Developmental evaluation of a potential non-steroidal estrogen: triclosan

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Abstract

Triclosan is an antibacterial agent commonly used in industry and often detected in wastewater effluent. The potential of triclosan to act as an endocrine disruptor was examined because its chemical structure closely resembles known non-steroidal estrogens (e.g. DES, bisphenol A). Japanese medaka fry (Oryzias latipes) were exposed for 14 days beginning 2 days post-hatch to triclosan (100, 10, 1 μg/l), 17-β estradiol (E2; 1 μg/l), or a solvent control (ethanol). Two months post-exposure, the phenotypic sex of each adult was assessed visually using sexually dimorphic fin shape and size. The proportion of females in each group was similar for triclosan-exposed animals and solvent-treated controls (ethanol 53%, 1 ppb 58%, 10 ppb 45%, 100 ppb 36%) although E2 treatment did produce 92% female adults. Sexually dimorphic fin traits were quantified to look for potential effects of triclosan and E2 on the development of secondary sexual characters. These results do not support the hypothesis that triclosan is potently estrogenic. However, changes in fin length and non-significant trends in sex ratio suggest triclosan is potentially weakly androgenic. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Antibacterial; Wastewater; Effects-fish; Reproductive cycle; Secondary sexual characters

1. Introduction

Triclosan is an industrial antibacterial agent in toothpaste, soap, and cleaners, and is frequently found in wastewater effluent. Measurements of triclosan from the effluent of municipal sewage treatment plants in Sweden determined levels to be as...
high as 500 μg/l (Paxeus, 1996). Samples analyzed from Rhode Island found 10–20 μg/l of triclosan in effluent and 80–100 µg/g of triclosan in sediment near the outfall of a wastewater treatment plant (Lopez-Avila & Hites, 1980). Recent studies have demonstrated biocidal effects of triclosan on fatty acid synthesis in Escherichia coli (McMurry, Oethinger & Levy, 1998). Triclosan is similar in chemical structure to several non-steroidal estrogens, suggesting a potential to act as an environmental estrogen. The goal of this study was to determine if triclosan would mimic the effects of estradiol, using the development of Japanese medaka as an endpoint.

Japanese medaka are a gonochoristic teleost species. Phenotypic sex can be determined visually by examination of sexually dimorphic fin characters (Yamamoto, 1975). Males have longer and morphologically distinct dorsal fins and longer anal fins than females. This species has been used extensively as a model for endocrine disruption because of their consistent (non-seasonal) reproduction and rapid development time (Metcalfe, Gray & Kiparissis, 1999). In medaka, sex is determined by sex chromosomes although early developmental exposure to estrogen can result in skewed sex ratios in the resulting populations (Nimrod & Benson, 1998) or intrasex morphology of the gonads (Gray & Metcalfe, 1997). A previous study (Nimrod & Benson, 1998) demonstrated that exposure to concentrations of 17-β-estradiol (E2) as low as 1 μg/l produced 100% females. In this study, we examined the effects of triclosan exposure on the development of Japanese medaka.

2. Materials and methods

Hatchling Oryzias latipes from a stock culture maintained at the University of Mississippi were exposed for 14 days, beginning at 2 days post-hatch, in 500-ml beakers containing balanced salt solution. Fish were maintained at 25°C and fed brine shrimp once a day during the exposure. A stock solution of 50 μl of ethanol (EtOH) containing triclosan (three doses), 17-β-estradiol (positive control) or nothing (solvent control) was added to each beaker daily following an 80% water renewal. The final nominal concentrations of each exposure were 1, 10, 100, 500 μg/l, and 1 mg/l of triclosan and 1 μg/l of E2. After 14 days of exposure, animals were transferred to a 30 l aquaria and allowed to mature. In aquaria, medaka were maintained at 27°C, fed brine shrimp twice daily and Tetramin flakes once a day. After 10 weeks, the sex of each individual was determined by visual examination of sexually dimorphic dorsal and anal fin morphology. As well, the length of the dorsal and anal fins was measured for each animal.

3. Results and discussion

Triclosan exposure at high concentrations was lethal to medaka fry. At lower concentrations, triclosan did not have a significant effect on the sex ratio of the resulting fish. However, triclosan’s effect on fin length and a trend in the sex ratio of adult animals suggest the chemical may be androgenic. A triclosan concentration of
1 mg/l was lethal to hatchlings within 24 h; 500 μg/l was lethal after 3 days of exposure. From this data, the 48-h LC50 for medaka fry was calculated to be 352±68 μg/l.

Triclosan treatment did not skew the sex ratio of animals grown to maturity (100 ppb: five females, nine males; 10 ppb: nine females, 10 males; 1 ppb: 11 females, eight males; EtOH: 10 females, nine males; Fig. 1). The 100-ppb treated group containing 64% males was not significantly different from the 47% male ethanol-treated group (2×2 contingency table, $\chi^2=0.373$, $P>0.25$). However, 1 ppb E2 treatment produced 92% females, which was significantly different than controls (2×2 contingency table, $\chi^2=6.25$, $P<0.05$).

Analysis of the fin lengths from the adult animals resulting from this exposure shows a similar pattern of effects (Table 1). As expected, males have significantly longer dorsal and anal fins than females in each treatment group [two-way analysis of variances (ANOVA), both $P<0.001$; pairwise post-hoc Tukey tests, all $P<0.05$). Among females, there were no differences in fin lengths between the treatment groups (AVOVARs, both $P>0.05$). Among males, animals treated with 100 ppb triclosan had longer dorsal and anal fins than those treated with 10 ppb (ANOVARs, both $P<0.03$; pairwise post-hoc Tukey tests, both $P<0.05$), the measurements of both triclosan-treated groups overlapped the measurements from control males.

![Fig. 1. Resultant sex ratio from exposed medaka fry.](image)

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<th>EtOH</th>
<th>1 μg/l</th>
<th>10 μg/l</th>
<th>100 μg/l</th>
<th>E2</th>
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<td><strong>Dorsal fin</strong></td>
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<td>Males</td>
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<td>4.00±0.33</td>
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<td>2.73±0.20</td>
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<td>2.83±0.18</td>
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<td><strong>Anal fin</strong></td>
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<tr>
<td>Males</td>
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<td>4.06±0.37</td>
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<td>Females</td>
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<td>2.70±0.31</td>
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</table>
Developmental exposure to triclosan did not result in a skewed sex ratio as was observed in response to early E2 exposure. The evidence in this preliminary study was not sufficient to determine if triclosan acts as an environmental estrogen to disrupt development. The marginal male bias found in the sex ratio of 100 ppb-treated animals and the differences in fin length between males from different treatment groups does however suggest triclosan may act as an environmental anti-estrogen or androgen. Further exposures will explore this possibility as well as look at other assays of estrogenicity.

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References