Toxicokinetics of 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane in juvenile brown trout (*Salmo trutta*) and effects on plasma sex hormones

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Introduction

It is anticipated that with tighter regulations on some bromine-based FRs increased production and usage of alternative ones might arise. One likely chemical whose production volumes could increase to meet the demand for flame retardancy in polystyrene foams, electrical cable coatings and in construction materials is 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane or tetrabromoethylcyclohexane (TBECH).

To date, two studies have reported on the detection of TBECH in the environment[1,2]. Tomy *et al*. measured the β-isomer in Canadian arctic beluga whales (*Delphinapterus leucas)* and. Gauthier *et al.* measured all four isomers of TBECH in herring gull (*Larus argentatus*) eggs from the Laurentian Great Lakes of North America. There are also some reports that TBECH, of which there are four, can illicit androgenic effects and induce prostate specific antigen *in vitro*[3,4].

In this study we examined the toxicokinetics and effects of TBECH on circulating plasma hormone levels in juvenile brown trout (*Salmo trutta*). Our experiment was designed to exposure fish to 3 separate doses of the β-isomer via their food followed by a phase in which fish were exposed to untreated food. Fish in a fourth tank were continually exposed to unfortified food. Fish sampling was done on prescribed days and β-TBECH was measured in the carcass (whole fish minus thyroid, gall bladder and whole-blood) while estradiol (E2), testosterone (T) and 11-ketotestosterone (11-KT) were measured in the plasma.

Materials and Methods

Four hundred juvenile brown trout (*Salmo trutta*, mean weight *ca*. 60 g) obtained from the Whiteshell Fish Hatchery (West Hawk Lake, Manitoba) were randomly separated into four 800 L fibreglass aquaria. Each tank received water at a constant flow of 1.5L/min of UV- and carbon-dechlorinated Winnipeg city tap water, at a temperature of 12–15º C and pH between 7.6 and 9.4. The dissolved oxygen was always at level of >90% saturation. A 12-h light and 12-h dark photoperiod was maintained throughout the experiment. Fish were acclimatized in their respective tanks for 7 days prior to the start of the experiment and fed a diet of control food. Fish were fed a ration diet of 1% of their body weight three times per week. Our experiment consisted of two phases: (*i*) an uptake phase lasting 56 days where fish were in three tanks were fed fortified food; fish in the fourth tank were fed unfortified food and, (*ii*) a depuration phase lasting 77 days where all fish

were fed unfortified food. Eight fish from each tank were sacrificed on days 7, 14, 21, 35, 49 and 56 of the uptake phase and days 63, 77, 91, 105 and 133 of the depuration phase. Fish were euthanized 48 hours after the previous feeding by an overdose of a pH buffered solution of MS-222 (0.5 g/L). Once operculum movement ceased (<5 min), fork lengths and weights were recorded and 1–3 mL of blood was removed *via* the caudal vein with a heparinised syringe, along with the liver, gall bladder and thyroid. Whole blood was centrifuged at 6000*g* for 10 minutes to separate plasma from red blood cells. Plasma along with other portions of the fish was stored at –80°C. Whole fish (excluding the liver, lower jaw and gonad) was used for calculating bioaccumulation parameters while circulating levels of the studied hormones were measured in the plasma.

HPLC/MS/MS. HPLC separations were done on an Agilent 1100 series HPLC system (Agilent Technologies Canada, Inc., Mississauga, ON, Canada) equipped with a vacuum degasser, binary pump and an autosampler. A 0.2 µm in-line filter (Agilent Technologies) was connected before the C_{18} analytical column (50 mm x 2.1 mm i.d., 4 µm particle size; Alltech Canada, Guelph, ON, Canada). A binary mobile phase of Optima grade water and Optima grade methanol (Fisher Scientific, Nepean, ON, Canada) was used at a flow rate of 300 µL/min. For the targeted steroids, an initial composition of 80:20 water/MeOH (v/v), held for 1 minute, ramped linearly to 100% MeOH in 9 minutes and held for 6 min was used. The column was allowed to equilibrate for 7 minutes between runs. A Sciex 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) was used in the electrospray ion mode. Native and mass-labelled estradiol (E2 and $^{13}C_2$–E2) were analyzed in the negative (–ve) ion mode, while the androgens, T, 11–KT and d_8-P were monitored in the positive (+ve) ion mode.

GC/MS. All analyses were performed on an Agilent 5973 GC-mass selective detector coupled to an Agilent 6890 gas chromatograph. Splitless injections of 2 µL were made by a 7683 Agilent autosampler onto a 15 m × 0.25 mm id Rtx-1614 column capillary column (0.10 µm film thickness, Restek, Brockville, ON, Canada). UHP helium was used as the carrier gas at a flow rate of 0.75 mL/min and the injector was set to the oven tracking mode. The temperature program used was: initial oven temperature 90ºC with no hold time, ramped at 20ºC/min to 310ºC, and held for 5 min. Both electron ionization (EI) and electron capture negative ionization (ECNI) were employed in our study. In EI the ion source temperature was set at 250°C while in ECNI the ion source temperature was 150°C. Quadrupole temperature was 150°C in both ionization modes. BDE–71 and BDE–126 were analyzed in ECNI, with methane as the buffering gas, in the selected ion monitoring mode (SIM) by monitoring the two intense fragment ions of Br^-, *m/z* 79/81. β–TBECH was analyzed in EI-SIM by monitoring the $[M–HBr–Br]^+$ m/z 265/267. BDE-71, -126 and β–TBECH were quatified using external standards.

Results and Discussion

The bioaccumulation and depuration of the β-isomer in our exposed fish at each of the three doses is shown in Figure 1 (a-c). The β-isomer was detectable after day 14 [arithmetic mean ± 1 × SE: 2.4 ± 0.6 pmoles/g (lw)] and peaked on day 21 (3.8 ± 0.4 pmoles/g (lw)) in fish from the low treatment group. Fish from the medium treatment group had detectable amounts of the β-isomer after just 7 days of exposure [1.89 ± 0.82

pmoles/g (lw)] with a peak occurring on day 49 [10.3 ± 2.7 pmoles/g (lw)]. The range of β-isomer in fish from these two dosing groups brackets those measured in fish from Lake Winnipeg [mean (*n=8*): 6 pmoles/g, (lw), G. Tomy, *personal communication*].

Using ANOVA, we tested to see if there were statistically significant differences in amounts of the β-isomer in fish between sampling points in each of the three treatment groups. For fish from the low group, there were no statistically differences ($p>0.05$) in measured amounts of the β-isomer at any sampling point. In fish from the medium treatment group, the amount of the β-isomer measured in fish sampled on day 7 was significantly smaller ($p<0.05$) in fish from the other sampling points. In fish from the high treatment group, there were no statistically significant differences ($p<0.05$) in amounts of β-isomer on days 35, 49 and 56. Likewise, there were no statistically significant differences ($p>0.05$) in the amounts of β-isomer in fish sampling on days 7, 14 and 21. However, there were statistically significant differences ($p<0.05$) in the measured amounts of the β-isomer between the first and last three sampling points.

The appearance of the uptake profiles and results of our statistical analysis implies that fish from all three treatment groups reached steady-state before the end of our uptake phase and that the time to steady state likely occurred after day 21. This is consistent with the fact that the time to steady-state is dependent only on the elimination rate.

The elimination of the β-isomer in fish from all three treatment dose groups was rapid with calculated half-lives of *ca*. 16 days. This is consistent with the work of Nyholm *et al*., who showed that *t*-TBECH was also cleared rapidly in zebrafish (Nyholm et al., 2009).

We were also unable to detect any metabolites arising from loss of Br_2 or HBr. If the β-isomer is being excreted rapidly or if metabolites are being formed but are eliminated before sequestration in tissue compartments then this might explain why we did not observe any metabolites in our samples. No detectable amounts of any other isomer of TBECH other than the β-isomer were observed in whole fish muscle samples or in composite liver samples.

Because androgenic effects have been implicated by molecular modeling and *in vitro* ligand-binding and receptor–activation bioassay we chose to examine whether under our dosing regimens an effect might be observed[3,4]. For male fish, E2 levels remained unchanged throughout the study period. There were no differences in E2 levels between treated males and control males except at day 91 where fish from the high treatment group had reduced E2 levels relative to the control group. There were also no differences in E2 levels in male fish within the three treatment groups. The overall trend in T levels in male and female fish from all groups were similar: T levels remain relatively constant throughout the exposure period except for day 133 where in all cases we see an increase in T levels. Not surprisingly, the trends in 11-KT in fish are similar to trend observed for T. Overall, while we did observe a few instances where the measured amount of E2, T and 11-KT differed in fish from the treated groups relative to controls, we did not see a

consistent or discernable trend. Further work on gonadal gland histopathology to see if we can discern any impacts on gonad development at a cellular level is ongoing.

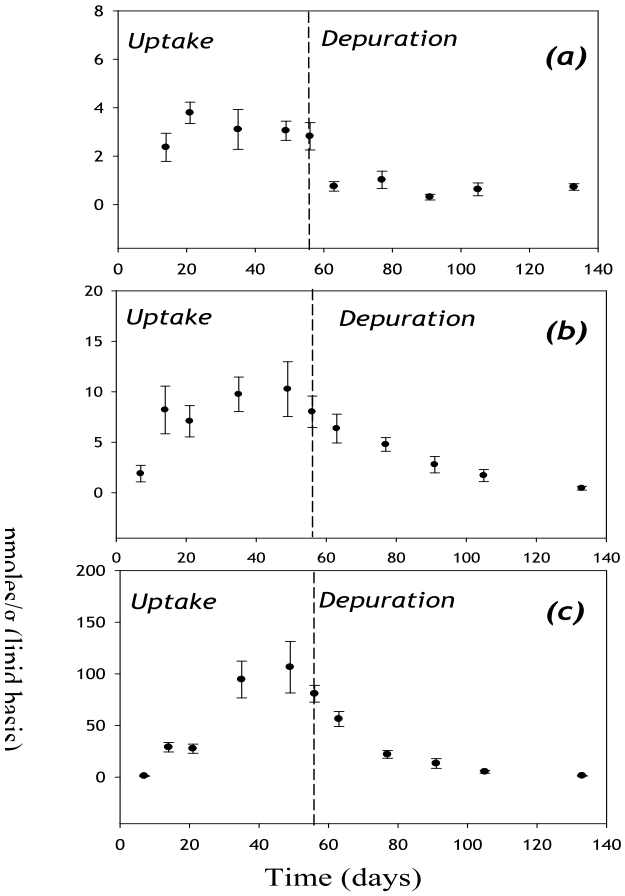

Figure 1 Uptake and depuration profiles of β - TBECH in juvenile brown trout from (a) low, (b) medium and (c) high treatment groups. Each point represents the arithmetic mean ± standard error (vertical bars) of eight fish from each group.

References:

1. Gauthier LT, Potter D, Hebert CE, Letcher RJ. (2009); *Environ. Sci. Technol. 43*, 312-317.
2. Tomy GT, Pleskach K, Arsenault G, Potter D, McCrindle R, Marvin CH, Sverko E, Tittlemier SA. (2008); *Environ. Sci. Technol. 42* (2), 543-549.
3. Khalaf H, Larsson A, Berg HA, McCrindle R, Arsenault G, Olsson PE. (2009) *Environ. Health Perspec.117*, 1853-1859.
4. Larsson A, Eriksson LA, Andersson PL, Ivarson P, Olsson PE. (2006); *J. of Med. Chem. 49*, 7366-7372.
5. Nyholm JR, Norman A, Norrgren L, Haglund P, Andersson PL. (2009); *Environ. Toxicol. Chem. 28* (5), 1035-1042.
6. Tomy GT, Palace VP, Halldorson THJ, Braekevelt E, Danell RW, Evans B, Brinkworth L, Fisk AT. (2004); *Environ. Sci. Technol.38*, 1496-1504.
7. Tomy GT, Palace VP, Pleskach K, Ismail N, Oswald T, Danell RW, Wautier K, Evans B. (2007); *Environ. Sci. Technol. 41* (14), 4913-4918.
8. Tomy GT, Thomas CR, Zidane TM, Muirson KE, Pleskach K, Hare J, Arsenault G, Marvin CH, Sverko E. (2008) *Environ. Sci. Technol.42*, 5562-5567.