Assessing the Toxicity of Polychlorinated *n*-Alkanes using Japanese Medaka (*Oryzias latipes*) Embryos and Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Introduction

Polychlorinated *n*-alkanes (PCAs), also known as chlorinated paraffins (CPs), are used for a variety of industrial applications including lubricating additives, flame retardants, adhesives, sealants and a number of other miscellaneous applications [1,2]. Industrial PCA formulations have carbon chain lengths between 10 and 30 and chlorine contents between 35-70% (by weight). Because they are produced with free radical chlorination, a single PCA formulation consists of thousands of different compounds with a range of physical-chemical properties [3]. Annual world production of PCAs is estimated at greater than 300 kilotonnes, and they remain one of the last high molecular weight organochlorines in production and use in North America and western Europe [4].

Despite high production and use, there is a lack of data regarding the toxic mechanism of action, the toxicity of PCAs of a single carbon chain length and chlorine content, and the sub-lethal effects of PCAs in aquatic organisms. The objective of this work was to study the effect of carbon chain length (C_{10}, C_{11}, C_{12} and C_{14}) and chlorine content on the toxicity of PCAs using the Japanese medaka (*Oryzias latipes*) embryo toxicity assay and dietary exposures using juvenile rainbow trout (*Oncorhynchus mykiss*). The medaka assay has been used to assess the toxicity of a number of hydrophobic organochlorines, such as chlorinated dioxins and PCBs [5,6,7], allowing a relative comparison of PCA toxicity. Juvenile rainbow trout were exposed to high dietary concentrations of PCAs to assess their short term (21 days) effects on behavior and their short and long term (82 days) effects on the histology of the liver, posterior kidney and thyroid.

Materials and Methods

Six PCAs were synthesized for this experiment; three by chlorination of an alkene starting material (1,5,9-decatriene; 1,10-undecadiene; and 1,13-tetradecadiene), and three by free radical chlorination of a ^{14}C-labeled alkane starting material (C_{10}, C_{12} and C_{14}). ^{3}H-labelled 2,3,7,8-TCDD was also used as a positive control in the medaka assay.

The protocols for the medaka assay were similar to past work [5,6,7]. Briefly: Eggs were collected from females and were placed into individual 1.8 ml GC vials containing rearing solution (1 ml) and various amounts of PCA, TCDD or no dose (control) (10 eggs per treatment). Four to 7 exposure concentrations were established for each PCA and TCDD. The PCA concentrations were based on their water solubility (WS) [8], as well as 1/100, 1/10, 10X and 100X the WS. Estimated concentrations accounted for partitioning of the PCA onto the glass vial. TCDD concentration were based on past Japanese medaka embryo toxicity tests [5,7]. Eggs were added to the vials on the day of fertilization, and were maintained at 25°C throughout the experiment,

and checked daily for 20 days. Water, egg and larvae concentrations of the ^{14}C-PCAs and the ^{3}H-TCDD were determined by LSC. Due to detection limit problems, concentrations of non-labeled PCAs were estimated based on the partition behavior of the ^{14}C-PCAs.

Juvenile rainbow trout were exposed to PCA spiked, and non-spiked (control), food for 21 days (3 concentrations) and 82 days (1 concentration). Ten rainbow trout were used for each treatment and were housed in separate 10, 20 or 40 L glass aquaria with flow-through dechlorinated water (11°C). After 21 days of exposure, 3 trout were designated for histological examination and 3 for determination of PCA concentrations. The remaining fish from the lowest exposure group were exposed for an additional 61 days (82 days in total). On day 85, 3 fish were sacrificed for histological examination and 3 for determination of PCA concentrations. PCA concentrations were determined by LSC [9] or by GC-ECD [10]. Behavioral monitoring was carried out daily during the 21 day exposures. Behavior data was compared with those outlined by McKim et al. [11]. For histological examination, tissues (liver, thyroid and posterior kidney) were processed in an automated tissue processor (IL MUP Tissue Processor) using an ethanol/butanol series and embedded in Tissue Prep II paraffin. Only tissue from the medium (21 days of exposure) and low (85 days of exposure) concentrations exposure trout were examined for histopathological effects and histomorphological measurements.

Results and Discussion

High aqueous concentrations of $C_{10}H_{15.5}C_{6.5}$, $C_{10}H_{15.3}Cl_{6.7}$, $C_{11}H_{18.9}Cl_{5.1}$ and $C_{12}H_{19.5}Cl_{6.5}$ caused mortality in medaka eggs or lethargic larvae (heart beat but no movement), but no mortalities or lesions were observed at lower concentrations, or in any eggs exposed to the C_{14}-PCAs. The aqueous concentrations in the exposures and the tissue concentrations of the larvae exposed to the C_{10}-, C_{11}- and C_{12}-PCAs were at levels that should elicit narcosis (Table 1). Concentrations of the C_{14}-PCAs in larvae did not reach narcotic levels, and larvae in these exposures appeared normal with no signs of narcosis. In contrast to the results for PCAs, TCDD was found to be extremely embryotoxic, consistent with past work using Japanese medaka eggs. The LOEC and NOECs for the PCAs and TCDD in medaka are presented in Table 1. The PCA LOEC were all > 30,000 times higher than the TCDD LOEC.

Rainbow trout exposed to the high dietary concentrations of PCAs, with the exception of trout exposed to $C_{14}H_{24.9}Cl_{5.1}$, exhibited a lack of, or a slowed startle response, loss of equilibrium and dark coloration. These responses are indicative of a narcotic mode-of-action. Histopathological lesions were observed in the liver of trout exposed to $C_{10}H_{15.3}Cl_{6.7}$ at dietary concentrations of 13 $\mu g \cdot g^{-1}$and tissue concentrations of 0.92 ± 0.24 $\mu g \cdot g^{-1}$. These multi-focal lesions included coagulative necrosis of hepatocytes with associated pigmented macrophage proliferation. These changes are an established biomarker of contaminant exposure in fish. No overt lesions were found in the liver of trout exposed to the other PCAs, or in any posterior kidney or thyroid tissue. Some changes in the histological morphometrics of the liver of rainbow exposed to PCAs were observed when compared with control rainbow trout (Table 2). No changes in kidney proximal tubule epithelium cell height or thyroid epithelium cell height were found in the PCA exposed rainbow trout.

Table 1: Estimated LC_{50} values based on K_{ow} assuming the chemical is a narcotic and tissue concentrations PCAs and TCDD required to cause a narcotic effect in Japanese Medaka eggs for

chemical	LC_{50}[A] ng·mL^{-1}	highest water conc. used ng·mL^{-1}	low estimate narcotic tissue conc. μg·g^{-1}[B]	highest tissue conc. measured μg·g^{-1}	Death or lethargic behavior	LOEC[A] ng·mL^{-1}	NOEC[A] ng·mL^{-1}
$C_{10}H_{15.5}Cl_{6.5}$	2500	9600	73	-	Y	460	62
$C_{10}H_{15.3}Cl_{6.7}$	2500	7700	74	3500	Y	370	50
$C_{11}H_{18.9}Cl_{5.1}$	2000	8900	58	-	Y	420	57
$C_{12}H_{19.5}Cl_{6.5}$	2700	270	78	460	Y	55	9.6
$C_{14}H_{25.9}Cl_{5.1}$	2500	3400	74	-	N	> 3400	3400
$C_{14}H_{23.3}Cl_{6.7}$	2900	1600	85	84	N	> 1600	1600
TCDD	2200	0.036	64	0.0081	Y	0.0017	0.0013

[A] The LC_{50} values were estimated based on the equation log LC_{50} = -0.94 log K_{ow} + 0.94 log (0.000068 K_{ow} +1) - 1.25 [12] and assuming the chemical is a narcotic.

[B] The low and high estimate of narcotic tissue concentrations are based on narcosis occurring at concentrations of 2 to 8 μmol·g^{-1} [13].

Table 2. Histological morphometrics (mean ± 1 standard error, n = 3) of liver from rainbow trout exposed to PCAs for 21 and 82 days. Treatment means which significantly differ from the control mean are indicated by an (* or **)(ANOVA, Dunnett pairwise comparison, * $p < 0.05$, ** $p < 0.1$).

PCA	exp. length (d)	exp. conc. (μg·g^{-1})	hepatocyte nuclear diameter (μm)	relative hepatocyte size (μm^2)	nucleus : cytoplasm area (μm^2)
control	21	-	5.51 ± 0.174	160 ± 1.27	0.175 ± 0.012
$C_{10}H_{15.5}Cl_{6.5}$	21	12	5.61 ± 0.0859	118 ± 6.47*	0.270 ± 0.0305*
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	21	13 ± 0.21	5.53 ± 0.293	135 ± 12.1	0.216 ± 0.0079
$C_{11}H_{18.4}Cl_{5.6}$	21	2.6	5.63 ± 0.134	149 ± 10.9	0.240 ± 0.0202
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	21	14 ± 0.11	5.30 ± 0.0361	123 ± 2.34*	0.219 ± 0.0036
$C_{14}H_{24.9}Cl_{5.1}$	21	0.78	5.58 ± 0.102	134 ± 12.6	0.228 ± 0.0215
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	21	29 ± 0.51	5.20 ± 0.162	116 ± 4.28*	0.226 ± 0.0184
control	82	-	5.81 ± 0.0470	183 ± 10.1	0.171 ± 0.0106
$C_{10}H_{15.5}Cl_{6.5}$	82	0.87	5.40 ± 0.165	155 ± 7.39	0.174 ± 0.00350
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	82	0.84 ± 0.65	5.30 ± 0.216**	146 ± 8.05**	0.179 ± 0.00610
$C_{11}H_{18.4}Cl_{5.6}$	82	1.8	5.41 ± 0.0751	160 ± 10.7	0.169 ± 0.0127
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	82	1.9 ± 0.042	5.48 ± 0.142	154 ± 8.41	0.184 ± 0.0214
$C_{14}H_{24.9}Cl_{5.1}$	82	0.082	5.63 ± 0.149	151 ± 13.8	0.200 ± 0.0112
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	82	5.7 ± 0.061	5.60 ± 0.0451	147 ± 0.693**	0.201 ± 0.00300

Summary

These are the first data on PCA toxicity using PCAs with a specific carbon chain length and chlorine content. The results of the Japanese medaka assay and feeding experiments using rainbow trout suggest that the acute mechanism of toxicity of short (C_{10-13}) and medium (C_{14-18}) carbon chain PCAs is narcosis (i.e., non-specific toxicity). The toxicity of PCAs appears to be inversely related to carbon chain length, consistent with past work [12,13]. However, because

water solubility is also inversely related to carbon chain length [8], the exposure rate in the medaka exposures may be greater for the shorter carbon chain length PCAs.

Histological lesions observed in the liver of rainbow trout exposed to high dietary concentrations of $C_{10}H_{15.3}Cl_{6.7}$ indicate that PCAs could have biochemical or physiological effects in fish. No effects were observed in the posterior kidney or thyroid, suggesting that the liver is a target for PCA toxicity. No other overt lesions were observed in the other treatment suggesting that toxicity is inversely related to carbon chain length, which has been observed in similar studies using mammals [12,13,14]. The $C_{10}H_{15.3}Cl_{6.7}$ standard was synthesized using free radical chlorination, and is unlikely to have a large number of PCAs with terminal Cl atoms. $C_{10}H_{15.5}Cl_{6.5}$ has terminal Cl atoms due to its synthesis from alkene strating material, and despite using similar dietary exposure concentrations as $C_{10}H_{15.3}Cl_{6.7}$ no liver lesions were found in rainbow trout exposed to this PCA. This suggests that PCAs with terminal Cl atoms may be less toxic. These results suggest that histopathological effects would only occur at extremely high exposure and tissue concentrations of PCAs, but at levels which are probably well beyond those observed in wild fish or invertebrates. However, more information is needed on the effects of long term PCA exposure.

Acknowledgments

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References

1. Government of Canada. **1993**; ISBN 0-662-0515 17E.
2. Willis, B., Crookes, M.J., Diment, J. and Dobson, S.D; **1994.** Garston, Watford, Building Research Establishment, 47 pp (Report TSD/19).
3. Tomy, G.T., Fisk, A.T., Westmore, J.B. and Muir, D.C.G; *Rev. Environ. Toxciol Chem.* **1998.** (*in press*).
4. Swedish National Chemicals Inspectorate; **1991.** KEMI Report No. 1. Washington DC.
5. Wisk, J.D. and Cooper, K.R; *Environ. Toxicol. Chem.* **1990**, 9, 1159.
6. Harris, G.E., Kiparissis, Y. and Metcalfe, C.D; *Environ. Toxicol. Chem.* **1994**, 13, 1405.
7. Metcalfe, C.D., Metcalfe, T.L., Cormier, J.A., Huestis, S.Y. and Niimi, A.J; *Environ. Toxicol. Chem.* **1997**, 16, 1749.
8. Drouillard, K.G., Hiebert, T., Tran, P., Tomy, G.T., Muir, D.C.G. and Friesen, K.J; *Environ. Toxicol. Chem.* **1998**, in press.
9. Fisk, A.T., C.D. Cymbalisty, A. Bergman and D.C.G. Muir; *Environ. Toxicol. Chem.* **1996**, 15, 1775.
10. Fisk, A.T., C.D. Cymbalisty, G.T. Tomy and D.C.G. Muir; *Aquat. Toxicol.* **1998**.(in press).
11. McKim, J.M., Bradbury, S.P. and Niemi, G.J; *Environ. Health. Perspect.* **1987**, 71, 171.
12. Veith, G.D., Call, D.J. and Brooke, L.T; *Can. J. Fish. Aquat. Sci.* **1983**, 40, 743.
13. McCarty, L.S; *Environ. Toxicol. Chem.* **1986**, 5, 1071.
14. Bucher, J.R., Alison, R.H., Montgomery, C.A., Huff, J., Haseman, J.K., Farnell, D., Thompson, R. and Prejean, J.; *Fund. Appl. Toxicol.* **1987**, 9, 454.
15. Serrone, D.M., Birtley, R.D.N., Weigand, W. and Millischer, R; *Food. Chem. Toxic.* **1987**, 25, 553.
16. Poon, R., Lecavalier, P., Chan, P., Viau, C., Hakansson, H., Chu, I. and Valli, V.E; *J. Appl. Toxicol.***1995**, 15, 455.