THE EFFECTS OF TRICLOSAN ON THE DEVELOPMENT OF RANA PALUSTRIS

Sowers AD, Klaine SJ

AUTHORS: Clemson University Institute of Environmental Toxicology, 509 Westinghouse Road Pendleton, SC 29670

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Extended Abstract:

Triclosan is a bisphenolic antimicrobial agent found in a variety of personal care products including, but not limited to, soaps, shampoos, deodorants, and toothpastes and is typically found at a concentration of 0.1-0.3%. A per capita daily usage rate has been estimated to range from 3-5mg/person/day (Mcavoy et al. 2002). Due to the nature of the personal care products containing triclosan, it can be assumed that considerable amounts of the compound will enter into municipal wastewater systems and may enter the environment in wastewater effluents. A study conducted by the United States Geological Survey detected triclosan in more than half of the 139 surface waters they sampled across 30 states indicating a widespread prevalence of triclosan in the aquatic environment (maximum level of 2.3 ug/L) (Kolpin et al. 2002).

The potential for triclosan to act as an endocrine disrupting compound in aquatic systems has been briefly examined in the literature. Foran et al. (2000) observed non-significant trends of increased male sex ratios and increased male anal fin lengths two months post-exposure after a 14-day larval exposure using Japanese medaka, suggesting that triclosan may be weakly androgenic. Ishibashi et al. (2004) observed an increase in vitellogenin production in male medaka after exposure to 20 and 100 ug/L triclosan and concluded that a metabolite of triclosan may be weakly estrogenic. In addition to interfering with sex-related steroid hormone homeostasis, the potential for triclosan to disrupt thyroid hormone (TH) homeostasis has been observed in a mammalian model. Oral administration of triclosan to Long-Evans rats resulted in a dose-dependent decrease in serum total thyroxine (T4) levels (Crofton et al. 2007). The reduction in circulating levels of T4 was hypothesized to be a result of activation of the pregnane-X-receptor (PXR) leading to an induction of biotransformation enzymes responsible for catabolizing THs. Induction of biotransformation enzymes, specifically glucuronidases, which catabolize THs is an area of concern for amphibian species because THs are the primary agents responsible for driving the metamorphic process in amphibians. Amphibians have not been shown to possess a form of PXR, but a nuclear receptor known as the benzoate-x-receptor (BXR) residing in the same subfamily as PXR has been identified in *Xenopus laevis*, although not much is known about the physiological effects of BXR induction (Blumberg et al. 1998).

The present work tested the hypothesis that aqueous exposure to triclosan would delay the metamorphic process in the pickerel frog, *Rana palustris*, by inducing biotransformation enzymes, presumably through activation of the BXR leading to an increased metabolism of THs and a longer time to reach metamorphosis.

Reconstituted moderately-hard water with a nominal hardness and alkalinity of 80 and 60 mg/L as $CaCO_3$, respectively, was created in the laboratory and used as the control test solution. Appropriate volumes of stock solution were added to the reconstituted moderately-hard water to form each treatment solution. A 3mg/L stock solution of triiodothyronine (T3) (Sigma-Aldrich, St. Louis, MO) was made by dissolving 3 mg of T3 in 15 mL of 50mM sodium hydroxide and then diluting to 1 L. Four hundred forty seven microliters of benzyl benzoate (Sigma-Aldrich, St. Louis, MO) were dissolved in 10 mL of 100% ethanol. Triclosan (Irgasan, Sigma-Aldrich, St. Louis, MO) stock solutions were made by dissolving 100 mg of triclosan in 10 mL of 50mM sodium hydroxide followed by either a 100 fold dilution, used for the two lowest triclosan concentrations, or a 1000 fold dilution, used for the two highest triclosan concentrations.

Two clutches of pickerel frog, *Rana palustris*, eggs were purchased from the Charles Sullivan Co. (Nashville, TN). Eggs were pooled and maintained in a thin layer of moderately-hard water. Upon becoming free swimming larvae (Gosner stage 25) organisms were separated into groups of 3 and stocked in 750mL glass jars containing moderately-hard water. Exposures began 14 days after stocking larvae. Experimental solutions consisted of a negative control containing moderately-hard water, a 3ug/L T3 positive control, a 5mg/L benzyl benzoate treatment to serve as a positive control for BXR activation, a range of triclosan treatments (0.25, 1.0, 10.0, and 30.0ug/L), and a combined 3ug/L T3 and 30ug/L triclosan treatment. Treatments were renewed every 48hrs. Each treatment consisted of 9 replicate 750mL jars containing 3 tadpoles apiece. All test containers contained 0.01% (v/v) ethanol. Tadpoles were maintained in a climate controlled test room on a 16:8 light:dark cycle and were continuously aerated.

Organisms were removed from exposure containers and euthanized upon reaching forelimb emergence (Gosner stage 42) and the time to reach this stage was recorded. A subset of stage 42 tadpoles from

each exposure container were moved to inclined aquaria containing the appropriate treatment solution and allowed to reach complete metamorphosis (tail length $\leq$ 2 mm). Complete metamorphs were used for the measurement of snout-vent length, weight, hepatosomatic index (ratio of liver weight to total body weight), hind limb length, and thyroid gland histological examination.

Heads from newly metamorphosed organisms were fixed in 10% neutral-buffered formalin overnight. Tissues were then dehydrated using a graded series of ethanol concentrations (50, 75, 90, 100%), cleared with xylene, and embedded in resin (Immunobed, Polysciences, Warrington, PA). Heads were sectioned at 3 um until a minimum of 10 sections containing intact thyroid gland tissue per organism were obtained. Slides were stained with azure II and basic fuchsin. Thyroid gland size, follicular hypertrophy, defined as an increase in the size of cells surrounding the follicles, and follicular hyperplasia, defined as an increase in the number of cells surrounding the follicles were assessed relative to controls. Five organisms were assessed per treatment.

Tadpoles exposed to the 1ug/L triclosan solution displayed a trend of accelerated metamorphosis when compared to the control. At the higher concentrations of triclosan (10ug/L and 30 ug/L) tadpoles did not display an accelerated metamorphosis when compared to control organisms. Benzyl benzoate treated organisms displayed a trend of delayed metamorphosis when compared to the control. Length, weight, hepatosomatic index, and hind limb length did not differ between control and triclosan treated organisms. Benzyl benzoate treated organisms displayed a greater length, weight, and hind limb length when compared to all other treatments. No differences in thyroid gland morphology were observed between any treatments.

The accelerated rate of metamorphosis at the intermediate triclosan concentration of 1ug/L suggests that triclosan may be acting as a thyroid hormone agonist, thus leading to an increased rate of thyroid-dependent metamorphosis. At the higher concentrations of triclosan, biotransformation enzymes may have been induced, thus leading to increased thyroid hormone metabolism and a decreased rate of metamorphosis. Additionally, the decreased rate of metamorphosis observed in benzyl benzoate treated organisms may have resulted from biotransformation enzymes being induced via BXR pathways. Benzyl benzoate may also have acted as a dietary supplement leading to the increased length and weight of treated organisms. Organisms in the benzyl benzoate treatment may have been reluctant to initiate metamorphosis in an effort to achieve the greatest possible size at metamorphosis.

Trilcosan alone does not appear to pose a significant threat to the successful metamorphosis of *R. palustris*. While some trends in metamorphic rate were observed among treatments, none were found to be statistically significant. Because triclosan typically exists in the environment in a complex matrix containing a variety of xenobiotics, the possibility exists that other compounds with a similar mode of action may work in accordance and cause effects greater than those of any individual compound.

Works Cited

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