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Response of Trout Hepatic Mixed-Function Oxidases to Experimental Feeding of Ten Known or Possible Chlorinated Environmental Contaminants

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Induction of the hepatic mixed-function oxidase (MFO) enzymes in fish has been proposed as a possible index of sublethal stress (ADDISON *et al.*, 1979, WALTON *et al.*, 1978). In this paper, we describe the effects on brook trout (*Salvelinus fontinalis*) hepatic MFOs of several organic compounds or mixtures of industrial importance, some of which are known or expected to be environmental contaminants.

MATERIALS AND METHODS

The following materials were studied:

Aroclor 5460, a mixture of chlorinated biphenyls manufactured for various industrial uses by the Monsanto Co., and which has been reported occasionally as an environmental contaminant, (FREUDENTHAL & GREVE, 1973). Sample from the Monsanto Co.

Chloralkylene 12, a mixture of isopropyl chlorobiphenyls manufactured by Prodelec, Inc., for possible use as a PCB replacement. Sample from Prof. O. Hutzinger.

Halowax 2141, a mixture of chlorinated naphthalenes manufactured by the Koppers Co., with possible use as a PCB replacement. Sample from Dr. U. Brinkman.

Mirex manufactured as an insecticide and fire-retardant by Hooker Chem. Co. Sample from I. Chu.

Hexachlorobenzene, a compound with various uses known to be an environmental contaminant arising from several sources, including electrolytic processes (MUMMA & LAWLESS, 1975). Sample from Fisher Scientific, Inc.

Hexachlorobutadiene, which has various uses and is expected to be an environmental contaminant from electrolytic processes (MUMMA & LAWLESS, 1975). Sample from Eastman Kodak.

Octachlorostyrene, known to be a contaminant from electrolytic processes (KUEHL *et al.*, 1976). Sample from Dr. E.B. Ofstad.

FireMaster BP-6, a brominated biphenyl-based fire retardant manufactured by Michigan Chem. Co. Sample from Dr. J. Bitman.

1,2,4-trichlorobenzene, which has various uses, including potential as a PCB replacement for electrical uses. Sample from Eastman Kodak.

All materials were used as received, without further refinement.

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Experiments were carried out essentially as described by ADDISON *et al.* (1977). Brook trout weighing 200-250 g were taken from the laboratory's stock of fish and divided randomly into two groups of six kept in separate tanks in flowing fresh water at 10°C. Control fish were each fed three times at 2-day intervals, a gelatin capsule containing organochlorine-free dogfish oil (ADDISON & ACKMAN, 1974); experimental fish were similarly fed capsules containing the oil fortified with the materials under study, at concentrations chosen to produce whole-body concentrations of 200 $\mu g.g^{-1}$, assuming complete retention of dose fed. (In the case of hexachlorobenzene, which was insoluble in dogfish oil, the vehicle used for both controls and experimentals was dimethylsulphoxide). Eighteen days after the first feeding, the fish were killed, weighed and analysed for indices of hepatic MFO activity, including liver microsomal protein content, microsomal "cytochrome P-450", microsomal aniline hydroxylase activity and ethoxycoumarin O-de-ethylase activity (ADDISON *et al.*, 1977). Microsomal benz(a)pyrene hydroxylase activity (WATTENBERG *et al.*, 1962) and aminopyrine N-demethylase activity were also measured. Aminopyrine N-demethylase was assayed by determination of formaldehyde produced using the COCHIN & AXELROD (1959) modification of the NASH (1953) procedure. The "soluble" fraction (supernatant from 100,000 x g centrifugation for 1 h) was also examined for MFO activity.

Data were analysed statistically by *t* test.

RESULTS

The effects of feeding the materials listed above on fish weight and weight gain over the 18-day experimental period were variable. Depending on season, and on the fishes' willingness to feed, different groups showed some weight gain or weight loss. Only in the case of feeding mirex, Halowax 2141, FireMaster BP-6 and hexachlorobutadiene did experimental fish show less gain (or more loss) than controls, but even in these cases, differences between experiments and their controls were not significant ($P > 0.05$).

Liver weight expressed as a percentage of total body weight varied in the range 1.3-1.6 g (depending on the weight of the fish: *c.f.* ADDISON *et al.*, 1981), but did not differ significantly between control and experimental groups ($P > 0.05$).

Microsomal protein content of the liver varied from 15-26 $mg.g^{-1}$. Differences between controls and experimentals were not significant ($P > 0.05$).

Microsomal MFO activity showed some response to treatments. In general, "Cytochrome P-450" concentrations varied around 1 nmole $(mg\ microsomal\ protein)^{-1}$, but showed no significant differences between experimentals and their controls. Aniline hydroxylase activities varied around 3 nmoles product formed $(mg\ protein)^{-1}.hr^{-1}$ and aminopyrine N-demethylase activities around 30 nmoles HCHO formed $(mg\ protein)^{-1}.hr^{-1}$; neither enzyme differed significantly in experimental

groups from their controls ($P > 0.05$). However benz(a)pyrene hydroxylase activity was significantly increased by feeding Aroclor 5460 and FireMaster BP-6 (Table 1). Ethoxycoumarin O-de-ethylase activity was increased significantly by Aroclor 5460, and depressed significantly by mirex, and was increased (though not significantly so) by FireMaster BP-6.

MFO activity was present in the "soluble" fraction but only at 5-10% of that in the microsomal fraction (expressed on a total protein basis); the low activities precluded reliable detection of the effects of treatment on this fraction.

DISCUSSION

The observation that fish weight and weight gain did not differ between experimentally treated fish and their controls indicates that the fish were not under any severe stress caused by the materials studied. Nevertheless, some materials had some effects on indices of MFO activity.

The more integrated indices of MFO induction (liver weight as a fraction of total body weight, liver microsomal protein content) did not vary with treatment, and confirmed our previous observations (ADDISON *et al.*, 1977, 1978, 1979) that these indices are not sufficiently sensitive to be useful. Similarly, "Cytochrome P-450" concentrations did not vary with treatment, also in agreement with previous work. Of the enzymes studied, neither aniline hydroxylase nor aminopyrine N-demethylase activities responded to treatment; however, these enzymes are more usually associated with MFO systems involving Cytochrome P-450, to which the trout MFO system seems not to respond (ADDISON *et al.*, 1978).

Benz(a)pyrene hydroxylase and ethoxycoumarin O-de-ethylase activities appeared to be the most useful indices of MFO induction, probably because they are associated with Cytochrome P-448 based systems which in trout liver are inducible (ADDISON *et al.*, 1978; ELCOMBE & LECH, 1978). FireMaster BP-6 induced benz(a)pyrene hydroxylase significantly, and caused an increase (though not a statistically significant one) in ethoxycoumarin O-de-ethylase activity. These results are in agreement with those reported by ELCOMBE & LECH (1978). Aroclor 5460 induced both ethoxycoumarin O-de-ethylase and benz(a)pyrene hydroxylase activities. Aroclor 5460 is known to be an inducer of MFOs in the rat (SOSA-LUCERO *et al.*, 1973). However, the stresses brought about by treatments were not always in the direction of induction of MFO activity, as is illustrated by the effects of mirex. Similar conclusions have emerged from field studies of MFO activity in response to environmental pollution (AHOKAS *et al.*, 1976).

In the limited terms of this sublethal bioassay, it would seem that the three potential PCB replacements (Chloralkylene 12, Halowax 2141 and 1,2,4-trichlorobenzene) would be acceptable; there is no evidence from this study that they cause MFO induction in fish.

TABLE 1. Effects of feeding various chemicals as described in text on trout (*Salvelinus fontinalis*) hepatic MFO activities[a]

Chemicals	Benz(a)pyrene Hydroxylase[b]		7-Ethoxycoumarin-O-De-Ethylase[c]	
	Control	Treated	Control	Treated
Aroclor 5460	0.59 ± 0.07	1.18 ± 0.14[d]	1.73 ± 0.21	4.98 ± 1.47[d]
Chloralkylene 12	0.79 ± 0.12	0.67 ± 0.18	1.89 ± 0.15	1.86 ± 0.40
Mirex	0.56 ± 0.20	0.21 ± 0.02	2.05 ± 0.17	1.35 ± 0.09[d]
Halowax 2141	0.64 ± 0.09	0.54 ± 0.08	2.11 ± 0.28	2.94 ± 0.48
Hexachlorobenzene	N.A.[e]	N.A.	2.04 ± 0.26	2.69 ± 0.34
FireMaster BP-6	0.48 ± 0.05	1.39 ± 0.26[d]	2.18 ± 0.38	3.09 ± 0.48
1,2,4-Trichlorobenzene	0.57 ± 0.07	0.55 ± 0.18	1.65 ± 0.10	1.88 ± 0.21
Octachlorostyrene	0.51 ± 0.10	0.39 ± 0.04	2.28 ± 0.24	1.49 ± 0.29
Hexachlorobutadiene	0.60 ± 0.20	0.60 ± 0.11	1.62 ± 0.26	1.21 ± 0.17

[a] Values given as mean ± SE
[b] Nmol-equivalents of 3-(OH) benz(a)pyrene formed/mg microsomal protein/h
[c] Nmol 7-(OH)coumarin formed/mg microsomal protein/h
[d] Difference between treated and controls significant ($P < 0.05$)
[e] Not analyzed

(There are of course other factors to be considered in accepting them as PCB replacements). The perchlorinated hydrocarbons (hexachlorobutadiene, hexachlorobenzene and octachlorostyrene) also appear not to be a problem in terms of MFO induction, though they may be undesirable on other grounds, such as that of persistence.

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