TOXICITY AND TISSUE SELECTIVE ACCUMULATION OF SEDIMENT WEATHERED AND UNMODIFIED TOXAPHENE

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Introduction

Technical toxaphene (TTX), a widely used organochlorine biocide until its ban in the U.S. in 1982, is a mixture of polychlorinated monoterpenes whose selective persistence and bioaccumulation in nature has been well documented[1]. Depending on specific conditions and spatial and temporal considerations relative to source input, the composition of toxaphene residues in the biosphere is a direct result of environmental processing ("weathering")[2-4]. In estuarine sediments heavily contaminated by industrial discharge of TTX, for example, the complex mixture of residue congeners is dominated by lower chlorinated (Cl_5-Cl_7) transformation products of unmodified TTX components[5].

Early toxicological work focused on the effects of individual components of TTX isolated from technical mixtures on mammalian systems[6,7]. Little is known, however, about the effects of weathering on the ecotoxicity of toxaphene residues in aquatic systems. The objective of this study was to compare the tissue-specific bioaccumulation and toxicity of sediment weathered vs. unmodified toxaphene by exposing an estuarine fish species to spiked food under laboratory-controlled conditions.

Materials and Methods

Experimental. Mummichogs (*Fundulus heteroclitus)* were collected from an uncontaminated tidal creek near the Skidaway Institute of Oceanography in June 2001. Eighty females [55-65 mm total length (TL)] were treated in 0.01% formalin before acclimation at 20°C, 25‰ salinity and pH 7.5-8.0 in 10-gal glass aquaria with active aeration and under gravel filtration (4 tanks, 20 fish/tank). One week prior to toxaphene exposure, water was gradually increased to 26°C to induce spawning. Water quality was measured daily and adjusted as needed using heaters and/or addition of deionized or filtered seawater. A minimum of food was administered during acclimation.

At day 0, commercial fish food (Tetramin) spiked with a hexane extract of sediment contaminated with ~500 μg/g weathered toxaphene residues[8] ("SED") or unmodified TTX (Hercules Inc. product standard; "TTX") at a nominal concentration of 50 μg/g food was administered at a dose of 0.1g/fish/d to each of 2 treatment tanks. A control group ("CONT") received a similar amount of unspiked food. Toxaphene-spiked food was administered every day for day 0-7 and 49-57, and every 3rd day for day 7-49. At day 0, 12/16, 31, 43 and 57, 5 fish per treatment were sacrificed. TTX treated fish were sampled on day 12 rather than day 16 (CONT and SED) due to concerns over their survival. Sacrificed fish were sedated using 3-aminobenzoic acid ethyl ester (MS-222), measured (TL and body weight) and gonads and hepatopancreas excised and weighed separately. Tissues and body cavities were frozen immediately in solvent-rinsed glass vials.

Toxaphene analysis by gas chromatography. Defrosted tissues were homogenized with Hydromatrix, spiked with recovery surrogates and extracted with CH_2Cl_2 using accelerated solvent extraction (ASE). After CH_2Cl_2 evaporation and gravimetric lipid determination, extracts were cleaned up using Florisil column chromatography (18.0 g, 1.0% water deactivated), with toxaphene residues eluted into 2 fractions (F1 and F2) of increasing solvent polarity[9]. Extracts were analyzed by narrow bore, capillary column gas chromatography with electron capture detection (GC-ECD). Total toxaphene (ΣTOX) was estimated by summing peak areas in a toxaphene retention time window for both fractions. Non-toxaphene interferences (primarily in F1s) were subtracted based on GC-ECNI-MS confirmatory analyses. Blanks, TTX-spiked tissue and recovery surrogates were incorporated into this study to ensure data of the highest quality[10].

Data Analysis. Gonado- and hepatosomatic indices (GSI and HSI, respectively) were computed as the ratio of tissue to body cavity weight. Mean parameters were compared across treatments (SED, TTX and CONT) and at various time points using ANOVA and Scheffe's multiple comparison test.

Results and Discussion

Growth and condition. Mean fish TL increased in all treatments throughout the experiment. By the end of the experiment (day 57), SED mean TL was 0.6 cm greater than TTX mean TL; $p<0.025$) (**Fig. 1**). Although the difference was not statistically significant, CONT fish were 0.2 cm longer on average than TTX fish. Time series mean HSI values did not differ among treatments except for day 57, where TTX>SED ($p<0.05$) and TTX>CONT ($p=0.06$) (**Fig. 2**). At the first sampling time (d12-16), the mean GSI for TTX fish was less than SED fish ($p<0.025$), and half of that for CONT fish (**Fig. 3**). Whereas mean GSI peaked early (~d16) for CONT and SED fish, it appeared to increase gradually with exposure time for TTX fish for the duration of the experiment.

Figure 1. Mean total length (TL). Error bars are 1σ.

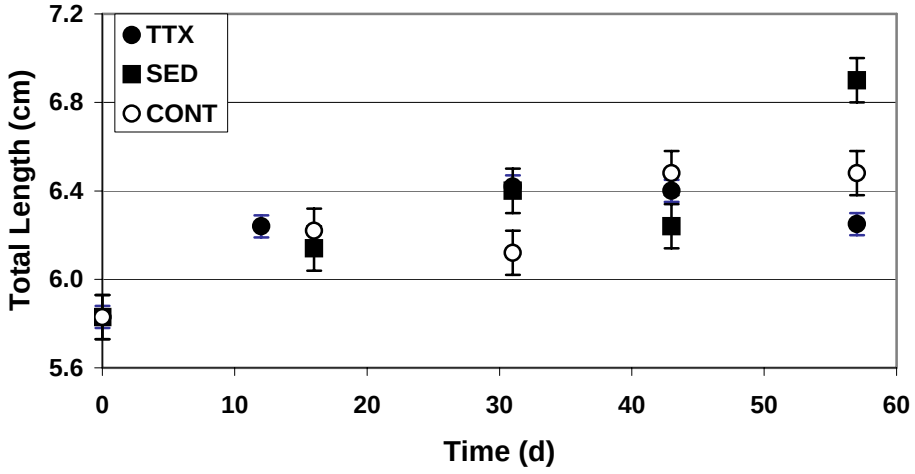


Figure 2. Mean Hepatosomatic index (HSI). Error bars are 1σ.

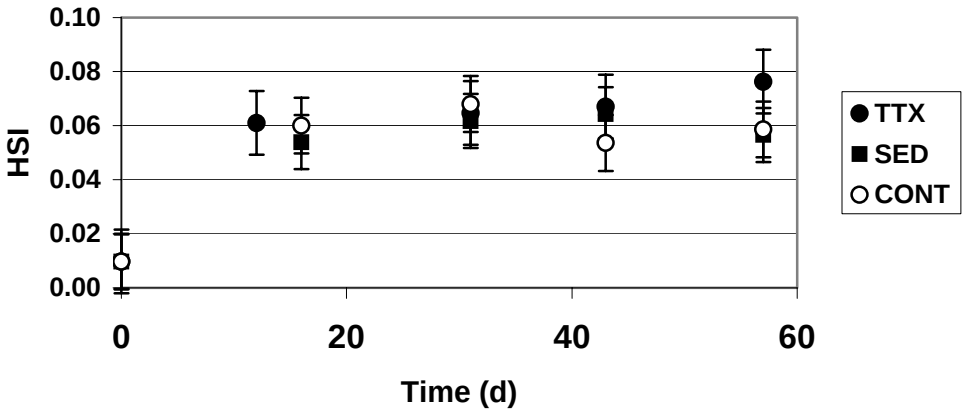
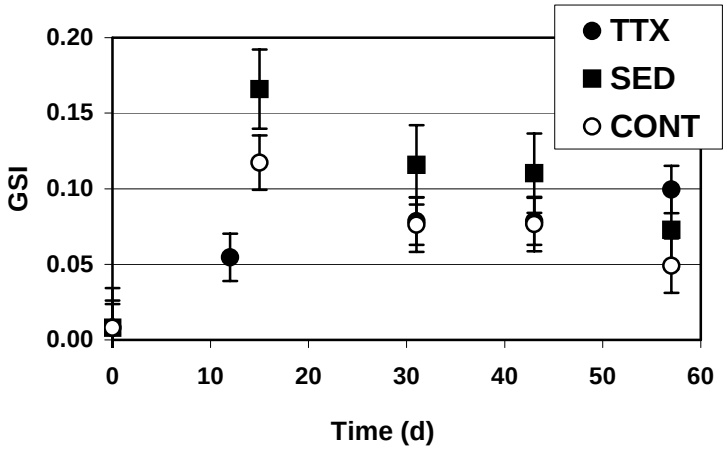


Figure 3. Mean gonadosomatic index (GSI). Error bars are 1σ.



Toxaphene exposure in various test organisms has resulted in reduced growth[11], increased relative liver weight[12] and purported reproductive effects[13]. The stunted growth and elevated HSI for TTX fish compared with the other treatments indicates a greater toxic effect. In wild *F. heteroclitus* populations, spawning cycles are linked to lunar/tidal stimuli resulting in increased egg production (and hence GSI) during new and full moon periods[14]. After spawning, GSI is expected to decline as the spawning season progresses, similar to what was observed in our SED and CONT groups. In contrast, the gradually increasing trend in GSI with time for TTX fish suggests reduced/retarded egg development.

Tissue-specific bioaccumulation. Gonad and hepatopancreas ΣTOX increased steadily for both toxaphene treatments with exposure time (data not shown). No statistically significant differences in ΣTOX between treatments were noted except for hepatopancreas at day 57, where SED (147±19.6 μg/g) > TTX (90.8±22.1μg/g) (p<0.005). Toxaphene bioaccumulation in gonads was consistently 2-7 times less than in hepatopancreas. Mean food concentrations as measured by GC-ECD (42 μg/g TTX; 62 μg/g SED) bracketed our target level of 50 μg/g, but differed by 20 μg/g.

Previous studies have reported on the hyperaccumulation of toxaphene residues in livers and other fatty organs of aquatic species[1,15]. Specimens of *F. heteroclitus* collected in a tidal creek impacted by discharge from a former toxaphene plant also contained very high ΣTOX (~150 μg/g); blood from these fish also exhibited elevated DNA damage relative to aquarium-kept and wild reference fish (unpubl. results). ΣTOX in wild fish from the general vicinity of this plant were also elevated (max ΣTOX ~ 30 μg/g)[10], and also exhibited greater accumulation in liver tissues[9].

Summary and Conclusions

- Adult fish treated with unmodified toxaphene (TTX) exhibited reduced growth, enlarged hepatopancreas, and delayed/reduced gonadal development compared with those exposed to sediment weathered residues
- Toxaphene accumulation from spiked food was much less efficient for gonads (20-40% accumulation efficiency) than for hepatopancreas (220-240% efficiency).
- For short term, chronic exposures, unmodified technical toxaphene appears to exert greater growth and reproductive toxicity than sediment weathered residues

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