Acute and Chronic Oral Toxicity of Chlorinated Dibenzofurans to Salmonid Fishes

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A median mortality of 120 ± 30 days occurred among juvenile Atlantic salmon (*Salmo salar*), fed dry fish food containing 2.7, 5.7, 2.8, and 9.1 μg/g wet weight of 2,8-di-, tri-, tetra-, and octachlorodibenzofuran, respectively (*1*). Only octachlorodibenzofuran was detected in the dead fish, and the level was 0.03 μg/g in the muscle and 0.21 μg/g in the gut (both values on wet weight basis). The fate of the lower chlorinated dibenzofurans was not known and additional experiments, described in this paper, were carried out with immature brook trout (*Salvelinus fontinalis*), fed relatively high levels of 2,8-dichlorodibenzofuran.

Experimental

The preparation of 2,8-dichlorodibenzofuran was carried out as described (*2*). Three crystallizations were necessary to remove tri- and tetrachlorodibenzofuran from the product.

Ten immature brook trout, tagged with colored tags were kept in running water (2 l./min) in a 200-l. Fiberglas tank and fed twice a day commercial fish food (Trout Chow, Ralston Purina Company) in the form of floatable pellets and in clear No. 3 gelatin capsules (E. Lilly & Company). Both pellets and capsules contained approximately 100 mg of food. Three fish, each fed on the average 700–800 mg of food per day, also received gelatin capsules containing crystalline 2,8-dichlorodibenzofuran. Once crystallized product, still containing a trichlorodibenzofuran and a small amount of a tetrachlorodibenzofuran was fed to one fish. By accident, one fish from the former group received one capsule containing the mixture of chlorinated dibenzofurans. The feeding schedule of 2,8-dichloridibenzofuran and the initial and final weights of the fish are presented in Figure 1. The fish were sacrificed as indicated and kept frozen (−14°C) until analysis. Fish R was isolated after the final capsule (10.3 mg) in a small tank containing 4 l. of water. Water was changed after 24, 48, 72, and 144 hr and processed as described (*3*) to detect possible hydroxylated metabolities of 2,8-dichlorodibenzofuran.

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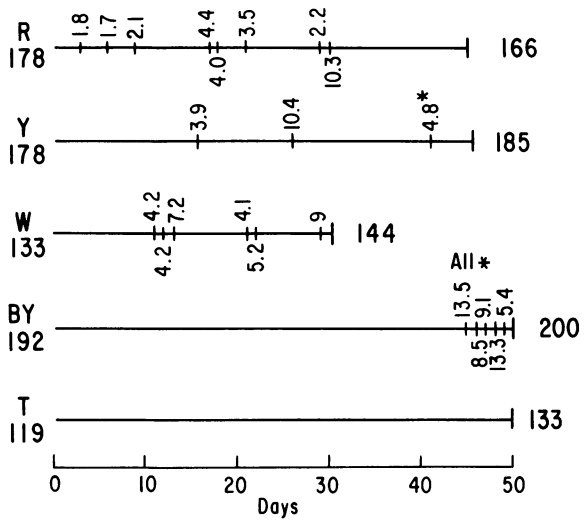

FIGURE 1. Feeding schedule. Initial and final weights (g) of fish are on the left and right margin, respectively. Administered amounts of 2,8-dichlorodibenzofuran (mg) are indicated by vertical lines. The asterisks (*) indicate administration of once-crystallized 2,8-dichlorodibenzofuran containing tri- and tetrachlorodibenzofuran.

Samples of the white lateral muscle, taken between the dorsal fin and the lateral line, and whole livers were extracted with hexane and the extracts were cleaned up by column chromatography on alumina and silica (*4*). Polychlorinated biphenyls (PCBs) were separated from chlorinated dibenzofurans by column chromatography on alumina (*5*) Gas chromatographic analysis was performed as described (*4*). A DuPont CEC 21-110B mass spectrometer was used to record mass spectra. Lipid concentrations refer to hexane-extractable lipid.

Results and Discussion

The levels of PCBs and lipid in the tissues, liver weight, and sex of the fish are summarized in Table 1. PCBs were of the Aroclor 1254 variety and had to be separated before the quantitation of chlorinated dibenzofurans. As an example, the GLC patterns of the Y liver extract are presented in Figure 2. The tracing *A* was obtained on the hexane eluate from the silicia column. After the removal of PCBs in the first fraction from the alumina column (2% methylene chloride in hexane, tracing *B*),

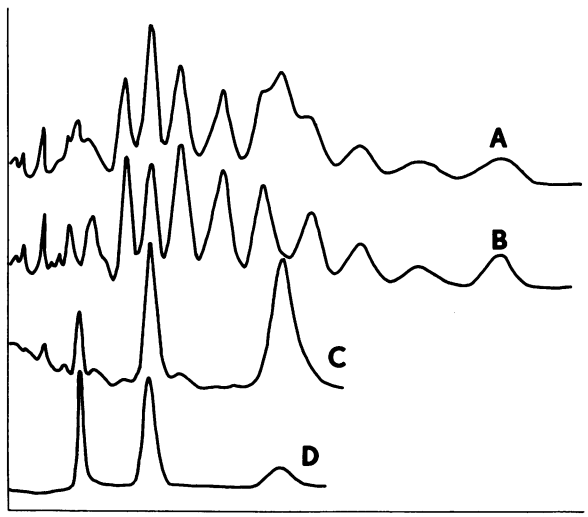

FIGURE 2. GLC patterns of Y liver extract: (*A*) hexane eluate from the silica column, containing a mixture of PCBs and chlorinated dibenzofurans and separated by chromatography on alumina to yield the PCB fraction (*B*) and the dibenzofuran fraction (*C*); (*D*) GLC pattern of once-crystallized 2,8-dichlorodibenzofuran (first peak from the left), containing tri- and tetrachlorodibenzofuran (second and third peaks, respectively).

Table 1. Chemical and biological characteristics of fish.

Fish	PCBs, μg/g wet weight [a]		Liver weight, % of body weight	Lipid, %		Sex (immature)
	Muscle	Liver		Muscle	Liver	
R	0.05	0.25	0.75	0.32	3.05	Male
Y	0.05	0.34	3.12	0.40	14.45	Male
W	0.15	0.35	1.86	2.98	8.01	Male
BY	0.08	0.12	1.77	0.42	2.71	Female
T	0.06	0.33	1.96	0.67	8.60	Female

[a] Aroclor 1254.

Table 2. Relative accumulation of 2,8-di-, tri-, and tetrachlorodibenzofurans.

Sample	Peak height ratios		
	2,8-Dichlorodibenzofuran	Trichlorodibenzofuran	Tetrachlorodibenzofuran
Fed preparation	1	0.92	0.18
Y muscle	1	0.40	0
Y liver	1	2.06	1.87
BY muscle	1	0.87	0.28
BY liver	1	1.04	0.36
T liver	1	1.11	1.16

chlorinated dibenzofurans appeared in the second fraction (20% methylene chloride in hexane, tracing *C*). The tracing *D* of the once-crystallized 2,8-dichlorodibenzofuran is presented for comparison.

Table 3. Administered and tissue levels of 2,8-dichlorodibenzofuran.

Fish	2,8-Dichlorodibenzofuran, μg/g wet weight		
	Administered	Found	
		Muscle	Liver
R	361	0.052	0.307
Y	107	0.048	0.150
W	254	0.340	0.400
BY	260 [a]	0.230	1.04
T	—	—	0.146

[a] Once-crystallized preparation

It can be seen that the relative concentration of the tri- and, particularly, of the tetrachlorodibenzofuran is higher in the isolated mixture than in the administered preparation. The relatively higher accumulation of these compounds was observed in both fish Y and BY, and also in the liver of fish T, which was not actually fed chlorinated dibenzofurans, but was present in the tank during the feeding of all fish (Table 2).

The administered doses of 2,8-dichlorodibenzofuran and the detected tissue residues are presented in Table 3. In all cases the tissue residues are very low, 0.01–0.13% of the administered dose in the muscle and 0.08–0.4% in the liver. The feeding history affects the residual levels. Thus fish W, sacrificed 1 day after dosing contains higher levels of 2,8-dichlorodibenzofuran than fish R, which received a similar last dose and a higher total dose, but was sacrificed only 15 days after the last dose.

Mass spectra of the crude mixtures of organic compounds isolated from the water, containing excreta of fish R, indicate the presence of a dichlorodibenzofuran (M^+236) and of a hydroxydichlorodibenzofuran (M^+ 252).

According to the feeding data, 2,8-dichlorodibenzofuran has a low acute toxicity to immature brook trout, since no mortality resulted even after administering a single dose at a level as high as 122 mg/kg. The low acute toxicity and residual levels of 2,8-dichlorodibenzofuran may be due to the poor absorption of the compound in the gut and to its excretion in the form of a conjugated hydroxy derivative.

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