gross lesions as well as sperm granulomas, leukocyte infiltration, aberrant cell types within the lumen, or absence of clear cells in the cauda epithelium.

In addition, thyroid glands from the control and 100–300 mg/kg groups were histologically evaluated. Thyroids were placed longitudinally in one block, embedded in paraffin and cut into 4- to 6-μm transverse sections. The central thyroid sections were stained with H&E, and three step-sections were subjectively evaluated for follicular epithelial height and colloid area as previously described (Capen and Martin, 1989), using a five-point grading scale (1 = shortest/smallest; 5 = tallest/largest, respectively, http://www.epa.gov/scipoly/oscpendo/index.htm) and any abnormalities or lesions noted. The range of scores was first established using the complete set of slides. The slides were then randomly mixed and evaluated blind for scoring based on the range established.

Hepatic Enzyme Activity

All chemicals used in enzyme assays were purchased from Sigma Chemical Co. (St Louis, MO) and were of the highest grade commercially available. Liver microsomal fractions were prepared as described previously (DeVito *et al.*, 1993). Microsomal protein concentrations were determined using a protein assay kit (Bio-Rad, Richmond, CA) with BSA as the standard. Hepatic microsomal UDPGT (uridine diphosphoglucuronosyl transferase) activity was assayed based on the method of Visser *et al.* (1993) as modified by Zhou *et al.* (2001), to determine any induced clearance of T4 by glucuronidation. ^{125}I-labeled T4 was purchased from NEN Life Science Products, Inc. (Boston, MA). The calculated UDPGT activity was expressed as pmol T4-G per mg protein per minute and graphically represented as percentage of control group mean activity. The limit of detection for UDPGT was 0.05 pmol T4-G/mg protein.

Hepatic microsomal EROD (a marker for CYP1A1 activity and induced by dioxin) and PROD (a marker of CYP2B activity) activities were assayed using a previously described method (DeVito *et al.*, 1993). All substrate concentrations were 1.5nM. Both EROD and PROD values were calculated as pmol resorufin per mg protein per min and graphically represented as percentage of control activity. Frozen microsomes from rats orally exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (10 μg/kg) plus 300 mg/kg Arochlor were thawed and used as positive controls for both induction of hepatic EROD and PROD (Craft *et al.*, 2002). The potential that triclosan can photodegrade into dioxin intermediates was of particular concern in this study as dioxin may influence the concentration of thyroid hormones and an increase in EROD may indicate dioxin contamination.

Statistics

Controls from each block were analyzed using a *t*-test to determine if the two groups were significantly different. When no differences existed, controls from both blocks were combined and blocks were analyzed together. When control means (from different blocks) were different, data were analyzed to its relative control and expressed as percent of control. All liver enzyme data were expressed as percent of control. All data were analyzed for age and treatment effects by ANOVA using the General Linear Model (GLM) procedures (SAS, version 8.1, SAS Institute, Inc., Cary, NC), and for homogeneity of variance using Bartlett's test (GraphPad InStat, GraphPad Software, San Diego, CA). When significant treatment effects ($p < 0.05$) were indicated by GLM, Dunnett's *t*-tests were used to compare each treatment group with the control.

To predict lower dose-effects on T4 and PROD, benchmark dose (BMD), and lower-bound confidence estimates were determined for alterations in serum T4 and enzymatic activity of PROD using U.S. Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS Version 1.40d). The Hill model was chosen to fit these continuous data according to the following equation:

$$y(x) = e_0 - \frac{e_{\max} x^n}{b^n + x^n}$$

where$y$ is the response; x is the dose; e_o is the estimated background response level; $e_{\max}$ is the maximal increase or decrease from background; b is the ED_{50}; n is the shape parameter, and is constrained to be greater than 0. The benchmark dose was set at a 20% decrease in T4 concentration (http://www.epa.gov/ncea/pdfs/bmds/BMD-External_10_13_2000.pdf) to balance biological and

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statistical significance and a 50% increase for hepatic enzyme activity (Zhou *et al.*, 2001). The 95% lower bound for the BMD (BMDL) was calculated as the lower end of a two-sided 90% confidence interval.

RESULTS

Weight and General Toxicity

No visible signs of toxicity (salivation, diarrhea, labored breathing, etc.) were observed in any of the treated animals following exposure to triclosan. In addition, we observed no treatment-related changes in growth or necropsy body weight (Fig. 3), and kidney and adrenal weights were not affected following triclosan exposure (Table 1). However, liver weights were significantly increased from 100 to 300 mg/kg (114–119% of control, $p < 0.05$, Table 1). Anterior pituitary weights were increased in a non-dose-dependent manner, with significance only at 3 and 300 mg/kg of triclosan ($p < 0.05$, Table 1).

Pubertal Development and Reproductive Endpoints

Triclosan exposure did not affect the age of onset of PPS at any of the doses evaluated (Fig. 4). In addition, the ventral prostate, seminal vesicles, LABC, epididymal and testicular weights of the triclosan-treated rats were not statistically different from the control (Table 1). However, there was a significant decrease in the serum testosterone concentration at 200 mg/kg, but not at 300 mg/kg, as compared with the controls ($p < 0.05$, Fig. 5A). Androstenedione was not significantly different in triclosan-treated animals as compared with controls (Fig. 4B); however the hormone profile was similar to that of testosterone. The serum and pituitary LH and PRL were not different from controls (data not shown). Histological evaluation did not reveal any significant treatment-induced lesions or alterations in either testes or epididymides following triclosan exposure. However, a few animals in the higher dose groups showed testicular degeneration, which is characterized by the presence of multinucleated giant cells (a marker of germinal cell degeneration) within the seminiferous tubule epithelium. This change was noted as minimal and was not correlated with decreased testosterone or testis weight in the individual animals.

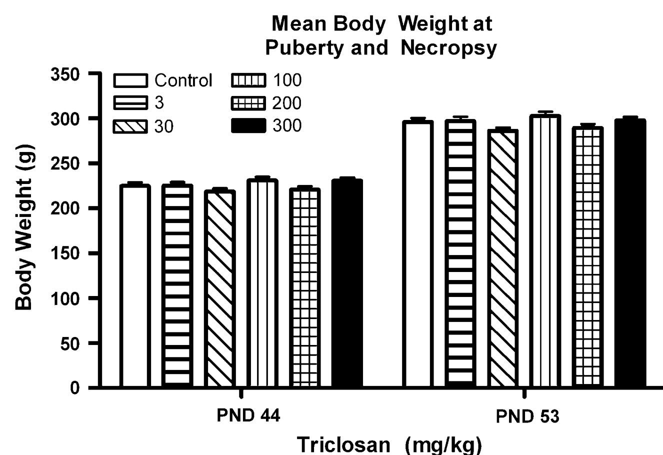

FIG. 3. The mean body weights at puberty (PND 44) and necropsy (PND 53) following postweaning exposure to triclosan in the male Wistar rat.

Thyroid Function

Thyroid hormones. The mean concentration of serum T4 was significantly decreased in a dose-dependent manner from 30 to 300 mg/kg following the 31-day exposure, with no effect at 3 mg/kg ($p < 0.05$, Fig. 6A). The control means of serum T4 were statistically different, therefore data are presented as observed in blocks (Fig. 6A) and are presented as percent of control (Fig. 6B) which displays the dose-response and was used for the BMD modeling. Although there were apparent decreases at 100 mg/kg and above, mean serum T3 was only significantly decreased at 200 mg/kg ($p < 0.05$, Fig. 6C). The 31-day exposure of triclosan did not result in a significant difference in serum TSH as compared with controls (Fig. 6D).

Histology. There was no significant change in the follicular epithelial height in any of the dose groups of triclosan following the 31-day exposure. There was a significant decrease in colloid area (colloid depletion) in the thyroid gland sections of the 300 mg/kg dose groups, but not in any other dose groups.

Enzyme activity assays. UDPGT activity was not significant different than controls (Fig. 7A). PROD activity was significantly increased at 300 mg/kg (400-fold, $p < 0.05$, Fig. 7B). In contrast, EROD activity was significantly decreased at all doses (3–300 mg/kg, $p < 0.05$, Fig. 7C).

BMD analysis. Analysis of T4 concentrations and PROD enzymatic activity was performed using EPA's BMDS software to determine the BMD and BMDL. Because the doses were run in two blocks, the datasets were combined after T4 was scaled to control values in each block. For a benchmark response (BMR) of 20%, the BMD was 14.51 mg/kg and the BMDL was 7.23. At 25% BMR, the BMD was 19.69 mg/kg, and the BMDL was 11.14 mg/kg. PROD analysis was only done on one block, and setting the BMR at 50%, the BMD was 14.31 mg/kg and the BMDL was 10.57 mg/kg.

DISCUSSION

The current study clearly demonstrates that triclosan exposure in the juvenile male rat suppresses T4 concentrations in a dose-dependent manner. The changes were dramatic, with a decrease of 47% at the 30 mg/kg dose and an 81% decrease at the 300 mg/kg dose, and were not accompanied by changes in body weight. T3 concentrations were not altered to the same extent as T4, and the 31-day exposure did not elicit a feedback increase in TSH. In the rat, TSH induces the thyroid to

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TABLE 1
Triclosan Exposure Effects on Growth of Reproductive Tract and Gross Body Tissue Weights

Tissue	Control	3 mg/kg	30 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg
Pituitary (mg)	8.26 ± 0.16	9.28 ± 0.34*	8.77 ± 0.34	8.44 ± 0.33	8.51 ± 0.28	9.23 ± 0.28*
L. testes (g)	1.52 ± 0.05	1.48 ± 0.03	1.46 ± 0.03	1.54 ± 0.05	1.61 ± 0.03	1.51 ± 0.05
L. epididymis (g)	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
Ventral prostate (g)	0.23 ± 0.01	0.24 ± 0.02	0.22 ± 0.01	0.28 ± 0.02	0.24 ± 0.02	0.23 ± 0.01
Seminal vesicle (g)	0.59 ± 0.02	0.56 ± 0.03	0.55 ± 0.04	0.66 ± 0.07	0.63 ± 0.03	0.58 ± 0.04
LABC (g)	0.53 ± 0.01	0.54 ± 0.03	0.50 ± 0.02	0.53 ± 0.02	0.55 ± 0.03	0.56 ± 0.02
L. kidney (g)	1.23 ± 0.02	1.20 ± 0.03	1.16 ± 0.03	1.28 ± 0.04	1.26 ± 0.04	1.28 ± 0.04
L. adrenal (mg)	25.05 ± 0.56	25.67 ± 1.11	23.68 ± 2.04	23.71 ± 1.08	22.97 ± 3.24	23.97 ± 1.66
Liver (g)	14.50 ± 0.27	15.40 ± 0.56	14.52 ± 0.33	17.39 ± 0.56*	17.18 ± 0.79*	16.53 ± 0.33*

Note. Mean tissue weight ± SEM, $*p < 0.05$ as compared with controls.

synthesize T4 which is then 5′-monodeiodinated to the more biologically active T3. The rate of TSH release is controlled by the amount of thyrotrophin-releasing hormone released by the hypothalamus, as well as by circulating concentrations of T3 and T4 (Capen, 1997). The colloid area was decreased in the high dose group (300 mg/kg), indicating there was a feedback effect resulting in increased release of active thyroid hormones into the circulation (Capen, 1997). It is unclear why T3 was significantly increased in the 3 mg/kg group, however, because there were no other corresponding hyperthyroidism signs (such as increased T4), this short-term exposure would not likely result in a biologically relevant effect. It is possible that there is a sufficient concentration of T4 available for deiodination to T3 at the time of necropsy, or a preferential increase in T4 catabolism and clearance of T4 and not T3 (Zhou *et al.*, 2002), but a more extended dosing regimen may result in decreased T3 and increased TSH.

A disruption in thyroid hormone homeostasis can be the result of altered T4 clearance. Thus, decreased serum T4 may reflect an increase in glucuronidation after conjugation by UDPGTs (Saghir *et al.*, 2008). In this study, triclosan exposure significantly increased liver weights at 100 mg/kg and above, which is indicative of induction of hepatic enzymes. For these reasons, we measured several hepatic enzymes, including UDPGT, in the second block of animals. T4 concentrations

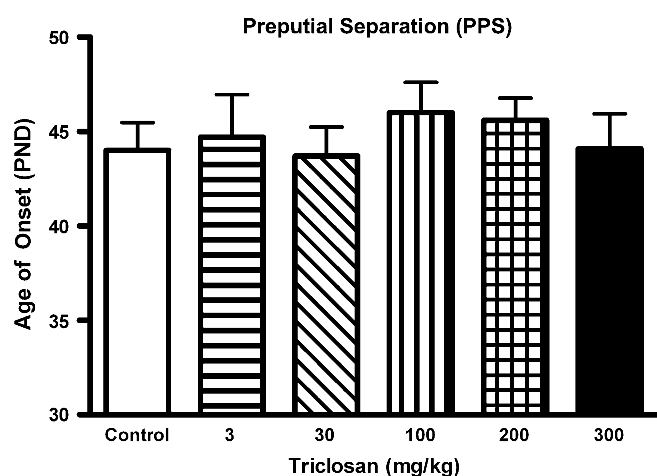

FIG. 4. The mean age of PPS following postweaning exposure to triclosan in the male Wistar rat.

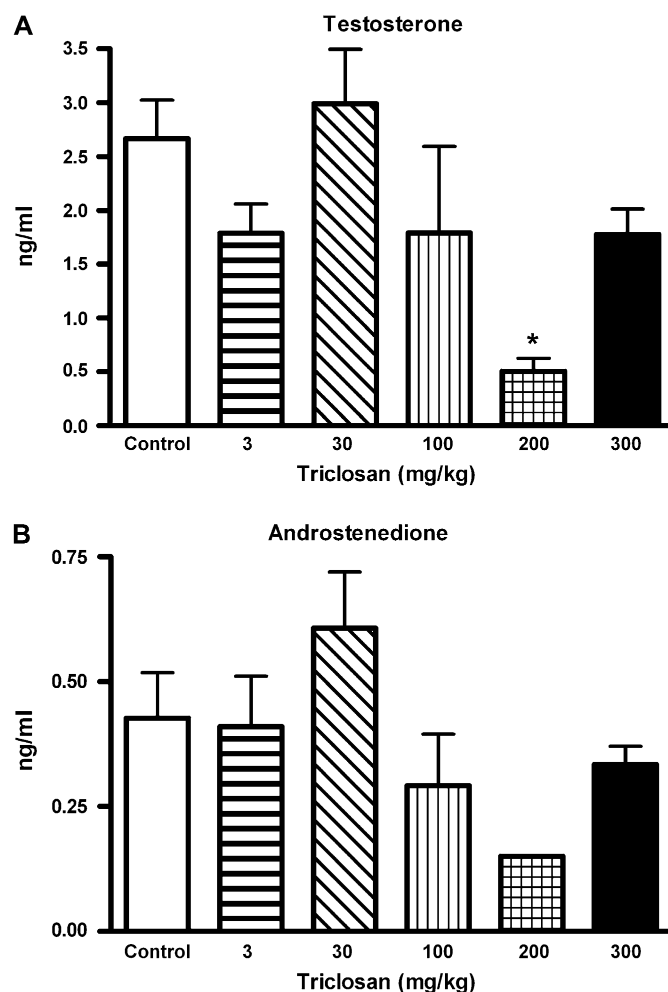

FIG. 5. The effect of a 31-day exposure to triclosan (mg/kg/day) on mean (A) total serum testosterone and (B) serum androstenedione concentrations in the male Wistar rat. $*p < 0.05$ as compared with control mean.

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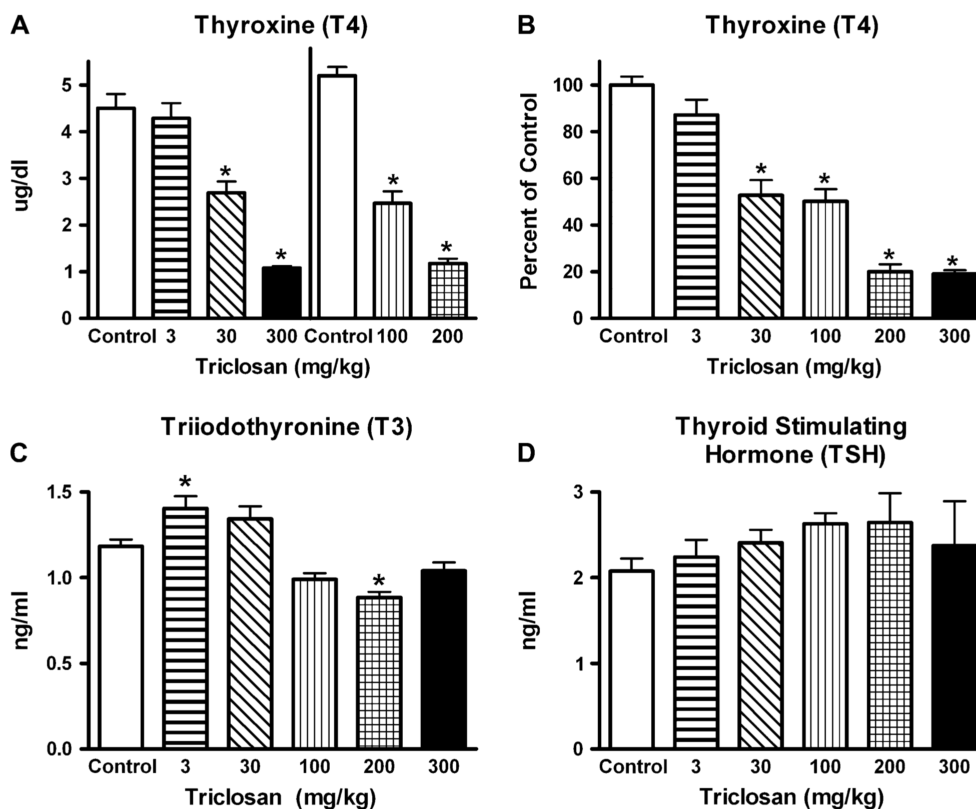

FIG. 6. The effect of a 31-day exposure to triclosan (mg/kg/day) on (A) mean total serum T4, (B) mean total T4 shown as percent of control, (C) mean total T3, and (D) mean serum TSH concentrations in the male Wistar rat. $*p < 0.05$ as compared with control mean.

were reduced by 81% at the 300 mg/kg dose compared with controls. We did observe that the mean activity of UDPGT activity nearly doubled compared with controls at this dose; however, this difference was not statistically significant. Thus, without further study we cannot conclude that differences in T4 metabolism contributed to the decreased T4. Also, the level of change in UDPGT at 30 mg/kg triclosan again indicated a discrepancy between the enzyme activity and the observed decrease in T4. This type of inconsistent relationship between UDPGT induction and T4 concentrations has been reported previously (Craft *et al.*, 2002; De Sandro *et al.*, 1992; Hood and Klaassen, 2000) and is likely due to the inherent variability in this assay.

We also examined the hepatic enzymatic activities of PROD and EROD, as others have reported changes following exposure to triclosan (Hanioka *et al.*, 1996; Jinno *et al.*, 1997). In this study, there was a significant non-dose-dependent decrease in EROD activity and a significant increase in PROD (a marker for CYP2B) activity at the highest dose. The lack of any increase in EROD activity suggests that the triclosan was not contaminated with dioxins, as dioxin activates the aryl hydrocarbon receptor (Schuur *et al.*, 1998). In addition, the decrease in EROD activity is in agreement with Hanioka *et al.* (1996), who concluded that triclosan acts as a competitive inhibitor of EROD activity.

Triclosan did not alter the age of onset of puberty or the development of the androgen-dependent tissues, even though there was a 60% decrease in serum testosterone at 200 mg/ml triclosan as compared with control levels. However, no decrease in serum testosterone was seen at 300 mg/kg dose. This discrepancy could be due to either the 200 mg/kg dose group not being biologically significant, and thus is why we did not observe any changes in androgen-dependent endpoints, or this could be due to block variability. The testosterone serum sample and tissue weights were taken on PND 53 and it is possible that these animals may have had sufficient concentrations of dihydrotestosterone (product of testosterone) for the development of androgen-dependent tissues (Blohm *et al.*, 1986). It is also conceivable that the testosterone concentrations were not sufficiently decreased at the time of pubertal onset, which allowed normal progression of puberty and reproductive tract development.

It is well known that when hypothyroidism is induced in the early neonatal period there are alterations in sexual maturation, testicular and reproductive tract development, and gonadotrophin and steroid concentrations (Francavilla *et al.*, 1991; Kirby *et al.*, 1992, 1997; Valle *et al.*, 1985). However, in the current study there were no apparent effects of triclosan on puberty or the development of the male reproductive tract, despite the fact that triclosan induced hypothyroxinemia in the males. This

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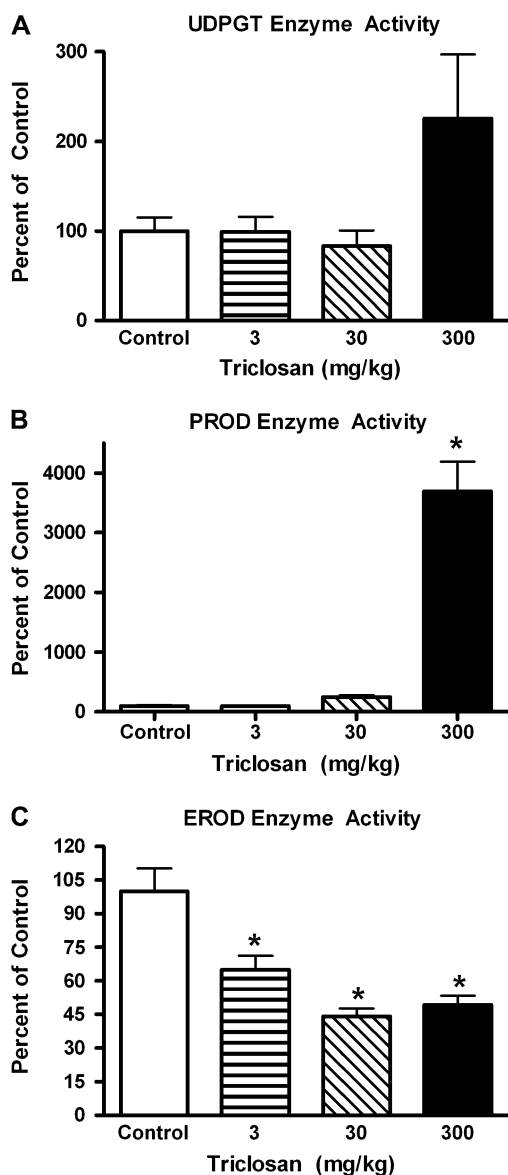

FIG. 7. Liver enzyme activity after 31-day triclosan (mg/kg/day) exposure in the male pubertal rat. (A) UDPGT, (B) PROD, and (C) EROD levels. *$p < 0.05$ as compared with control means.

agrees with studies which show that testicular function does not appear to be affected if hypothyroidism is induced during the postweaning period or in adulthood (Francavilla *et al.*, 1991; Kalland *et al.*, 1978; Maqsood, 1954; Stoker *et al.*, 2006). Thus, the period of life at which hypothyroidism is present, relative to the maturity of the reproductive system, is key in explaining the difference of effect on the male reproductive system.

In conclusion, this study clearly demonstrates that triclosan suppresses serum T4 concentrations in a dose-dependent manner in the juvenile male rat. Because triclosan is present in such a variety of personal care and household products, in the ecosystem, and in human body fluids, there is a potential concern for adverse effects on human health. Although interspecies differences exist, effects on the thyroid of this magnitude, particularly T4, should be carefully evaluated due to thyroid hormone status of pregnant women on the future neuropsychological development of the child (Haddow *et al.*, 1999; Morreale de Escobar *et al.*, 2000). Further studies are in progress in our laboratory examining the effects of triclosan on steroidogenesis in vitro and on female reproductive development.

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