Submitted to the Committee, Mt. Desert Island Biological Laboratory

ACUTE TOXICITY OF A MIXTURE OF POLYCHLORINATED BIPHENYLS (AROCLOR 1221) AND DDT IN A MARINE TELEOST (Fundulus heteroclitus) AND EFFECT ON SERUM OSMOLALITY, Na^+ AND K^+.

Louise S. Merkens and William B. Kinter, State University of New York Upstate Medical Center, Syracuse, New York.

Polychlorinated biphenyls (PCBs) are a group of chlorinated hydrocarbon compounds which are similar in structure to DDT and share the same properties of persistence, stability with respect to heat and acid, and lipid solubility which make DDT a threat to the environment. PCBs are industrial compounds which have a wide spectrum of applications including ballast for fluorescent fixtures, plasticizers, heat transfer agents, and carbonless reproducing paper (Environ. Sci. Tech., 4, 814-819, 1970). Aroclors (Monsanto Company Trademark) are mixtures of PCBs. The last two digits of the number which identify each of the mixtures indicate the weight percent of chlorine, for example, Aroclor 1221 contains 21% chlorine. Even though PCBs have been available commercially for 40 years it was not until 1966 that they were identified and reported in the environment (New Scien., 32, 612, 1966). Since then they have been reported in many organisms (BioSci., 20, 958-964, 1970) and (Bull. Environ. Contam. Tox., 5, 171-180, 1970) although there is little published data on the toxicity of these compounds to marine life. It has already been suggested (Science, 173, 1146-1148, 1971) that the sensitivity of teleosts to DDT involves impairment of osmotic regulation by inhibition of the Na^+, K^+-activated adenosine triphophatase (Na, K-ATPase). The present study explores the toxicity of a mixture of polychlorinated biphenyls in a marine teleost and the effect on osmoregulation as compared to DDT.

DSW 033664

STLCOPCB4017626

For each experiment ten sea-water-adapted Fundulus heteroclitus (5 males and 5 females, each about 5 grams) were placed in an aluminum or enameled metal container holding 2 liters of sea water and maintained at 14-16° C throughout the exposure time. In the control containers 2 ml of 100% ethanol was added. In the experimental containers Aroclor 1221 (kindly supplied by Monsanto Co., St. Louis) or p,p'-DDT (Aldrich Chemical Co., Milwaukee, 99+% pure) was dissolved in 2 ml of 100% ethanol and then added to the sea water. These solutions formed a cloudy suspension when added to the sea water and in addition some separation of the Aroclor was noted making actual concentrations unknown. Aroclor 1248 could not be tested in this system due to a much greater degree of separation when added to sea water. Blood was drawn by cardiac puncture with heparinized glass capillary tubes. Whole blood from 3 to 5 fish was pooled and then centrifuged. The serum was diluted and the osmolality (freezing point depression) and concentration of Na^+ and K^+ (flame photometry) were measured.

The toxicity data, Table 1, show that with respect to the acute lethal level in sea water the Aroclor was less toxic than DDT by a factor of about 100. There is a "dose-response" in terms of the theoretical concentration in sea water and death for both Aroclor 1221 and DDT but the actual dose which the fish received is unknown since the concentration of the compounds in the tissues was not measured.

The osmolality and Na^+ concentration of the serum (Table 2) were increased after exposure to either Aroclor 1221 or DDT for periods of 6 and 24 hr. The Aroclor and DDT concentrations selected for these serum composition studies were those that were potentially lethal to most of the fish but low enough to have killed less than half at the time samples were taken from those still

STLCOPCB4017627

surviving. The increase in serum osmolality were significant except for those fish exposed for 24 hours to 25 ppm Aroclor 1221 or 0.075 ppm DDT. The increases in Na^+ concentration were significant for those fish exposed for 6 hours to 0.25 ppm DDT and 24 hours to 75 ppm Aroclor 1221. The serum osmolality and concentration of K^+ were higher in all the ethanol treated fish whether or not they received the experimental compounds ($p<0.05$, except for the osmolality of the 24-hour controls). It is not known if this difference is due to the ethanol or to the confinement of the fish in the experimental containers. Preliminary data (Table 3) show that in the extreme case, when blood was sampled before the fish died but after they had completely lost their righting ability and were continuously oriented sideways, the serum osmolality and concentrations of Na^+ and K^+ were increased well above normal. It should be noted that the osmolality changes (Tables 2 and 3) cannot always be accounted for by the changes in measured electrolytes.

Table 3.

Over all, the results indicate a decreased ability to osmoregulate since both osmolality and Na^+ concentration increased toward that of sea water in all experimental cases. Impairment of osmoregulation is possibly due to inhibition of Na, K-ATPase by the experimental compounds since this enzyme is believed to be involved in the transport of sodium across cell membranes and appears to function in the osmoregulatory process of marine teleosts to maintain tissue hypotonicity. Using a crude homogenate of King O'Norway (Hemitripterus americanus) intestinal mucosa and methods of Janicki and Kinter (Science, 173:1146-1148, 1971) we showed that 250 ppm of Aroclor 1221 inhibited the Na, K-ATPase by 51% and 22% in two preliminary experiments. More extensive work by Yap, Desaiah, and Cutkomp (Nature, 233, 61-62, 1971) shows that several of the Aroclor mixtures inhibit the ATPase enzymes in

STLCOPCB4017628

different fish tissues (brain, liver, muscle, kidney). In conclusion, one PCB mixture, Aroclor 1221, affected serum osmolality and Na^+ of a marine fish in a manner similar to DDT and enzymatic data suggest that the mechanism may be the same, Na, K-ATPase inhibition.

This work was supported by USPHS Grant AM 06479.

DSW 033667

STLCOPCB4017629

Table 1

COMPARISON OF THE ACUTE LETHAL TOXICITY OF AROCLOR 1221 AND DDT IN F. heteroclitus IN A STATIC SYSTEM

Compound	Initial conc.*	Total number of fish	% Dead (cumulative)			
			Day 1	Day 2	Day 3	Day 4
Control (ethanol)		10	0	0	0	0
Aroclor 1221	7.5 ppm	10	0	0	0	0
	25 ppm	10	50	80	80	80
	75 ppm	50	88	96	98	100
DDT	0.025 ppm	10	0	0	0	0
	0.075 ppm	10	40	50	50	50
	0.25 ppm	10	60	80	90	90
	0.75 ppm	10	100	100	100	100

*See methods

DSW 033668

STLCOPCB4017630

Table 2

EFFECT OF AROCLOR 1221 AND DDT ON SERUM OSMOLALITY

Na^+ AND K^+ OF F. heteroclitus

Compound and initial conc.*	mosmols/kg	Na^+ mEq/l	K^+ mEq/l
Untreated	352.0 ± 2.2 (18)	173.0 ± 1.9 (16)	3.61 ± 0.16 (16)
6-Hour Exposure			
Control	365.3 ± 2.5 (6)	170.7 ± 1.6 (6)	4.28 ± 0.23 (5)
Aroclor 1221 75 ppm	395.9 ± 7.4 (9)†	173.2 ± 2.6 (9)	4.38 ± 0.25 (9)
DDT 0.25 ppm	388.5 ± 8.5 (8)†	177.8 ± 4.5 (9)‡	4.40 ± 0.28 (9)
24-Hour Exposure			
Control	361.0 ± 7.2 (6)	175.8 ± 2.2 (6)	5.31 ± 0.51 (6)
Aroclor 1221 25 ppm	373.5 ± 6.8 (6)	178.9 ± 3.6 (6)	4.54 ± 0.30 (6)
75 ppm	406.5 ± 10.2 (4)†	188.7 ± 4.9 (4)†	5.06 ± 0.39 (4)
DDT 0.075 ppm	367.8 ± 2.9 (7)	180.3 ± 5.2 (5)	4.14 ± 0.35 (6)

Values are means ± SE (number of pooled samples)

* See methods

† $p<0.05$

‡ $p<0.1$

DSW 033669

STLCOPCB4017631

Table 3

OSMOLALITY, NA^+ AND K^+ FROM FOUR INDIVIDUAL SAMPLES OF SERUM FROM F. heteroclitus

Compound and initial conc.	Duration hr	mosmols*	Na^+ mEq/l	K^+ mEq/l
Aroclor 1221 75 ppm	23-32	550 428	208.2 215.6	8.12 7.28
DDT 1 ppm	9	430 440	206.6 209.6	8.30 8.92

Each sample contains serum pooled from about 3 fish.

* See methods

DSW 033670

STLCOPCB4017632