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Effect of Aroclor 1254 on the Biological Fate of 2,6-Dimethylnaphthalene in Coho Salmon (*Oncorhynchus kisutch*)

Tracy K. Collier, Edward H. Gruger, Jr., and Usha Varanasi

Biochemical Effects Task, Environmental Conservation Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, 2725 Montlake Boulevard East, Seattle, WA 98112

Polychlorinated biphenyls (PCBs) are widely distributed throughout the world's oceans. These persistent environmental contaminants are readily accumulated by many marine organisms (Peterson and Guiney 1979) and are known to be toxic to fish (Sangalang et al. 1981). PCBs can also affect the toxicity of other chemicals to fish. For example, PCBs can either enhance or inhibit aflatoxin-induced chemical carcinogenesis in rainbow trout (Salmo gairdneri) depending on the timing of exposure (Shelton et al. 1983). It has also been shown that exposure of rainbow trout to PCBs induces in vitro hepatic metabolism and DNA binding of benzo(a)pyrene (Egaas and Varanasi 1982), and there are considerable data which show that PCBs induce many of the enzymes responsible for the metabolism of polycyclic aromatic hydrocarbons (PAH) in aquatic species (Forlin 1980, Gruger et al. 1977, James and Little 1981). However, there is a lack of data on how PCBs affect the in vivo disposition of xenobiotics in marine organisms.

In the current study, we have investigated how exposure of coho salmon (Oncorhynchus kisutch) to PCBs can affect the subsequent biological fate of 2,6-dimethylnaphthalene (DMN). An alkylated PAH was chosen because, as a class, they are prominent constituents of the water-soluble fractions of fuel oils and crude oils (Roubal et al. 1977), and are acutely toxic to marine organisms (Dixit and Anderson 1977).

MATERIALS AND METHODS

2,6-Dimethyl[4-^{14}C]naphthalene (5.9 mCi/mmol) was synthesized by Wizard Laboratories (Davis, CA) and was found to be greater than 99% pure by thin-layer chromatography (TLC). 2,6-Dimethyl-3-naphthol, trans-3,4-dihydro-3,4-dihydroxy-2,6-dimethylnaphthalene, 2,6-dimethyl-3,4-naphthoquinone and 6-methyl-2-naphthalenemethanol were synthesized as described by Gruger et al. (1981). The PCB mixture used was Aroclor 1254®[1] (provided by Monsanto Chemical,

[1]Mention of trade names is for information only and does not constitute endorsement by the U.S. Department of Commerce.

St. Louis, MO). α-Naphthyl-β-D-glucuronic acid and α-naphthyl sulfate were obtained from Sigma Chemical Co. (St. Louis, MO).

Seawater-adapted coho salmon (93 ± 11 g) of mixed sex were purchased from DomSea Farms (Bainbridge Island, WA). Fish were acclimated to experimental conditions (11-12°; 27-30°/oo salinity) for at least one week prior to being used. Fish were fed daily to satiation with Oregon Moist Pellet (Moore-Clark Co., LaConner, WA) but were not fed for 24 hr prior to PCB or DMN exposure. Fish were held in 70L glass aquaria with flow-through seawater.

Coho salmon were injected ip with 0, 5, or 100 mg PCBs/kg body wt, dissolved in 100 uL corn oil. These groups are hereafter designated control, T_5, and T_{100} respectively. Four days later, they were force-fed 20 uCi of ^{14}C-DMN dissolved in 50 uL corn oil. This constituted a dose of 5.7 mg ^{14}C-DMN/kg body wt. Five fish from each group were collected at 24, 48, 96, and 168 hr after DMN exposure. Brain, liver, light muscle (from anterior dorsal area), bile, and blood samples were taken from each fish and stored at -60° until analyzed.

Brain (entire organ), liver (entire organ), and muscle (1 g) samples were homogenized in chilled saline (4 mL/g wet wt) using an Ultra-Turrax stainless steel homogenizer (Model SDT, Tekmar Co, Cincinnati, OH) set at 50. Blood samples (0.5-2 mL) were homogenized without added liquid. Aliquots (25-100 uL) of each homogenate, or bile, were added to 15 mL of InstaGel scintillation cocktail (Packard Instrument Co., Downers Grove, IL) and assayed for ^{14}C in a Packard 300C Tri-Carb Liquid Scintillation Spectrometer.

DMN and DMN metabolites were extracted from the homogenates using an automated extractor-concentrator (Prep I; DuPont, Wilmington, DE) as described by Krahn et al. (1982). The organic extract was analyzed for DMN metabolites by TLC as follows: 25 uL was applied to an LK6DF TLC plate (Alltech, Deerfield, IL) and developed to 15 cm in hexane. Under these conditions, the R_f of DMN was 0.55, and the DMN metabolite standards and conjugated naphthalene standards all had R_f values below 0.10. The adsorbant below R_f 0.2 was then scraped off and analyzed for ^{14}C by liquid scintillation spectrometry as described above, except that only 5 mL cocktail was used. Aliquots of bile (10 uL) were analyzed for DMN metabolites using a similar TLC method.

Two-way analysis of variance (SPSS 9.1) was used to examine the effects of time and PCB dose on DMN disposition in individual tissues and bile. In order to rank tissues according to levels of DMN-derived ^{14}C, data were converted to a dry wt basis. These data were first analyzed by one-way analysis of variance (Minitab 81.1) using all time points. Student-Newman-Keuls (SNK) test (Zar 1980) was then used to determine differences between tissues. The biological half-life of DMN-derived ^{14}C in blood was calculated from linear regression of log-transformed data.

RESULTS AND DISCUSSION

The levels of total DMN-derived radioactivity in bile, liver, brain, muscle, and blood of fish fed ^{14}C-DMN, with and without prior exposure to Aroclor 1254, are given in Table I. For all tissues and bile, regardless of PCB dose, levels of DMN-derived radioactivity decreased from 24 to 168 hr ($p<0.01$). The five sites examined fell into four groups with regard to concentration of total DMN-derived ^{14}C; bile > liver > muscle $\approx$ brain > blood ($p<0.05$), irrespective of either PCB exposure or time. The effects of PCB exposure and time on DMN disposition in individual sites appeared to be independent of each other, as no significant interactions were shown by two-way analysis of variance.

Concentrations of total ^{14}C in the bile (Table I) increased as a result of PCB exposure ($p<0.001$). This increase was primarily due to exposure at the high dose (T_{100}). TLC of the bile showed that virtually all (>90%) of biliary ^{14}C was in the form of DMN metabolites.

In the liver, there was no effect of PCB exposure on levels of unmetabolized DMN, total metabolites, or total ^{14}C. The proportions of DMN metabolites were not as high in the liver as in the bile, but still exceeded 40% of the total ^{14}C in the liver at 24 hr after DMN feeding, and at later times ranged from 65% to 82% (Figure 1).

An apparent effect due to PCB exposure was seen in both muscle ($p<0.10$) and brain ($p<0.10$). This effect, for both tissues, was primarily due to a decrease in total ^{14}C in the T_{100} fish (Table I). The proportions of total DMN metabolites in the muscle and brain ranged from 15-35% and 15-20%, respectively, at 24 and 48 hr (Figure 1). These values were not affected by either PCB exposure or time. After 48 hr, the levels of ^{14}C were too low to measure metabolites accurately.

In the blood, PCB exposure significantly affected levels of total ^{14}C ($p<0.05$) with the effect seen as a decrease in this value for T_{100} fish (Table I). Metabolite levels in the blood were too low to measure accurately. The biological half-life of total ^{14}C in the blood of control fish was 43 $\pm$ 10 hr, whereas in T_5 and T_{100} fish, it was 28 $\pm$ 2 and 27 $\pm$ 2 hr, respectively.

The results show that the biological fate of ^{14}C-labeled DMN in coho salmon can be substantially altered by prior exposure of the fish to PCBs. For example, biliary levels of DMN metabolites were increased in T_{100} fish as compared to controls. However, we did not observe any increase in either the amount or the proportion of DMN metabolites in the liver of PCB-exposed fish. PCBs are known to induce hepatic metabolism of xenobiotics by fish (Forlin 1980). Accordingly, our data suggest that induction did occur in the liver, but that DMN metabolites were readily excreted from this organ into the bile, where increased levels of DMN-derived ^{14}C were found after PCB exposure. Statham et al. (1978) found that

Table I: Effects of PCBs and time on levels of DMN-derived ^{14}C in coho salmon

	PCB Dose (mg/kg body wt)[a]		
	0	5	100
Bile*	4400±1000(15)[b]	4800±1100(15)	9100±1800(16)
Liver	160±24(17)	140±27(15)	170±31(18)
Brain*	44±8.6(19)	50±11(19)	29±6.6(18)
Muscle*	40±9.2(19)	49±11(18)	24±5.4(18)
Blood*	11±2.9(16)	12±2.9(17)	8.8±2.0(18)

	Time (hr)[c]			
	24	48	96	168
Bile†	8500±2500(12)	7400±1400(10)	6600±1100(12)	2000±650(11)
Liver†	260±34(14)	180±21(13)	120±14(14)	52±9.9(11)
Brain†	88±11(14)	46±4.7(15)	19±3.6(15)	6.8±1.2(12)
Muscle†	83±13(14)	35±4.1(15)	22±5.9(14)	6.6±1.4(12)
Blood†	25±3.1(11)	11±1.6(15)	5.0±1.0(14)	1.1±0.3(11)

a Data shown are for all times combined.
b x ± sem (n); units are DPM/uL or DPM/mg wet wt
c Data shown are for all doses combined.
* Significant effect due to PCB exposure. See text.
† Significant effect due to time. See text.

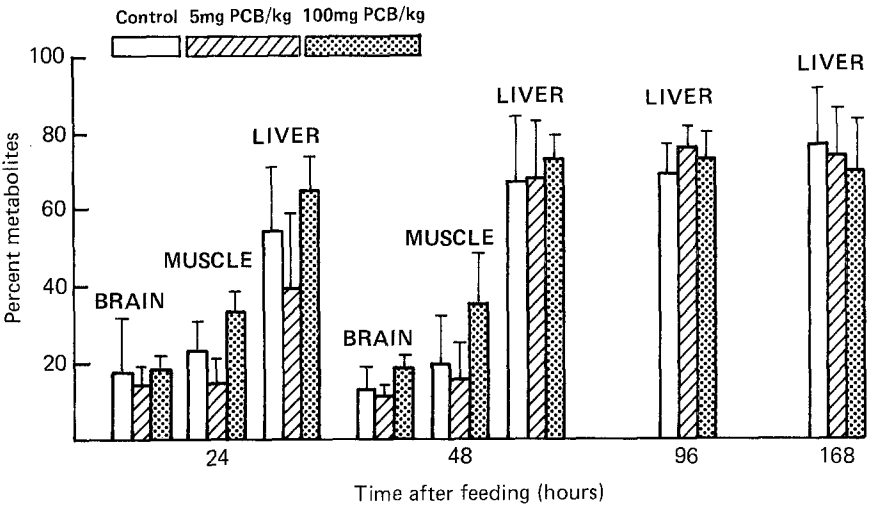

Figure 1. Proportion of total radioactivity found as DMN metabolites in coho salmon exposed to ^{14}C-DMN, with and without prior exposure to Aroclor 1254.

pretreatment of rainbow trout with 2,3-benzanthracene (ip injection) enhanced the metabolism and biliary excretion of ^{14}C-labeled 2-methylnaphthalene administered via waterborne exposure, but pretreatment also increased hepatic levels of methylnaphthalene-derived radioactivity. However, this increase was only seen for 6 hr after exposure to methylnaphthalene.

Analyses of the extrahepatic tissues provided further evidence for enhanced excretion of DMN or its metabolites from PCB-exposed coho salmon. Muscle, brain, and blood all showed decreases in DMN-derived radioactivity as a result of PCB exposure at the high dose. Also in the blood, the biological half-life of DMN-derived ^{14}C was reduced by approximately 40% after PCB exposure at both doses. In contrast to our results with extrahepatic tissues, the work of Statham et al. (1978) did not show any effect of 2,3-benzanthracene on the levels of methylnaphthalene-derived radioactivity in the muscle or blood of exposed fish. It is possible that if they had sampled at times beyond 12 hr, they may have seen an effect. However, the difference may also be due to the different routes of exposure used in the two studies. Thus, the present results demonstrate that prior exposure to Aroclor 1254, at least at the higher of the two doses used in this study, can significantly affect several aspects of the *in vivo* disposition of DMN in coho salmon. The overall effect of PCBs appears to be increased levels of DMN metabolites in the bile, resulting in generally lower levels of DMN or its derivatives in extrahepatic tissues. Further studies should address whether this altered disposition is correlated with altered toxicity.

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