

Short communication

Short-term *in vivo* exposure to the water contaminant triclosan: Evidence for disruption of thyroxine[☆]

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Abstract

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a chlorinated phenolic antibacterial compound found as an active ingredient in many personal care and household products. The structural similarity of triclosan to thyroid hormones and recent studies demonstrating activation of the human pregnane X receptor (PXR) and inhibition of diiodothyronine (T_2) sulfotransferases, have raised concerns about adverse effects on thyroid homeostasis. The current research tested the hypothesis that triclosan alters circulating concentrations of thyroxine. The hypothesis was tested using a 4-day oral triclosan exposure (0–1000 mg/kg/day) in weanling female Long-Evans rats, followed by measurement of circulating levels of serum total thyroxine (T_4). Dose-dependent decreases in total T_4 were observed. The benchmark dose (BMD) and lower bound on the BMD (BMDL) for the effects on T_4 were 69.7 and 35.6 mg/kg/day, respectively. These data demonstrate that triclosan disrupts thyroid hormone homeostasis in rats.

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1. Introduction

Triclosan is a chlorinated phenolic antibacterial compound used as an active ingredient in many personal care and household products (Bhargava and Leonard, 1996; Dayan, 2007). The bactericidal activity of triclosan results from inhibition of the enoyl-acyl carrier protein reductase (ENR) of Gram-negative and Gram-positive bacteria, thereby preventing bacterial lipid biosynthesis (McMurry et al., 1998; Levy et al., 1999; Heath and Rock, 2000). The ubiquitous usage in consumer products has led to widespread environmental contamination evidenced

by detection of triclosan in wastewater effluent in the US, UK, Japan, and other countries (Kanda et al., 2003; Loraine and Pettigrove, 2006; Nakada et al., 2006). Triclosan has been found in fish exposed to wastewater treatment plant effluents (Adolfsson-Erici et al., 2002), in human breast milk samples (Adolfsson-Erici et al., 2002; Dayan, 2007), and in urine samples from young girls (Wolff et al., 2007).

The structural similarity to thyroid hormones (THs, Fig. 1) and evidence for human exposures have increased concern about possible endocrine disrupting effects of triclosan (Veldhoen et al., 2006). Foran et al. (2000) studied the potential estrogenic effects of triclosan in *Oryzias latipes* (medaka) and concluded that it is not estrogenic, but may be a weak androgen. Vitellogenin induction, a biomarker for endocrine activity, has been found in medaka and *Xenopus laevis* (clawed frog) and was attributed to weak estrogenic activity of a triclosan metabolite (Ishibashi et al., 2004). There is also evidence that triclosan may disrupt thyroid mediated development. Exposure of *Rana catesbeiana* (North American bullfrog) to triclosan resulted in accelerated thyroid hormone-dependent metamorphosis and

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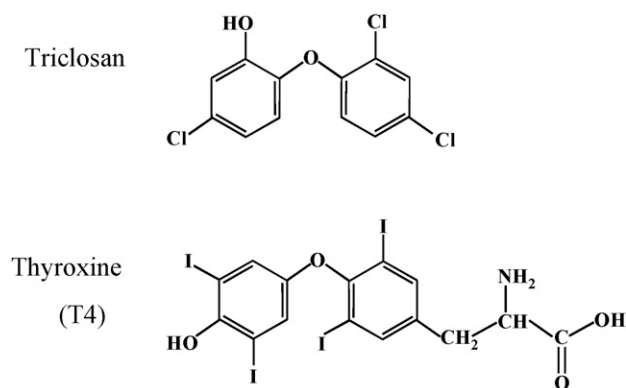


Fig. 1. The structures of triclosan and thyroxine. Both chemicals are halogenated biphenyl ethers.

decreased expression of thyroid receptor beta mRNA (Veldhoen et al., 2006). Triclosan also affects a number of biochemical processes important for thyroid hormone homeostasis. Triclosan induces hepatic EROD and PROD activity, and cytochromes P450 2B1/2 (Hanioka et al., 1996; Jinno et al., 1997). Inhibition of iodothyronine sulfotransferase activity was demonstrated in both rat and human hepatic microsomes (Schoor et al., 1998; Wang et al., 2004). Furthermore, triclosan activates pregnane-X receptor (PXR)-mediated transcription in transient transfection studies with human PXR in a human hepatoma cell line (Jacobs et al., 2005). PXR activation leads to increases in Phase I and II hepatic biotransformation enzymes, including glucuronidases that catabolize thyroid hormones (THs) (You, 2004). These enzymes are important in maintaining euthyroid hormone concentrations (Capen, 1994; Hill et al., 1998; DeVito et al., 1999). Developmental disruption of circulating concentrations of THs leads to adverse outcomes including altered neurodevelopment (Zoeller and Crofton, 2000; Mitchell and Klein, 2004; Morreale de Escobar et al., 2004; Koibuchi and Iwasaki, 2006). Currently it is unknown whether triclosan affects circulating concentrations of thyroid hormones.

The present work tested the hypothesis that *in vivo* triclosan exposure alters circulating levels of thyroxine (T₄). T₄ has been shown to be a sensitive biomarker for disruption of thyroid homeostasis in rats (DeVito et al., 1999). A 4-day exposure to rats was employed as this protocol has been previously used to detect thyroid disrupting chemicals (Zhou et al., 2001; Craft et al., 2002; Crofton, 2004), and triclosan upregulated rat hepatic microsomal enzymes after a 4-day exposure (Hanioka et al., 1996).

2. Materials and methods

2.1. Animals

Long-Evans female rats at 21–23 days of age were obtained from Charles River Laboratories Inc. (Raleigh, NC). These 80 rats were allowed 2–4 days of acclimation in an American Association for Accreditation of Laboratory Animal Care (AALAC)-approved animal facility prior to treatment. Animals were housed two per standard plastic hanging cage (45 cm × 24 cm × 20 cm), with heat sterilized pine shavings bedding (Northeastern Products Corp., Warrenburg, NY). Colony rooms were maintained at 21 ± 2 °C with 50 ± 10% humidity on a photo-period of 12-h light:12-h dark (0600–1800 h). Food (Purina Rodent

Chow #5001, Barnes Supply Co., Durham, NC) and tap water were provided *ad libitum*. All animal procedures were approved in advance by the Institutional Animal Care and Use Committee of the National Health and Environmental Effects Research Laboratory of the US EPA.

2.2. Chemicals and treatment

Triclosan (5-chloro-2-(2,4-dichloro-phenoxy)phenol, 99.6%, CAS#3380-34-5, Lot#06415CD) was obtained from Aldrich Chemical Company (St Louis, MO, Cat#524190-10G). The dosing solutions at concentrations of 0, 10, 30, 100, 300 and 1000 mg/kg/day were prepared by mixing triclosan with corn oil and sonicating for 30 min at room temperature. The 1000 mg/kg/day dose precipitated within 12 h, therefore, it was sonicated daily before use. Dosing volume was 1.0 ml corn oil/kg body weight. Rats at 27–29 days of age were exposed via oral gavage for 4 consecutive days ($n = 16$ for all groups except 10 and 1000 mg/kg/day where $n = 8$ /group). All dosing was conducted between 0900 and 1000 h. Young female rats were chosen to be consistent with previous work in our lab using the short-term 4-day dosing model (Zhou et al., 2001; Craft et al., 2002; Crofton, 2004). Rats were randomly assigned to treatment groups by counter-balancing body weights. Body weights were recorded and dosing volumes adjusted daily. Approximately 24 h after the final treatment rats were moved into a holding room. Prior to termination, rats were weighed, acclimated for 30 min, and then taken to a separate room with a separate air supply and killed by decapitation (0900–1100 h). Trunk blood was collected, after decapitation, into serum separator tubes (Becton Dickinson, 36-6154). Serum was obtained after clotting whole blood for 30 min on ice, followed by centrifugation at 1278 × *g* at 4 °C for 30 min and stored at –80 °C until analysis. Livers were weighed, quick frozen in liquid nitrogen and stored at –80 °C for future analyses.

2.3. Thyroxine assay

Serum total T₄ was measured in duplicate by standard solid-phase Coat-A-Count radioimmunoassay (RIA) kits (Siemens Medical Solutions Diagnostics, formerly Diagnostic Products Corporation, Los Angeles, CA). Intra-assay coefficient of variation for the triclosan data was 6.51%. The inter-assay coefficient-of-variance for six separate assays over a 1-year-period of time was 7.68%.

2.4. Data analysis

All data were analyzed as ng T₄/ml serum. The mean (±S.E.) for the control group is listed in the figure legend. Statistical significance was tested with a one-way ANOVA followed by Duncan's Multiple Range Test, with acceptable significance level set at $p > 0.05$. All data are represented as mean ± S.E. ($n = 16$ for 0, 30, 100, and 300 mg/kg/day doses and $n = 8$ for 10 and 1000 mg/kg/day doses). No-observed-effects-levels (NOELs) were defined as the lowest dose without a significant effect.

Benchmark dose (BMD) and lower-bound confidence limit (BMDL) estimates were determined for alterations in serum thyroxine concentrations using USEPA Benchmark Dose Software (BMDS Version 1.40d). The Hill model was chosen to fit these continuous data according to the following equations:

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

where y is the response; x is the dose; e_0 is the estimated background response level; e_{\max} is the maximal increase or decrease from background; b is the ED₅₀; n is the shape parameter. The benchmark response was set at a 20% decrease in T₄ concentration (Zhou et al., 2001). The BMDs were calculated from the Hill model fits to the data. The lower-bound confidence limit (BMDL) was calculated as the 95% lower confidence interval.

3. Results and discussion

No clinical signs of toxicity were observed in any animals following the 4-day oral triclosan exposure. No treatment-

Table 1
Doses, group sizes, body weights, and liver weights

Triclosan dose (mg/kg)	N	Body weight gain ^a , g (mean ± S.E.)	Liver weight, g (mean ± S.E.)	Liver-body weight ratio ^b (mean ± S.E.)
0 (vehicle)	16	26.6 ± 0.73	4.85 ± 0.18	0.050 ± 0.0006
10	8	26.1 ± 1.05	4.46 ± 0.26	0.053 ± 0.0007
30	16	28.5 ± 0.99	4.97 ± 0.23	0.051 ± 0.0005
100	16	26.7 ± 1.18	4.75 ± 0.25	0.050 ± 0.0004
300	16	27.2 ± 2.01	5.04 ± 0.26	0.052 ± 0.0006
1000	8	28.0 ± 1.31	6.26 ± 0.14*	0.056 ± 0.0008*

^a Notes: Weight gain between Day 1 and Day 5.

^b Absolute liver weight divided by absolute body weight.

* Significantly different from vehicle control group, $p < 0.05$.

related effects on body weight gain were found [$F(5,74) = 0.40$, $p > 0.8443$], Body weight gains were between 26 and 28 g for all treatments. There was a significant increase in liver weight [$F(5,74) = 4.23$, $p > 0.002$] of 15% in the 1000 mg/kg group (Table 1). No other treatments were different than control.

Serum thyroxine concentrations were reduced in a dose dependent manner (Fig. 2). Serum T_4 was decreased 28, 34 and 53% following treatment with 100, 300, and 1000 mg/kg/day triclosan, respectively. This shows a significant main effect of treatment [$F(5,74) = 18.32$, $p > 0.0001$]. The NOEL for triclosan was 30 mg/kg/day. The goodness-of-fit p -value for the Hill model was 0.2232. The BMD was 69.7 mg/kg/day and the BMDL was 35.6 mg/kg/day.

These data clearly demonstrate for the first time that triclosan decreases circulating concentrations of T_4 in rats. The mechanisms by which triclosan reduces serum T_4 are presently unknown, however, previous research suggests more than one possible mechanism. Evidence that PXR is activated by triclosan (Jacobs et al., 2005) suggests that decreases in T_4 may result from increases in the sulfonation or glucuronidation activity (Visser, 1996) via PXR-linked genes (Kretschmer and Baldwin, 2005). This hypothesis is consistent with triclosan-induced up-

regulation of P450 2B isozymes (Hanioka et al., 1996; Jinno et al., 1997). Activation of PXR is known to result in induction of cytochrome P450s and uridine diphosphoglucuronyl transferases (UGTs). UGTs are a family of isozymes, some of which glucuronidate THs, with subsequent excretion of the TH-glucuronide into the bile (Chen et al., 2003; Mackenzie et al., 2005; Zhou et al., 2005). This is not consistent with a report that triclosan is a weak inhibitor of acetaminophen and bisphenol A glucuronidation (Wang et al., 2004). However, morphine glucuronidation was not affected, suggesting an isoform-specific inhibition (Wang et al., 2004). PXR activation has also been shown to regulate hepatic transporters (Klaassen and Slitt, 2005). These transporters can increase influx of T_4 into the liver, and efflux of free and glucuronidated T_4 into the bile (Friesema et al., 2005). A combination of mechanisms may explain changes in TH levels, specifically the dose-dependent decrease in T_4 observed in this study. Future research on triclosan should characterize its effects on thyroid homeostasis and the relevance of these findings to humans.

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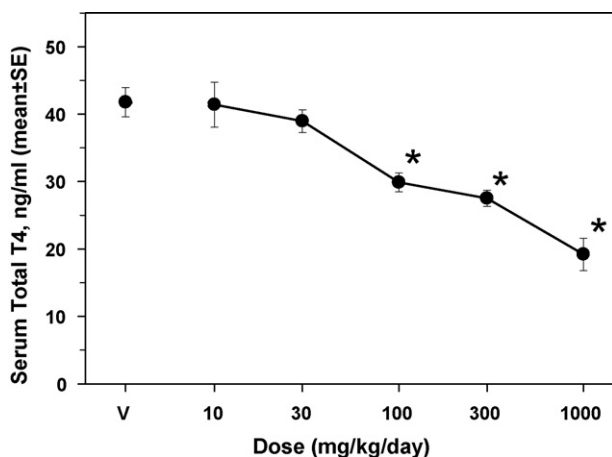


Fig. 2. Short-term oral exposure to triclosan decreased serum total T_4 concentrations. Data are expressed as group mean values (\pm S.E.). V, corn oil vehicle-only control. Absolute T_4 group mean value for the control group (0 mg/kg/day triclosan) is 41.8 ± 2.2 ng total T_4 /ml serum. The symbol (*) shows significant difference from the respective control ($p < 0.05$); $n = 16$ for all groups except 10 and 1000 mg/kg/day where $n = 8$ /group.

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