The above nomogram extends that of McLean (Physiol. Rev., 18:495, 1938) to zero pCO_2 and HCO_3^-, and uses the solubility data of CO_2 in elasmobranch plasma at 17°C from Albers and Pleschka (Resp. Physiol., 2:261, 1967). The solubility is 0.73 that in pure water, whence α = 0.045 mM CO_2 per pCO_2 as mm Hg.

At 17° and pH 7.5, the pK_a' of HCO_3^-/CO_2 in elasmobranch plasma (about 1000 mOsm) is 6.1 (Albers and Pleschka, ibid.). This pK_a' is the same as that for mammalian blood (300 mOsm) at 37° (Severinghaus et al., J. Appl. Physiol., 9:197, 1956). The reason for this is that pK_a' rises with falling temperature; and pK_a' falls with rising ionic strength (Harned and Bonner, J. Am. Chem. Soc., 67:1026, 1945). The present nomogram can therefore be used for any situation in which the pK_a' is approximately 6.1; the far right column could then be appropriately altered for differing solubilities of CO_2 when desired.

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1971 #31

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^+.

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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 biological environment. These compounds have a wide spectrum of industrial applications such as ballast for fluorescent fixtures, plasticizers, heat transfer agents, and carbonless reproducing paper (Environ. Sci. Tech., 4, 814-819, 1970). Aroclors (Monsanto Company Trandmark) 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, Arochlor 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 its effect on osmoregulation as compared to DDT.

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 were 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 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.

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

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 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 surviving. The increases in serum osmolality were significant except for those fish exposed for 24

Table 2

EFFECT OF AROCLOR 1221 AND DDT ON SERUM OSMOLALITY

Na^+ AND K^+ OF F. heteroclitus

Compound and initial conc.*	mosmols/l	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

† $p<0.05$

‡ $p<0.1$

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

Table 3

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

Compound and initial conc.	Duration hr	mosmols/l	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

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.

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 (Nautre, 233, 61-62, 1971) shows that several of the Aroclor mixtures inhibit the ATPase enzymes in 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.

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1971 #32

AXONAL TRANSPORT OF PROTEINS IN ASYMMETRIC OPTIC NERVES OF FLATFISH

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During development, teleosts of the order Heterosomata acquire a laterally compressed shape and both eyes migrate, each to a different extent, onto the lateral (now dorsal) surface of the head. This differential migration of the eyes produces an asymmetry in the optic nerve so that one nerve is consistently longer than the other. The purpose of this study was to compare axonal transport of proteins in the optic system of these animals to determine whether rates of transport are related to or independent of length of axon.

Three species of flatfish were used; the yellowtail flounder, *Limanda*, and the winter flounder, *Pseudopleuronectes*, in which both eyes are on the right side and the right nerve is elongated, and the sand dab, *Scophthalmus*, in which the reverse is true.

Optic nerves and optic tracts were measured in 47 animals (Table I).

Table I

Length of Nerve		Length of Tract	Total Length
Long Nerve	13.9 mm	5.4 mm	19.3 mm
Short Nerve	11.4 mm	5.2 mm	16.6 mm
% Difference	23%	3%	16%

Virtually the entire asymmetry is due to an elongation of the optic nerve. A sign test indicates no consistent difference in length of optic tract between the two sides. In all cases, the optic nerve and its associated tract could easily be separated from the opposite nerve and tract, indicating no mixing of fibers at the chiasm.

For transport studies, 10 Ci of ^{3}H-proline (specific activity 40 C/mM) was injected into each eye of an unanesthetized fish and after an interval of 3 hr. to 6 weeks, the animal was decapitated and the optic system fixed in situ in 5% paraformaldehyde in 0.2 M phosphate buffer, pH 7.2. The optic nerves, tracts and tecta were then dissected free, and immersed in cold 10% TCA for 24 hours. The nerves, tracts and tecta were then separated, weighed and measured, and the nerves were further cut into 3mm segments. The tissue was dissolved in Soluene-100 and counted in a toluene based scintillation fluid.

To estimate the transport rates, the mean radioactivity/mgm tissue from both tecta of all animals at each time point was plotted (Fig. 1). Two peaks occur, one at 18 hours after injection representing a rapid component traveling at 1-3 mm/hr., and one at 21 days, the slow component,