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Environmental Dose of Toxaphene Does Not Affect the Growth, Stress Response, and Selected Physiological Parameters in Juvenile Arctic Charr, (*Salvelinus alpinus*)

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Toxaphene is a major persistent organic pollutant in Arctic fish (Braune et al. 1999; Muir et al. 1999) including Arctic charr (Chan and Yeboah 2000). The Arctic charr (*Salvelinus alpinus*) is found throughout the Arctic and subarctic regions of North America, Europe and Asia, and is one of the most economically and culturally important fish species in the circumpolar north (Johnson 1980). Toxaphene is highly toxic to fish, and salmonids are especially susceptible (Mayer and Mehrle 1977). Toxaphene is neuro- and hepatotoxic (Saleh 1991), and chronic exposure in fish has been associated with bone and vertebral defects; decreased growth, particularly among young fish; and impaired reproduction (Saleh 1991). These effects have typically been noted at high dose levels but the effects of toxaphene on fish physiology at environmentally realistic levels have not been examined. The objectives of this study were (1) to develop an oral method to deliver toxaphene to Arctic charr; (2) to determine the effects of environmentally relevant doses of toxaphene on fish growth and organosomatic indices, and (3) to investigate possible effects on the physiology of the fish by measuring the circulating levels of cortisol, vitamin A and vitamin E.

MATERIALS AND METHODS

Forty-eight juvenile (1+) hatchery Arctic charr (Fraser River Strain, Pisciculture St. Damien, Quebec) were divided into twelve experimental groups of four fish. The range of weight and length within groups did not exceed 0.5 g and 10 mm respectively. Individual aquaria were randomly assigned one of three treatments; 'control', 'low dose' and 'high dose'. Single Torpac® gelatin capsules (size #5; capacity 0.13 ml; Fairfield, New Jersey, USA) were filled with a stock solution of toxaphene (manufactured by Hercules Co. and supplied as a gift by T.F. Bidleman, Environment Canada) in corn oil and administered to fish by gavage while the fish were anesthetized in 0.1g/L buffered tricaine methane sulphonate (TMS; Syndel, Vancouver, BC). The nominal doses were calculated using the mean wet weight of all fish in each treatment group. Thus ' control' fish (n = 16) received corn oil alone, while 'low dose' (n = 16) and 'high' dose (n = 16) fish received approximately 0.5 μg toxaphene/g wet weight and 5 μg toxaphene/g wet weight respectively. The fish were then maintained for 60 days, in accordance with the Canadian Council on Animal Care (CCAC) guidelines. Fish were fed 1% of their

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biomass of high quality feed daily (3.5 mm sinking feed, Moore-Clarke, Bayside, New Brunswick). After 60 days all fish were confined within a net for 30 seconds to elicit a stress response (Hontela 1998), killed by anesthetic overdose (1 g/L buffered tricaine methane sulphonate), weighed and the fork length measured; fish condition was estimated using the formula, Fulton's K = 100 × g wet weight × mm fork length $^{-3}$. Blood was drawn from the caudal vein using a heparinized syringe, centrifuged for 5 min at 10,000 rpm, and the plasma placed on dry ice until cortisol and vitamin quantification. The fish were eviscerated and the carcass, liver, spleen, gonad and visceral fat weighed to 0.001 g. The carcass was frozen at –30°C until toxaphene quantification.

Plasma cortisol was measured using a standard radioimmunoassay (RIA) kit (ICN Biomedicals, Irvine, California, USA, catalogue number 07221106). Vitamin A and E were quantified in the plasma using a micromethod. The detection limit for cortisol was 1ng/mL. Briefly, plasma samples (50 – 100 µL) were brought to a final volume of 250 µL with isotonic saline. An aliquot (250 µL) of ethanol was added to free vitamins bound to protein. The vitamins were then extracted using three extractions with petroleum ether. The petroleum ether extract was dried under nitrogen and reconstituted with 450 µL of methanol plus a known quantity of Vitamin K_1 as an internal standard. Vitamin A (α-*trans*-retinol) and E (α-tocopherol) were then quantified using HPLC. The detection limit for both vitamins was 0.05 µg/100g. For toxaphene quantification, dorsal muscle tissue from all fish per treatment group was pooled and homogenized. From each pooled sample, 5 g of tissue were prepared for toxaphene analysis, and toxaphene quantification performed using GC- MS/MS as described by Chan and Yeboah (2000). Toxaphene peaks were monitored at *m/z* 89, 99 and 273. Two blank samples, two replicates of reference material (NIST SRM 1588 cod liver oil) and spiked samples (non-dosed char tissue spiked with 50 ng of toxaphene congener 26) were processed concurrently for quality control. The detection limit for toxaphene was 0.5 ng/g fish tissue. No toxaphene was detected in the blank samples. The average total concentration in SRM was 3450 ng/g which agreed with the previously reported data (Chan and Yeboah 2000). The recovery rate for the spiked sample was 87%.

All statistical tests were conducted using SAS v.8 (SAS Institute 1999). ANOVA and a Bonferroni post hoc test were used to examine differences between the means of treatment groups. Differences in organ weights between treatments were verified using ANCOVA, with body mass minus organ mass as the covariate as recommended by Christians (1999). The level of statistical significance was set at α=0.05.

RESULTS AND DISCUSSION

Initial fork lengths and weights were 143 ± 6 mm and 32.2 ± 6.3 g respectively, and there were no significant differences between groups (ANOVA; n = 48, p > 0.05). Data from two of the four 'control' tanks were discarded because of

mortality due to a fungus outbreak; thus the final n for this group was 8. It was noted during the course of the experiment that fish in the 'high dose' group displayed more agonistic behaviour towards tank-mates during routine maintenance, and were less inclined to feed. However, the feeding efficiency was not recorded.

Results are presented in Table 1. Wet weight gain (12.6 ± 9.5 g), condition (1.10 ± 0.15) and visceral organ weights (liver 0.759 ± 0.359 g; spleen 0.039 ± 0.016 g; visceral fat 0.628 ± 0.449; gonad 0.175 ± 0.414 g) were not significantly different among treatment groups. Average toxaphene levels in 'control', 'low dose' and 'high dose' fish muscle were 0.03, 0.13 and 0.92 μg/g wet weight respectively (N=2). The gas chromatograph - mass spectrometry chromatograms of toxaphene congeners are presented in Figure 1. A trace amount of toxaphene was found in the control group and there was no major difference in congener profiles between the treatment groups. There was no treatment effect on plasma Vitamin A ($F= 0.918$, $p = 0.475$) or Vitamin E ($F= 1.438$, $p = 0.268$). The apparent dose-dependent decrease in plasma cortisol was not statistically significant ($F= 1.842$, $p = 0.173$).

Table 1. Growth parameters, organ weights, vitamin A, E and cortisol concentrations in Arctic charr 60 days after a single oral dose of toxaphene.

		Treatment		
		Control	**Low Dose**	**High Dose**
N		8	16	16
Growth	**g ± SD**	14 ± 6.9	12.5 ± 10.9	11.9 ± 9.7
Condition	**K ± SD**	1.11 ± 0.08	1.09 ± 0.16	1.10 ± 0.17
Liver	**g ± SD**	0.860 ± 0.264	0.740 ± 0.417	0.728 ± 0.348
Spleen	**g ± SD**	0.043 ± 0.014	0.036 ± 0.014	0.040 ± 0.018
Visceral Fat	**g ± SD**	0.747 ± 0.448	0.525 ± 0.460	0.672 ± 0.446
Gonad	**g ± SD**	0.099 ± 0.049	0.070 ± 0.087	0.170 ± 0.244
Vitamin A	**ng/ml ± SD**	169.5 ± 42.5	155.1 ± 59.2	181.3 ± 45.5
Vitamin E	**ng/ml ± SD**	16893 ± 6976	22551 ± 7920	17219 ± 10623
Cortisol	**ng/ml ± SD**	18.69 ± 25.08	7.82 ± 12.81	6.06 ± 8.20

Most of the experimental toxaphene toxicity studies in the literature used a single high dose administered usually by injection (e.g. Delorme et al. 1993). To study the toxicokinetics of pollutants it is more desirable to deliver the chemicals orally. The use of gelatin capsules to administer organic pollutants to fish has shown

considerable promise as an oral delivery method for a variety of OC contaminants (Niimi and Oliver 1988), but to our knowledge had never been attempted with toxaphene. The clear dose-related increase in toxaphene concentration in fish muscle (Figure 1) indicates that gelatin capsules are effective tools for the oral administration of toxaphene to juvenile Arctic charr. The trace amount of toxaphene found in the control fish suggests that there may be cross-contamination of water supply in the aquaria. However, the body concentrations in the control group were much lower than the treatment groups and should therefore not affect the interpretation of the results. After 60 days the retention of toxaphene in the treated fish (0.13 and 0.93 μg/g) was approximately 27% and 18% of the administered dose (0.5 and 5.0 μg/g); approximately 29% of this decrease was due to growth dilution. The final toxaphene levels in fish muscle were consistent with those reported in the fish collected in the field (Chan and Yeboah 2000). We found no effect of toxaphene on fish growth, condition or organ weights. Similar negative observations were reported by Fisk et al. (1998); this suggests that toxaphene may not pose a major threat to the health of Arctic charr.

The toxaphene congener profile in the fish muscles did not differ significantly from the technical mixture, suggesting that toxaphene doses may have saturated the first pass metabolism or toxaphene was not metabolized during the 60 day experimental period. The technical grade toxaphene used in this experiment is a mixture containing over 600 individual congeners. In natural situations, Arctic charr are exposed to fewer congeners due to selective environmental degradation of the industrial compound. However, as there is little information on the toxicity of specific congeners, one can only assume that the toxicity of the environmentally prevalent congeners is similar to that of the industrial mixture. Further research on the toxicity of specific congeners is needed.

Behavior may have been a confounding factor in this experiment. Although fish were size-sorted at the outset of the experiment, theoretically minimizing growth differences due to heredity or developmental conditions (Abbott and Dill 1989), the final weight range within individual tanks ranged from 9.4 g to 47.2 g. Every tank had at least one clearly subordinate fish that spent much of its time oriented vertically near the surface, a standard submissive behavior. This suggests the existence of dominance hierarchies within tanks. Arctic charr form dominance hierarchies (Noakes 1980), particularly when kept in small groups of two to nine individuals (Øverli et al. 1999). These dominance hierarchies may have had a strong confounding effect on many of the parameters studied. For instance, Øverli et al. (1999) noted that cortisol production in response to stress was higher in larger, dominant Arctic charr. Previous studies suggest that prolonged stimulation of the adrenal system due to chronic organochlorine exposure depresses the ability of the fish to respond appropriately to danger (e.g. Hontela 1998). Although the apparent decrease of cortisol secretion in the 'high dose' Arctic charr was not significant, further study is clearly warranted because dominance hierarchies may have produced considerable within-tank variability, thereby masking treatment effects.

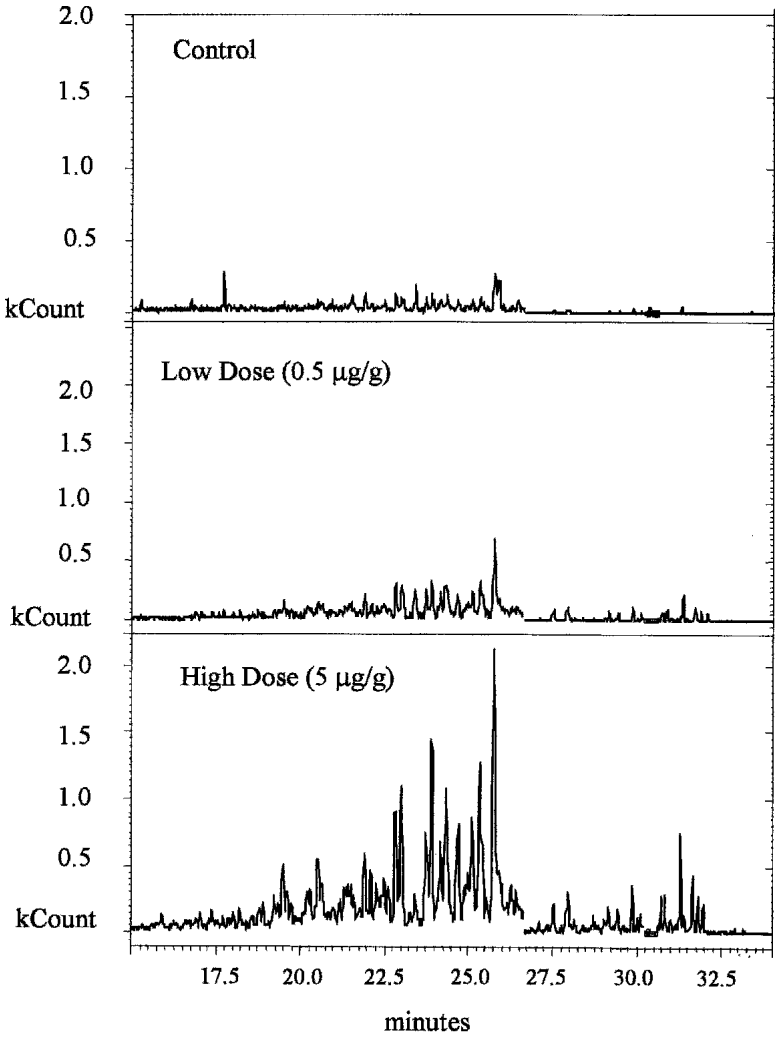

Figure 1. GC-MS/MS chromatogram of toxaphene in muscle tissue from Arctic charr in (from top to bottom) control, low dose and high dose exposure groups.

The effects of behaviour on the outcome of toxicological studies are rarely considered and merit further examination.

A final objective was to examine possible toxicological endpoints for future toxaphene studies. Lockhart et al. (1993) recommended looking at a number of physiological biomarkers, including circulating levels of Vitamin A, E and C. Vitamin C deficiency is considered a well-established diagnostic biomarker of toxaphene intoxication in fish (Saleh 1991) and was thus not examined here. vitamins A and E are implicated in important physiological processes; Vitamin A (retinol) is essential for vision, maintenance of epithelial tissues, growth and reproduction, while Vitamin E (tocopherol) is an antioxidant that protects cellular and subcellular membranes (Palace and Brown 1994). Decreased levels of both vitamins have been noted in fish exposed to organic contaminants including PCBs and dioxin (Palace and Brown 1994), but the effects of toxaphene were not known. There were no significant effects on plasma Vitamin E or A content in this study despite the high toxaphene levels achieved, indicating that Vitamin A and E are not suitable biomarkers of toxaphene exposure in Arctic charr.

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