Reproductive Toxicology 65 (2016) 394–401

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Triclosan elevates estradiol levels in serum and tissues of cycling and peri-implantation female mice

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ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 17 August 2016
Accepted 9 September 2016
Available online 13 September 2016

Keywords:
Estradiol
Estrogens
Sulfotransferase
Triclosan
Uterus

ABSTRACT

Triclosan, an antimicrobial agent added to personal care products, can modulate estrogenic actions. We investigated whether triclosan affects concentrations of exogenous and endogenous estradiol. Female mice were given injections of triclosan followed by 1 μCi tritium-labeled estradiol. Mice given daily 2-mg triclosan doses (57.9 mg/kg/dose) showed significantly elevated radioactivity in tissues and serum compared to controls. A single dose of 1 or 2 mg triclosan increased radioactivity in the uterus in both cycling and peri-implantation females. We also measured natural urinary estradiol at 2–12 h following triclosan injection. Unconjugated estradiol was significantly elevated for several hours following 1 or 2 mg of triclosan. These data are consistent with evidence that triclosan inhibits sulfonation of estrogens by interacting with sulfotransferases, preventing metabolism of these steroids into biologically inactive forms. Elevation of estrogen concentrations by triclosan is potentially relevant to anti-reproductive and carcinogenic actions of excessive estrogen activity.

1. Introduction

Triclosan (CAS 3380-34-5) is a synthetic biocide designed to inhibit bacterial reproduction by interacting with enoyl-acyl carrier protein reductase enzymes [1]. It is added to many consumer and household products, including soaps, dish sponges, cosmetics, deodorants, toothpastes, mouthwashes, clothing, and children's toys [2–4]. Dermal contact with these products leads to rapid absorption of triclosan into the body through the skin [5,6], while oral ingestion leads to uptake through the gastrointestinal tract [7]. Based on the 2003–2004 U.S. National Health and Nutrition Examination Survey (NHANES), 74.6% of the 2517 human urine samples contained detectable levels of triclosan, with concentrations ranging from 2.4 to 3790 μg/l [8]. Detection frequency of urinary triclosan in the U.S. population reached a peak between 2007 and 2008 at 80.8%, but has since fallen to 72.0% as of 2011–2012 [9]. Similarly, mean urinary triclosan concentrations in the U.S. population peaked in 2005–2006 at 18.8 μg/L but fell to 12.46 μg/l as of 2011–2012 [9]. Triclosan has also been detected in human serum [10,11], plasma [12], breast milk [12,13], and adipose and liver tissue [14].

Triclosan has known estrogenic effects, including stimulating breast and ovarian cancer cell growth *in vitro* [15,16] and magnifying the effects of ethinyl estradiol in rodent uterotrophic assays [17,18]. However, the mechanisms underlying these effects are not well understood. Triclosan binds to both conventional estrogen receptor (ER) subtypes, ERα and ERβ [19,20]. Thus, exposure to triclosan may induce estrogenic effects by directly activating ER. Triclosan also potently inhibits hepatic sulfotransferase activity [21–23], thereby reducing sulfonation of endogenous estrogens such as 17β-estradiol (E_2) and xenoestrogens such as bisphenol A (BPA) [23]. Thus, exposure to triclosan may potentiate *in vivo* estrogenic effects by preventing metabolism of estrogens to their biologically inactive forms.

Previous work in this laboratory demonstrated *in vivo* interactions between triclosan and BPA. When mice were given a single dose of triclosan ranging from 0.2–18 mg, greater levels of ^{14}C-BPA were detected in serum and tissues including the heart, lung, muscle, uterus, ovaries, and epididymides, than in animals given ^{14}C-BPA alone [24]. Other studies indicated that either triclosan or BPA can disrupt blastocyst implantation in inseminated female mice [25–29], and that doses of BPA or triclosan that were insufficient on their own to have effects could disrupt implantation when the two substances were given concurrently [29]. These findings are consistent with the notion that triclosan inhibits BPA conjugation [23], permitting higher levels of BPA to interact with ER in tissues such as the uterus.

Whereas BPA is a weakly estrogenic environmental chemical, E_2 is the most potent natural estrogen. Any deviations from normal E_2 levels might lead to adverse health effects, as estrogen levels

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http://dx.doi.org/10.1016/j.reprotox.2016.09.004
0890-6238/

are tightly regulated and play critical roles in development, fertility, and behavior [30]. Of especial importance to human health is the consistent finding that elevated E_2, often through hormone-replacement therapy, is associated with an increased risk of breast [31], endometrial [32], and ovarian [33] cancers. Also, in inseminated females, minute elevations in estrogen activity can impede blastocyst implantation, leading to pregnancy failure [30,34]. Given the fact that triclosan exposure is ubiquitous, its potential capacity to modulate estrogen levels or activity *in vivo* is worthy of investigation. Here we investigated the impact of single or repeated triclosan injections on concentrations of exogenous tritium-labeled estradiol (^{3}H-E_2) and endogenous urinary E_2. We hypothesized that a single injection of triclosan would elevate ^{3}H-E_2 levels in reproductive tissues of cycling and peri-implantation female mice, and that this effect would be more pronounced with repeated triclosan injections over multiple days. We also hypothesized that triclosan administration would increase endogenous E_2 concentrations as measured in urine.

2. Materials and methods

2.1. Animals and housing

Female mice aged 3–5 months were of CF–1 strain and obtained from Charles River (Kingston, NY). Animals were housed in standard polypropylene cages measuring $28 \times 16 \times 11$ ($l \times w \times h$) cm with wire tops allowing *ad libitum* access to food (8640 Teklad Certified Rodent Chow; Harlan Teklad, Madison, WI) and water, except where otherwise stated. The colony was maintained at 21 °C with a reversed 14 h light:10 h darkness cycle. All procedures adhered to the standards of the Canadian Council on Animal Care and were approved by the Animal Research Ethics Board of McMaster University.

2.2. Chemicals and materials

Triclosan (CAS 3380-34-5, 5-chloro-2-[2,4-dichlorophenoxy]phenol, ≥97% purity) was obtained from Sigma-Aldrich, St. Louis, MO. SOLVABLE solubilization cocktail, Ultima Gold scintillation cocktail, 8 ml midi-vial scintillation vials, and [2,4,6,7-[^{3}H](N)]-E_2 (stock solution in ethanol, 1.0 μCi/μl, 81.0 Ci/mmol) were obtained from PerkinElmer, Waltham, MA. E_2 and creatinine standards were obtained from Sigma-Aldrich, Oakville, ON, Canada. E_2 antibodies and HRP conjugates were obtained from the Department of Population Health and Reproduction at the University of California, Davis, CA.

2.3. Experiment 1: measurement of ^{3}H-E_2 in cycling females after repeated triclosan doses

At the onset of darkness on the first day of the experiment, 21 cycling female mice with regular estrous cycles were weighed (39.4 ± 5.6 g), individually housed, and each given a subcutaneous (sc) injection of 0, 1, or 2 mg triclosan (corresponding to 0.0 ± 0.0, 23.2 ± 2.5, or 57.9 ± 3.9 mg triclosan/kg bodyweight, respectively) dissolved in 0.05 ml peanut oil (n = 7 per dose). These injections were repeated at the same time on days 2 through 7, such that each mouse had a total of 7 injections of the same dose. We rotated injection locations of triclosan among the neck, right flank, and left flank to prevent irritation of the injection site. At 24 h after the final triclosan dose, each animal was given an intraperitoneal (ip) injection of 1 μCi ^{3}H-E_2 (corresponding to 3.36 ng E_2) in 9 μl phosphate-buffered saline (PBS). At 1 h after ^{3}H-E_2 administration, each animal was anesthetized with isoflurane and blood was collected via cardiac puncture. Each animal was perfused with 15 ml PBS and tissues were collected in pre-weighed scintillation vials. Tissue samples taken include the heart, lung, liver, superficial adductor muscle from the hind leg, the whole uterus, both ovaries, and a cross-section of the kidney encompassing both the medulla and cortex. Vials were re-weighed following tissue collection to determine the sample wet mass.

We administered triclosan via sc injection in all experiments, as the presence of triclosan in personal care products can lead to transdermal absorption. Percutaneous penetration is incomplete compared to sc injection; one estimate suggests that transdermal absorption of triclosan is 6.3 ± 1.1% in humans and 22.8 ± 4.6% in rats within 24 h [5]. The 1 and 2 mg triclosan doses were chosen based on the lowest effective doses in a previous study showing an interaction between triclosan and ^{14}C-BPA [24]. ^{3}H-E_2 was given ip to facilitate systemic distribution of the steroid [35], and the 1 μCi dose of ^{3}H-E_2 represents just a small fraction of the animals' endogenous E_2 [35,36]. Finally, the 24-h latency between triclosan injections and between the final triclosan injection and ^{3}H-E_2 administration, were chosen to exceed the 10.8 ± 6.3 h terminal elimination half-life of dermally administered triclosan [6].

Blood and tissue samples were processed for liquid scintillation counting following previously published procedures used in this laboratory [24,37]. In brief, blood samples were centrifuged at 1,500*g* for 10 min and 10 μl serum was added to a scintillation vial containing 5 ml Ultima Gold. Tissue samples were solubilized by adding 1 ml SOLVABLE to each vial and placing vials in a 50 °C water bath for 4–5 h until completely dissolved. Following the addition of 5 ml Ultima Gold, vials were agitated to promote mixing of the sample and scintillation cocktail. Each vial was stored in the darkness chamber of a TriCarb 2910 TR Liquid Scintillation Analyzer (PerkinElmer) for 5 min to eliminate background noise in the form of heat and luminescence. Radioactivity was measured for 5 min per vial and final adjusted estimates for the amount of radioactivity per sample in disintegrations per minute (dpm) were automatically calculated by the accompanying Quanta-Smart software package. The dpm measures were then reported as either dpm/mg tissue or dpm/μl serum. A reported concentration of 1 dpm/mg tissue or 1 dpm/μl serum is equivalent to 1.52 pg/g tissue or 1.52 pg/ml serum, respectively.

2.4. Experiment 2: measurement of ^{3}H-E_2 in cycling females after a single triclosan dose

At the onset of darkness, 20 cycling female mice were weighed (40.7 ± 4.1 g), individually housed, and each given a single sc injection of 0, 0.6, 1, or 2 mg triclosan (respectively 0.0 ± 0.0, 14.6 ± 1.7, 23.6 ± 2.9, or 53.9 ± 3.4 mg/kg) dissolved in 0.05 ml peanut oil (n = 5 per dose). At 30 min after triclosan administration, each animal was given an ip injection of 1 μCi ^{3}H-E_2 (3.36 ng E_2) in 9 μl PBS. At 1 h after ^{3}H-E_2 administration, each animal was anesthetized with isoflurane. All other procedures, including perfusion, blood and tissue collection, sample processing, and scintillation counting were identical to those of Experiment 1. The 30-min latency between triclosan and ^{3}H-E_2 and the 1-h latency between ^{3}H-E_2 administration and tissue collection were chosen based on a previous study demonstrating an interaction between triclosan and ^{14}C-BPA [24]. A 1-h latency between ^{3}H-E_2 administration and tissue collection permits systemic distribution of ^{3}H-E_2 [38].

2.5. Experiment 3: measurement of ^{3}H-E_2 in inseminated females after a single triclosan dose

We examined the influences of triclosan upon peri-implantation inseminated females given the potential relevance to blastocyst implantation failure. This should also produce less within-condition variance as endogenous E_2 dynamics are less variable than among cycling females. Sexually naïve females were each