Does dissolved organic carbon reduce the developmental toxcitiy of triclosan?

Sarah Grandstrand[1], Nadia Carmonsini[2] and Tisha C. King-Heiden[1]

[1]Department of Biology; [2]Department of Chemistry

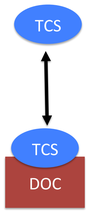
University of Wisconsin La Crosse

ABSTRACT

A variety of organic compounds contained in personal care products have emerged as environmental contaminants of concern. One such contaminant is triclosan, a broad-spectrum synthetic anti-microbial agent that is a widely used in antimicrobial soaps, deodorants, cosmetics, and other products. Triclosan (TCS) is released in effluents from wastewater treatment plants, and has been shown to be a persistent environmental contaminant with potential for bioaccumulation. Previous studies by our research group have determined that a waterborne exposure to triclosan causes subtle developmental toxicity in zebrafish larvae (i.e. pericardial and yolk sac edema). Taken together, this suggests that bioaccumulation of triclosan could pose a risk to wild fish populations; however, sorption to particles within the water column could impact its bioavailability. For example, we have shown that triclosan adsorbs to dissolved organic carbon (DOC), which may make it less bioavailable to aquatic organisms. I hypothesize that co-exposure to triclosan in DOC will reduce triclosan's bioavailability and therefore its toxicity to zebrafish larvae. Zebrafish are exposed to triclosan alone, DOC alone, or triclosan and DOC together during early larval stages of development (8-120 hours post fertilization) using static waterborne exposure. Development, hatching, and mortality are recorded daily to assess toxic endpoints. Lateral images are taken of a subsample of larvae to quantify developmental toxicity. We have established concentrations of DOC that do not induce toxicity, and on-going studies will allow us to better understand how environmental factors may influence the bioavailability and toxicity of triclosan. This study will further our understanding of the risks of triclosan to wild fish populations.

BACKGROUND

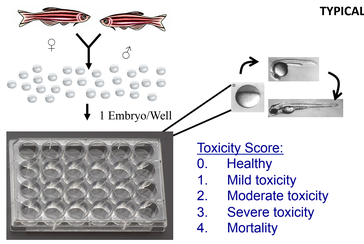
- Personal care products & anti-microbial agents are of emerging toxicological concern
- The anti-microbial agent triclosan (TCS) was shown by previous studies to induce developmental toxicity
- The DOC leonardite humic acid was found to sorb to triclosan with strong affinity



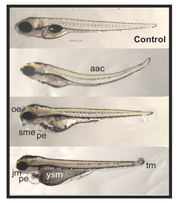
HYPOTHESIS

Exposure to TCS in the presence of DOC will reduce the bioavailability of TCS and therefore reduce its toxic effects.

EXPERIMENTAL DESIGN



TYPICAL ENDPOINTS OF TOXICITY IN ZEBRAFISH:



Developmental Toxicity Assays:

•Zebrafish embryos were exposed to each contaminant (or combination thereof) in 24-well cell culture plates (one embryo/well; n=24) with 2 ml of solution via static waterborne exposure from 8-120 hours post fertilization (hpf). Three replicate experiments were performed.

- •DOC exposure: 0 (fish water control), 10, 25, and 50 mg/l
- •TCS exposures: 0 (acetone vehicle control), 100, 300, 500, 700, and 900 µg/l
- •TCS and DOC exposures: 0 (fish water & acetone control), 100, 300, 500, 700, and 900 µg/l TCS in 10mg/l DOC; equilibrated 24h.

•Embryos/larvae were screened daily for mortality, hatching rate, and developmental toxicity. Each fish is scored for toxicity (see above).

•At 5 dpf, lateral photographs of eight representative fish per dose were taken to measure quantify incidence developmental toxicity.

• 2-way ANOVA was used to analyze the data

LEONARDITE HUMIC ACID HAS A HIGH AFFINITY FOR TCS AND IS NOT OVERTLY TOXIC

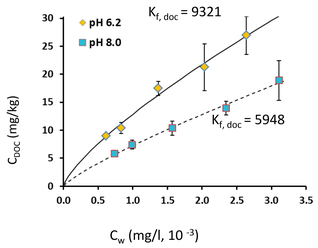


Figure 1. Affinity of DOC to TCS at pH of 6.2 and 8.0. This was run over a 24 hour period using dialysis tubing of 0.45µm. The Freunlich Isotherm Model was used to calculate affinity. Notice the high affinity to TCS even at low doses.

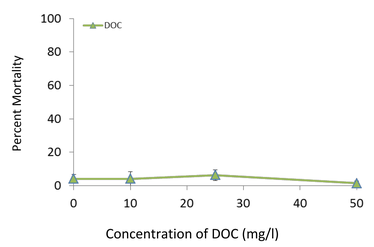


Figure 2. Cumulative mortality at 5dpf. Zebrafish embryos exposed to DOC from 8-120 hpf showed no significant mortality.

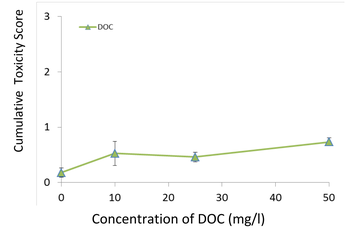


Figure 3. Cumulative sublethal at 5dpf. Zebrafish embryos exposed to DOC from 8-120 hpf showed minor sublethal toxicity. There was an increased incidence of bent spine seen in all concentrations (38% at 10mg/l, 21% at 25mg/l and 69% at 50 mg/l)

CONCLUSIONS & FUTURE DIRECTIONS

- Leonardite humic acid (DOC) has a strong affinity for TCS
- DOC is not overtly toxic to developing zebrafish
- 10 mg/l was identified as the ideal concentration to use for co-exposure studies
- Future studies will include desorption assays of TCS with DOC to determine if chemical affinity dissipates after 24 hours

DISSOLVED ORGANIC CARBON PARTIALLY REDUCES TCS TOXICITY

Figure 4. Percent mortality at 5dpf. Presence of DOC did not significantly reduce TCS toxicity; however, mortality was reduced at 500 µg/l. Letters denote significant differences.

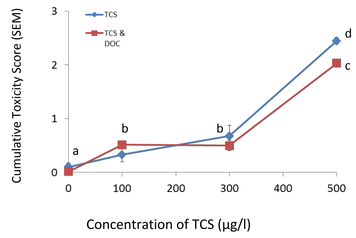


Figure 5. Cumulative sublethal toxicity at 5dpf. DOC provided limited protection from TCS sublethal toxicity. At 700 µg/l, toxicity onset appeared delayed, but still resulted in 100% mortality (data not shown).

Figure 6. Representative micrographs of larvae exposed to 500 µg/l TCS with or without DOC at 5dpf. Exposure to TCS induces "blue sac syndrome" in larval zebrafish. Severity of blue sac syndrome is slightly reduced with co-exposure to DOC. Abbreviations: cfm = craniofacial malformations; pe = pericardial edema; ysm= yolk sac malformations; yse= yolk sac edema

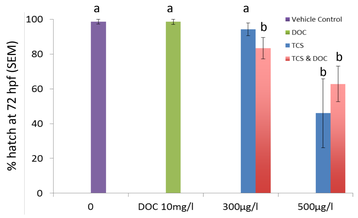


Figure 7. Percent of hatching at 72hpf. While TCS shows a dose-dependent influence on hatching success, it is not clear whether the presence of DOC influences hatching. Letters denote significant differences.

Figure 8. Incidence of specific endpoints of toxicity following exposure to 500 µg/l TCS with and without DOC. The presence of DOC does not influence the toxic endpoints observed. However, we noted a difference in the severity of some responses (as reflected in the mean sublethal toxicity score, see Figs 5&6). Abbreviations: BS = bent spine; YSE = yolk sac edema; PE = pericardial edema.

CONCLUSIONS and FUTURE DIRECTIONS

- 10 mg/l DOC does not significantly reduce the toxic effects of TCS; however, we do see a subtle shift in the dose-response to TCS when DOC is present
  - Currently quantifying sublethal toxicity to include incidence & severity of blue sac syndrome
- Does TCS desorb from DOC and become bioavailable?
  - We will evaluate sorption and desorption over longer period of time
- Is TCS still bioavailable, even if bound to DOC?
- Will increased concentration of DOC be sufficient to reduce bioavailability and rescue toxicity?
  - We will repeat experiments using greater concentration of DOC
- Since recent studies indicate that TCS may degrade into dioxin following UV exposures, and TCS toxicity resembles that of dioxin toxicity, we will use markers for dioxin toxicity (EROD assay) to evaluate whether TCS toxicity results from dioxin metabolites

ACKNOWLEDGEMENTS

We thank Courtney Schneider, Drew Revak, Trevor Cyphers, and Yer Lor for assistance with fish husbandry.

Funding was provided by: UWL Undergraduate Research and Creativity Grant, UWL Faculty Research Grants, and the UWL River Studies Center