Analysis of a Chlorinated Terphenyl (Aroclor 5460) and Its Deposition in Tissues of Cod *(Gadus Morhua)*

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SUMMARY

An analytical procedure for Aroclor 5460, a chlorinated terphenyl, is described. Cod fed Aroclor 5460 deposited this material in various tissues. Absorptive and excretory efficiency seemed poor. Some selectivity in absorption, deposition or excretion of Aroclor 5460 was observed.

INTRODUCTION

Chlorinated terphenyls (designated the Aroclor 5400 series) are available from their manufacturer for various industrial applications (1). So far as we are aware, no information about the analysis of these materials or their persistence in the environment has been reported. Some recent reports (2-4) have suggested that Aroclor 5460, a chlorinated terphenyl containing 60% Cl may exert some effect on metabolic processes. This paper describes the analysis of Aroclor 5460 and its retention by cod (*Gadus morhua*) after feeding.

EXPERIMENTAL

Analysis of Aroclor 5460

Aroclor 5460 was extracted from cod tissue by homogenising with *n*-hexane (Fisher Scientific, redistilled from glass). A suitable amount of tissue (except blood, see below) (1-10 g depending on lipid content) was homogenised at high speed for 2 mins in a stainless

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steel apparatus (Sorvall "Omnimixer") fitted with teflon gaskets and cooled in ice. The homogenate was filtered (through hexane-washed Whatman #41 paper) and allowed to separate, if necessary, into aqueous and organic phases. The hexane layer was recovered, dried over anhydrous Na_2SO_4 and made to volume. An aliquot was evaporated to dryness under N_2 for gravimetric lipid determination.

Blood samples were extracted with chloroform-methanol by the method of Plack *et al* . (5) since attempts to extract blood with hexane generated unbreakable emulsions. The chloroform-methanol phase was reduced to dryness by rotary evaporation, redissolved in hexane, and filtered if necessary to obtain a clear hexane phase.

An aliquot of the hexane phase containing <0.5 g lipid was reduced to 5 ml approx. by rotary evaporation. This was applied to a 15 x 2 (i.d.) cm column consisting of 25 g Florisil (60-100 mesh, Fisher Scientific) activated overnight at 110°C, cooled over P_2O_5 in a desiccator, slurried in *n*-hexane and topped with a few mm of anhydrous Na_2SO_4. Elution with 200 ml *n*-hexane brought off Aroclor 5460, PCBs and some *pp'*-DDE. The eluate was reduced to a known volume (generally 1-10 ml) by rotary evaporation prior to GLC.

Aroclor 5460 was estimated by GLC using a Hewlett-Packard 5750 instrument fitted with a ^{63}Ni pulsed electron capture detector. Conditions were as follows:

Column: 6' x ¼" (i.d.) coiled glass, packed with 3% Dexsil 300 on 100-120 mesh Gas-chrom Q, operated at 310°C.

Carrier gas: 10% methane in argon, 40 ml/min.

Detector: temp, 325°C; 150 µsec pulse interval

Injection temp: 320°C.

Runs were recorded on a Moseley 7127 stripchart recorder fitted with a Disc Instruments, Inc., integrator. Injections were made with Hamilton #701N syringes. Under these conditions, Aroclor 5460 eluted in 20 min (Fig. 1a).

Graphs of total area under curve v. load were linear in the load range 7-56 ng. Minimum level of detectability was approx. 2.5 ng.

The estimation of Aroclor 5460 by fluorescence spectroscopy and by TLC was briefly investigated.

Fluorescence spectra in *n*-hexane, recorded in a Perkin-Elmer MPF-2A instrument, showed an emission maximum at 330 nm (excitation at 255 nm). Minimum detectable concentrations were of the order of 0.5 ppm. However, these emission peaks were masked by other emissions in hexane extracts of cod tissue cleaned up on Florisil, and so the procedure was not used in the present study. In any case, emission spectra of Aroclor 5460 were similar to those of PCBs (6) and so differentiation of the two groups would have been difficult.

TLC of Aroclor 5460 was carried out on silica gel G plates activated at 130°C for 2 hr and developed in *n*-hexane or *n*-heptane. Spots were viewed in UV light after silver nitrate spraying (7). Aroclor 5460 ran just behind *pp'*-DDE (R_f. Aroclor 5460 0.45; R_f. *pp'*-DDE, 0.5) and could be detected at levels of down to approx. 1 µg. In view of the relatively poor sensitivity (and selectivity) of this procedure it was not adopted for the present analyses.

Feeding experiments

Two experiments were carried out in which Aroclor 5460 was fed to cod of commercial size (approx. 1-2 kg). In both experiments, cod were kept in groups of four in 60 gallon tanks, through which seawater at approx. 1°C flowed at 1 gall/min.

In Experiment 1, 2 ml of a herring oil, containing 1 g of Aroclor 5460 (prepared by mixing appropriate amounts of Aroclor dissolved in hexane and herring oil, and removing solvent by rotary evaporation), were fed to each of four mature cod which had been starved for two weeks up to this point. The oil was deposited by pipette in the stomach of the fish. Four control fish were fed 2 ml herring oil similarly. After one week during which they were starved, all fish were killed by free bleeding from the tail. Blood was collected in heparinised beakers and other tissues were removed by dissection. Gut contents were squeezed out into a beaker; and gut tissue was rinsed in running water to remove remaining traces of gut contents. Other tissues were rinsed briefly in running water to remove any blood present. Analyses for Aroclor 5460 and lipid were carried out as described above.

In Experiment 2, eight mature cod (starved two weeks) were fed 2 ml of a herring oil solution containing 1 g Aroclor 5460, as described above. The fish were kept 70 days and were fed chunks of herring every two or three days after an initial 7 day period of starvation. At the end of 70 days, the four surviving fish (four others having died or been killed at intervals in between) were killed and analysed.

Table 1

Levels of Aroclor 5460 in tissue (µg/g wet weight) and in tissue lipid (µg/g lipid) in cod fed Aroclor 5460

	Fish A		B		C		D			
Wt (kg)	0.625		1.815		1.362		1.700		Mean ± SD	
Sex	F		M		F		M			
	5460/ tissue	5460/ lipid	5460/ tissue	5460/ lipid	5460/ tissue	5460/ lipid	5460/ tissue	5460/ lipid	5460/ tissue	5460/ lipid
Liver	8.3	92.2	14.4	22.1	31.9	409	12.4	16.9	16.75±10.4	135±186
Muscle	0.06	18.2	0.11	21.2	0.12	27.3	0.14	35.9	0.11±0.03	25.7±7.8
Brain	0.45	2.32	2.25	42.4	1.7	11.3	0.36	4.6	1.19±0.93	15.2±18.6
Blood	11.0	2075	7.9	470	ND*	ND*	3.2	271	5.52±4.88	704±934
Gall bladder	1.43	27.6	2.0	62.5	2.28	79.7	5.15	358	2.72±1.66	132±152
Gill	0.6	65.9	8.5	548	0.86	68.2	1.64	193	2.9±3.76	218±227
Spleen	0.84	35.0	2.76	251	1.43	13.0	3224	120	2.07±1.12	105±108
Kidney	3.1	70.4	14.5	580	3.1	91.2	3.6	62.1	6.08±5.62	201±253
Heart	0.75	22.9	2.1	233	0.6	109	0.62	6.3	1.02±0.72	92.8±104
Gonads	0.17	5.98	0.22	5.02	0.42	30.2	0.29	16.9	0.28±0.108	14.5±11.8
Intestine	145	18590	612	149269	3.6	1385	7.8	1560	192±287	42701±71502
Stomach	1.10	314	0.36	133	0.22	34.9	2.13	426	0.95±0.87	227±176
Pyloric caeca	96.4	1890	41.5	2024	11.8	337	9.6	800	39.8±40.4	1263±825
Aroclor 5460 (g) in intestinal contents and stomach contents										
	1.05		0.63		0.0065		0.0025		0.422±0.512	

*ND = not detected

RESULTS & DISCUSSION

Recovery experiments with Aroclor 5460 showed that extraction efficiency (from cod liver and muscle samples spiked by injection with acetone solutions of Aroclor 5460) was better than 90%. Recovery from Florisil columns was in the range 95-105%. The figures in the following tables are not corrected for the small losses in extraction efficiency. It should be emphasized, however, that injection of Aroclor 5460 in acetone solution into a tissue does not necessarily simulate its distribution in tissue after deposition from feeding. Extraction efficiencies from experimental samples may therefore be below 90%, and so subsequent figures should be regarded as minimal. No recovery experiments were carried out on blood extraction methods.

The results from Experiment 1 are shown in Table 1. Aroclor 5460 was found in all tissues of those fish fed the material, except for one blood sample. No Aroclor 5460 was found in any tissues of control fish, and for sake of clarity analyses of controls are omitted from Table 1. Typical chromatograms of liver extracts from Aroclor fed and control fish are shown in Figs. 1b and 1c, respectively.

The amounts of Aroclor 5460 remaining in gut contents one week after feeding varied widely. Most of it was probably in the intestine, as no oil was observed in the stomach. High levels of Aroclor 5460 were found in pyloric caecal and intestinal tissue; these may have been due to contamination by unabsorbed material not effectively rinsed out after dissection, or, in the case of intestinal tissue, to incomplete absorption through the intestinal wall. Absorption efficiency seemed highly variable with, in one case (Fish A), approximately all the fed Aroclor 5460 apparently remaining in the gut. Even in the two fish which had low gut content Aroclor 5460 levels, (C and D) absorption efficiency (or retention) appeared low, as in each fish a total of 1-10 mg Aroclor 5460 was calculated to be distributed through all tissues out of 1 g fed. Catabolic and/or excretory efficiency may have been high in these fish — and the presence of Aroclor 5460 in the gall bladders suggests that some excretion did take place — but in view of the expected metabolic inertia of chlorinated terphenyls (by analogy with PCBs: eg. 8) this seems a less likely explanation of low tissue levels than poor absorption.

Aroclor 5460 levels varied widely between different fish probably as a result of (among other

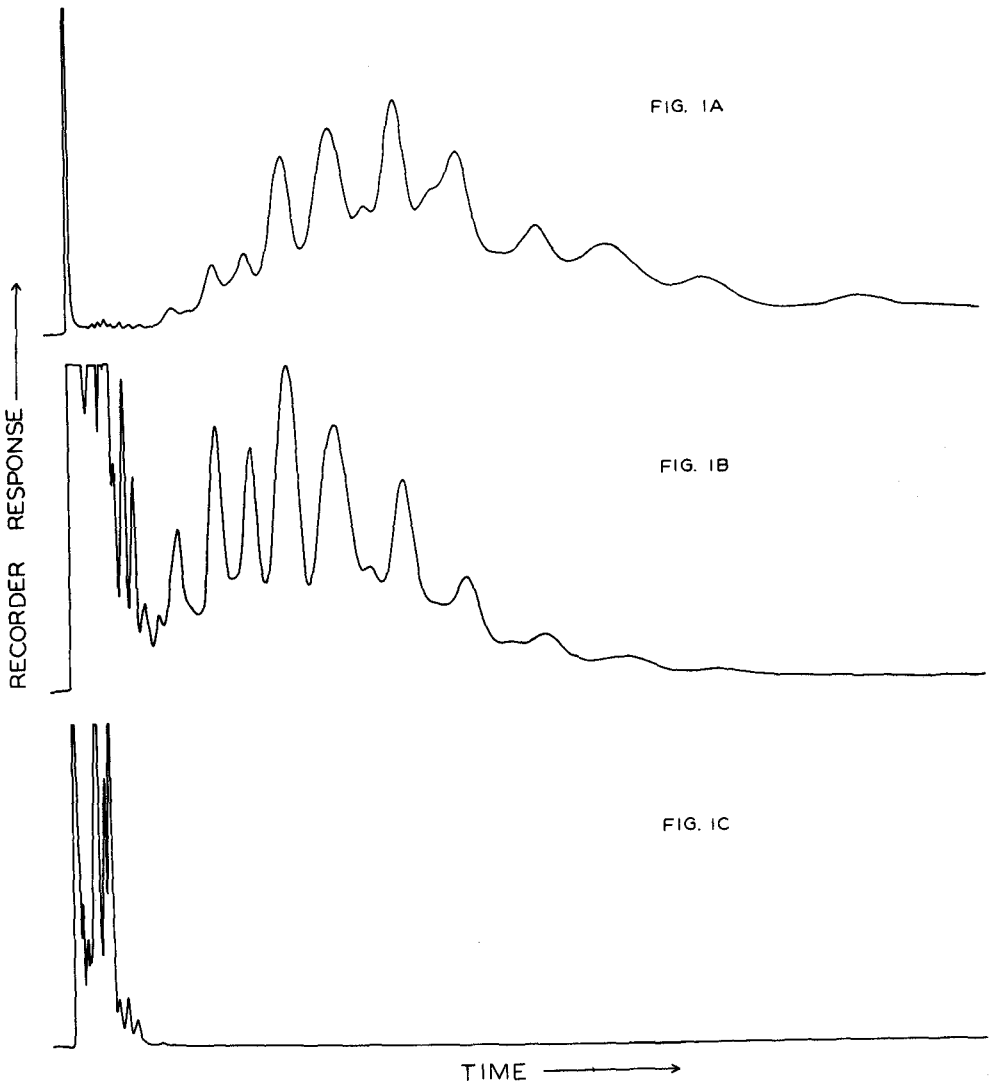

Fig. 1 Chromatograms of Aroclor 5460 run under conditions described in text. Fig. 1a, standard Aroclor 5460; 1b, hexane extract (cleaned up on Florisil) of liver from cod fed Aroclor 5460; 1c, hexane extract (cleaned up on Florisil) of liver from control cod.

Table 2

Aroclor 5460 levels in tissue (µg/g wet weight) and in tissue lipid (µg/g lipid) from cod starved 70 days after feeding Aroclor 5460

	Fish									
	H		J		K		L			
Wt (kg)	0.823		0.908		1.22		0.738		Mean ± SD	
Sex	F		M		M		F			
	5460/ tissue	5460/ lipid	5460/ tissue	5460/ lipid	5460/ tissue	5460/ lipid	5460/ tissue	5460/ lipid	5460/ tissue	5460/ lipid
Liver	0.63	31.9	11.96	20.1	1.53	21.9	6.36	13.6	5.12±5.21*	21.9±7.6
Gonad	0.21	31.3	0.28	2.38	0.04	10.3	0.38	26.6	0.23±0.14	17.6±13.6
Brain	0.29	2.24	0.43	6.72	0.24	2.1	0.67	6.63	0.41±0.19	4.42±2.6
Blood	0.55	112	0.71	34.1	0.60	31.3	0.22	17.6	0.52±0.21*	48.8±42.8*

* significantly lower ($P<0.05$) than comparable values in Table 1.

factors) inefficient absorption. It is therefore difficult to draw any but the most general conclusions from these results. It is clear, however, that Aroclor 5460 was distributed widely throughout the fish, being found in all tissues analysed, and was presumably transported *via* the bloodstream. It is perhaps worth noting that it was found to enter brain and gonadal tissue. In one batch of sperm, taken from a mature male fish, a level of 0.085 µg/g wet tissue (4.45 µg/g lipid) was observed. Liver was the organ richest in Aroclor 5460, and probably contained the bulk of the absorbed material.

Comparison of chromatograms of Aroclor 5460 extracts from cod tissue with standard Aroclor 5460 runs showed some differences in peak intensities (Figs. 1a, 1b). In general, the cod tissues contained more of the earlier peaks (probably lower MW and lower chlorine content since Dexsil behaves as a typical apolar stationary phase: 9) and fewer of the later peaks than did the standard. This would imply either some selective absorption and deposition of earlier peaks or selective catabolism and excretion of later peaks: of the two possibilities, the former seems more likely. Analogous results have been noted in the feeding of PCBs (10).

Aroclor 5460 is readily fat soluble, and is probably deposited in fat analogously to other chlorinated hydrocarbons. Levels expressed as concentrations in fat were widely spread; blood contained the highest levels of Aroclor 5460/lipid and muscle the lowest. These differences may reflect variations in the rates at which tissue lipids are turned over; thus, muscle lipid is believed to be essentially structural (11) and might not be expected to be metabolised particularly rapidly.

The results of the second experiment are shown in Table 2. It is clear that after a period of 70 days following feeding of Aroclor 5460, appreciable amounts remained in tissues. Excretory efficiency therefore seems poor. Mean levels were considerably lower than those in Expt. 1, but variance was such that reduction in Aroclor 5460 levels was often not significant (Student's t test) over this period. However, Aroclor 5460 levels in liver (µg/g tissue and µg/g lipid) were significantly lower ($P<0.05$) than comparable data for Expt. 1. The reduction in blood levels may be attributable to cessation of absorption of Aroclor 5460 through the gut 70 days after feeding and to partitioning of Aroclor 5460 into other tissue. The reduction in liver levels (µg/g tissue) may be influenced by the fact that liver lipid levels were considerably, though not significantly, reduced

in Expt. 2. An overall reduction in liver lipid might be expected to result in some mobilisation of Aroclor 5460 from liver tissue and possibly in its excretion from the fish.

Of the eight fish which were started in Expt. 2 three died (at intervals of 13, 22, 35 days) and a fourth fish was killed at 35 days. It is not clear whether these deaths were directly attributable to Aroclor 5460 feeding. There were, however, no obvious differences in appearances in internal organs of the dead fish as compared to the one fish killed at 35 days, or to other fish in our experience. No controls were run, as the experiment was designed to investigate the possible disappearance of Aroclor 5460 after feeding rather than the possible toxicity of the material.

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REFERENCES

1. Technical Bulletin O-FF/1 Monsanto Organic Division.
2. URRY, F. M. WAGSTAFF, D. J., BLAU, A. D. American Chem. Soc. Division of Pesticide Chemistry Paper No. 17, 158th meeting (1969)
3. CECIL, H. C., BITMAN, J., HARRIS, S. J. American Chem. Soc. Division of Pesticide Chemistry, Paper No. 1, 160th meeting (1970)
4. YAP, H. H., DESAIAH, D., CUTKOMP, L. K. and KOCH, R. B. Nature 233 61 (1971)
5. PLACK, P. A., MILLER, W. S., WARD, C. M. Biochem. J. 99 71 (1966)
6. ZITKO, V. Fisheries Research Board of Canada Manuscript Report No. 1083 (1970)
7. Pesticide Analytical Manual Vol 1 U. S. Dept. Health Education & Welfare, Food and Drug Administration (1969)
8. HANSEN, D. J., PARRISH, J. R., LOWE, J. I., WILSON, A. J., WILSON, P. D. BULL. Env. Contam. Toxicol. 6 113 (1971)
9. BOWMAN, M. C., BEROZA, M. J. Assoc. Offic. Anal. Chem. 54 1088 (1971).
10. GRANT, D. L., PHILLIPS, W. E. J., VILLENEUVE, D. C. Bull, Env. Contam. Toxicol. 6 102 (1971)
11. ADDISON, R. F., ACKMAN, R. G., HINGLEY, J. J. Fish Res. Bd. Canada 25 2083 (1968)