Uptake of Chlorinated Paraffins and PCB from Suspended Solids and Food by Juvenile Atlantic Salmon

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High molecular weight chlorinated paraffins were recently suggested as PCB substitutes in many applications [DOVER CHEMICAL CORPORATION 1972a]. Chlorinated paraffins have been marketed at least since 1946 [HOOKER CHEMICAL CORPORATION 1946] and used as fire retardants, plasticizers, lubricants, antistatic agents, and additives in paints, machine tool oils, and tanning compositions. In 1969 the U.S. annual production of chlorinated paraffins was 28×10^6 kg [UNITED STATES TARIFF COMMISSION 1971].

There are no data on the behaviour and fate of chlorinated paraffins in the environment and little is known about their toxicological properties. Chlorinated paraffins were not irritant to human skin [DOVER CHEMICAL CORPORATION 1972b]. The single-dose oral LDO was 25 and 50 g/kg in guinea pigs and rats, respectively [DOVER CHEMICAL CORPORATION 1972c].

Methods for the determination and confirmation of chlorinated paraffins have been recently published [ZITKO 1973, 1974].

This paper describes the uptake of chlorinated paraffins with 40 and 70% chlorine and of PCB with 54% chlorine, by juvenile Atlantic salmon (*Salmo salar*). The preparations were administered adsorbed on silica and in food.

EXPERIMENTAL

Chlorinated paraffins Cereclor 42 (I.C.I., 42% chlorine) and Chlorez 700 (Dover Chemical Corporation, 70% chlorine), and a PCB preparation Aroclor 1254 (Monsanto, 54% chlorine) were used.

Suspended solids were simulated by SilicAR CC7 (Mallinckrodt, 200-325 mesh). SilicAR (50 g) was suspended in hexane (150 mℓ) in a 500-mℓ round-bottom flask, chlorinated paraffin or Aroclor 1254 (50 mg in 10 mℓ hexane) was added, the suspension was shaken on a

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wrist-action shaker for 1 h, and hexane was evaporated on a rotatory evaporator at 30°C.

Dry fish food (Trout Chow, Purina) was contaminated as described [ZITKO and HUTZINGER 1972] to contain Cereclor 42 and Chlorez 700 at levels of 10 and 100 µg/g.

Uptake from suspended solids. Juvenile Atlantic salmon were kept for up to 144 h in 3-ℓ Erlenmeyer flasks (two 5-7 g fish per flask) in 2 ℓ of aerated fresh water at 7-8°C. The flasks contained contaminated SilicAR in a concentration of 1 g/ℓ. One flask contained uncontaminated SilicAR. Fish were sacrificed at different exposure times and kept frozen (-20°C) until analysis.

Uptake from food. Juvenile Atlantic salmon were kept in 20-ℓ fiberglass tanks (20 fish per tank) in running fresh water (500 mℓ/min) at 10-15°C, 16 h light, 8 h dark photoperiod and fed manually several times a day, 5 days a week. The amount of food added weekly to a tank was approximately 8 g. The contaminated diet was fed for 181 days, followed by 74 days on a control diet. Fish in one tank were fed the control diet throughout the entire experiment. At times one fish from each tank was sacrificed and kept frozen until analysis.

Analysis. PCB and chlorinated paraffins were determined as described [ZITKO 1971, 1973]. Two fish were analyzed at each exposure time in the suspended solids experiment, one fish in the feeding experiment. The analysis was always carried out on the whole fish. The concentration of chlorinated paraffins in fish is expressed as chlorine, µg/g wet weight, and can be converted into chlorinated paraffin concentration by multiplying by 2.38 and 1.43 for Cereclor 42 and Chlorez 700, respectively. The concentration of PCB is expressed as Aroclor 1254, µg/g wet weight.

RESULTS AND DISCUSSION

Uptake of chlorinated paraffins and PCB from suspended solids

Juvenile Atlantic salmon accumulated a relatively large quantity of PCB, but very little, if any chlorinated paraffins from suspended solids (Table 1). The origin of chlorine in the control fish is not known. None of the fish contained measurable amounts of p,p'-DDD, p,p'-DDT, and dieldrin which would be eluted in the same fraction as chlorinated paraffins in the analysis. Even in the case of fish exposed to chlorinated paraffins it is not certain that the detected levels of chlorine are due to these compounds, since the concentration is too low for the currently available confirmatory test [ZITKO

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1974]. The increase of chlorine concentration with increasing exposure time may be an indication of a small uptake of chlorinated paraffins.

TABLE 1

Uptake of chlorinated paraffins and PCB from suspended solids by juvenile Atlantic salmon.

Preparation	Time of exposure,h	Concentration in fish, chlorine, µg/g	Lipid %
Control	48	0.34	0.99
Cereclor 42	48	0.44	1.10
	144	0.75	1.33
Chlorez 700	48	0.22	1.56
	144	0.46	2.10
Aroclor 1254	24	19.9*	1.52
	48	28.3*	1.86
	144	134 *	1.78

*expressed as Aroclor 1254

It is not likely that the low concentration of chlorinated paraffins in fish is due to metabolic dechlorination of these compounds. The exposure times were quite short and fish are known to metabolize most lipid-soluble foreign compounds only very slowly. The uptake of chlorinated paraffins may be limited by their higher average molecular weight (579-922) as compared to that of Aroclor 1254 (327). In addition, chlorinated paraffins are more strongly adsorbed on silica than PCB. For example, 10% ether in hexane is needed to elute chlorinated paraffins from alumina and silica columns, whereas PCB is in both cases eluted with hexane [ZITKO 1973].

It is interesting to note that the GLC pattern of Aroclor 1254, taken up by the fish was significantly different from that of the standard (Table 2) and indicated a relatively higher uptake of the components with shorter retention times. The differences in the relative uptake were somewhat less pronounced at longer exposure times. Higher water solubility or volatility of chlorobiphenyls with shorter retention times may be responsible for this effect. On the other hand, no change of the PCB pattern was observed during the

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feeding of Aroclor 1254 contaminated food to juvenile Atlantic salmon [ZITKO and HUTZINGER 1972].

TABLE 2

Changes of GLC pattern of Aroclor 1254 taken up from suspended solids by juvenile Atlantic salmon.

Sample	Normalized peak heights*					
Aroclor 1254	7.78	11.4	9.09	10	8.25	6.96
Fish exposed 24 h	16.6	17.1	13.2	10	6.55	3.92
" " 48 h	15.1	15.9	12.6	10	6.68	4.48
" " 144 h	14.9	15.7	12.6	10	6.65	4.55

*Peak heights of the 6 major Aroclor 1254 peaks in order of increasing retention time, corrected to a height of 10 units for peak No. 4.

Uptake of chlorinated paraffins from food

Chlorinated paraffins did not accumulate in juvenile Atlantic salmon fed a diet containing 10 and 100 µg/g of Cereclor 42 and Chlorez 700 (Table 3). Residual levels of Aroclor 1254 obtained under similar feeding conditions [ZITKO and HUTZINGER 1972] are included for comparison. The levels of chlorine detected after 33 days of feeding may be partly due to chlorinated paraffins, but were again too low for confirmation. Chlorine was not detectable after 109 and 181 days of feeding. In the case of Aroclor 1254 the residues corresponding to the dietary level of 10 µg/g reached an equilibrium within 30 days, whereas at 100 µg/g an equilibrium was not reached within more than 200 days of feeding. It is possible that the high molecular weight of chlorinated paraffins slows down or completely inhibits their absorption in the digestive tract. However, the metabolism of chlorinated paraffins, for example, by dechlorination cannot be excluded.

Oral toxicity of chlorinated paraffins to juvenile Atlantic salmon

Mortalities occurred among fish fed chlorinated paraffins, but also among fish on the control diet. Nutritionally deficient food is very likely responsible for the mortalities in the control group. It was noticed late in the experiment that the crude lipid content of the fish food decreased to 2.47% from the normal value of 5-8%. This decrease was probably caused by autoxidation

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TABLE 3

Uptake of chlorinated paraffins and PCB from food by juvenile Atlantic salmon.

Days of feeding		33		109		181	
Diet		Residue*	Lipid %	Residue*	Lipid %	Residue*	Lipid %
Control		0.30	1.03	nd+	0.65	nd+	0.47
Cereclor 42,	10 µg/g	0.11	1.30	nd	0.69	nd	0.49
	100 µg/g	0.51	1.22	nd	0.49	nd	0.34
Chlorez 700	10 µg/g	0.29	1.13	nd	0.40	nd	0.29
	100 µg/g	0.49	1.30	nd	0.56	nd	0.92
Aroclor 1254,	10 µg/g	3.86**	5.09	3.80**	3.10	3.88**	2.07
	100 µg/g	13.9	5.30	24.0	2.73	30.0	2.69

*expressed as chlorine, µg/g wet weight, unless stated otherwise

**expressed as Aroclor 1254

+not detectable, <0.05 µg/g

of the lipids and oxidized lipids are toxic to fish [KING 1973]. No additional mortalities occurred in any of the groups during the final 74 days when a control diet with normal lipid content was fed. The LT50 values of fish fed chlorinated paraffins are much lower than that of fish on the control diet (Table 4). This result indicates that chlorinated paraffins are probably

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toxic to juvenile Atlantic salmon.

TABLE 4

LT50 of juvenile Atlantic salmon in the feeding experiment.

Feeding	LT50, days
Control	138
Cereclor 42, 10 µg/g	47
100 µg/g	80
Chlorez 700, 10 µg/g	71
100 µg/g	39

The toxicity of both chlorinated paraffin preparations is approximately the same. Cereclor 42 was more toxic at 10 than at 100 µg/g. The feeding experiment did not allow the determination of the amount of food actually ingested by the fish. It is possible that fish could taste Cereclor 42 and fed less on food containing higher concentration of this preparation.

CONCLUSION

In contrast to PCB, chlorinated paraffins are much less, if at all, accumulated by juvenile Atlantic salmon when the fish are exposed to chlorinated paraffins adsorbed on silica or fed contaminated food. Chlorinated paraffins at 10 and 100 µg/g in food are very likely toxic to juvenile Atlantic salmon, but additional experiments are required to confirm this conclusion.

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