Studies on the Mechanism of Toxicity of DDT and Polychlorinated Biphenyls (PCBs): Disruption of Osmoregulation in Marine Fish

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In 1970 studies were initiated at the Mount Desert Island Biological Laboratory to explore mechanisms of toxicity underlying the high sensitivity of bony fish, teleosts, to organochlorine pollutants. The starting point was several reports that DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] inhibited Na,K-ATPase (Na^+, K^+-activated adenosine triphosphatase), as well as the knowledge that this enzyme appears to play a central role in osmoregulation by marine teleosts. In the face of a desiccative environment, these fish maintain body fluid hypotonicity (Fig. 1) by drinking sea water, absorbing water coupled with salts across the intestinal epithelium, and eventually secreting the NaCl across the gill epithelium while retaining the free water. The primary driving mechanism in both intestine and gill is the Na pump with which Na,K-ATPase appears to be intimately involved. For fuller discussion, see (2) and (3).

After demonstrating that DDT did, in fact, inhibit Na,K-ATPase activity in homogenates of intestinal mucosa and gill filaments from several marine teleosts (2), attention was focused on the sea water-adapted eel (*Anguilla rostrata*), in which osmoregulation has been well investigated. This eel can also adapt to fresh water and can be used for future DDT studies.

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Materials and Methods

Eels, *Anguilla rostrata*, and killifish, *Fundulus heteroclitus*, were captured in estuaries along the Maine coast. They were adapted to and maintained in sea water for about three weeks before use. Non-radioactive purity grade p,p'-DDT was procured from Aldrich Chemical Co., Milwaukee, Wis. ^{14}C-DDT was obtained from Amersham/Searle, Arlington Heights, Ill. and had a specific activity of 24 mCi/mM.

Aroclor 1221 was donated by Monsanto Co., St. Louis, Mo. Plasma milliosmolarity was determined by a standard freezing point depression technique, while Na^+ and K^+ were analyzed by means of a flame photometer. Levels of ^{14}C-DDT were determined as described previously (4). The fractional ^{22}Na space of isolated gills was measured according to the procedure of Kamiya (5) where eel gills are incubated for 1 hr at 15° in oxygenated sea water. Control gills were incubated with the solvent 0.5% N,N-dimethylformamide (DMF), and experimental gills were exposed to 50 ppm DDT in 0.5% DMF. Gill homogenates were assayed for Na,K-ATPase activity as described previously (2). Acute toxicity and osmoregulatory studies were conducted in killifish using a simple static system. For each experiment ten sea water-adapted fish (5 males and 5 females, each weighing about 5 g) were placed in an aluminum or enameled metal container holding 2 liters of sea water and maintained at 14–16° throughout the exposure time. In control containers 2 ml of ethanol were added to the sea water; in experimental containers Aroclor 1221 (mixture of PCBs

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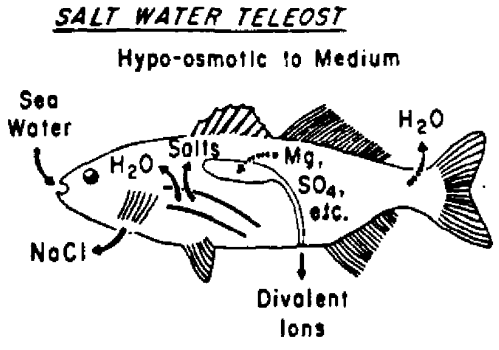

FIGURE 1. Water and salt transport in a salt water teleost. Typically the milliosmols/L are 1,000 for sea water and 350 for fish body fluids. Adapted from (1).

with 21% chlorine content) or p,p'-DDT was first dissolved in the ethanol. These solutions formed a cloudy suspension when added to the sea water, and in addition some separation of the Aroclor was noted, making the actual concentrations unknown (Aroclor 1248 was not tested due to much greater separation). Blood was drawn by cardiac puncture with lightly heparinized glass capillary tubes. Whole blood from 3 to 5 fish was pooled and centrifuged. The 0.05–0.1 ml of serum was diluted, and the osmolarity and concentration of Na and K ions were measured as for the eel.

Results

Ability to osmoregulate was followed in individual eels (about 100 g) placed in 15° sea water (2 liters) containing 0.1% ethanol and 1 ppm DDT, a level which is fatal by 10 hr. In Table 1 it is seen that, at 6 hr, plasma milliosmolarity of

Table 1. Effect of p,p'-DDT on plasma osmolarity, sodium and potassium in sea water adapted eels (*Anguilla rostrata*).*

Treatment	Osmolarity (mosmols./L)	Na^+ (mEq/L)	K^+ (mEq/L)
Control	358±10	163±2	2.4±0.3
p,p'-DDT	462±10†	192±3†	5.4±0.1†

* Mean±SE for 5–6 animals exposed to 1 ppm of DDT for 6 hr.
† Significantly different ($P<0.01$) from controls.

Table 2. ^{14}C-DDT levels in sea water adapted eels (*Anguilla rostrata*).*

Tissue	DDT levels, ppm
Plasma	17.2±3.0
Gill	22.2±2.2
Gut mucosa	6.8±0.8
Brain	11.2±1.0

* Mean±SE for 6 animals exposed to 1 ppm of ^{14}C-DDT for 6 hr.

Table 3. The effects of DDT on sodium space and Na,K-ATPase in gills from sea water adapted eels (*Anguilla rostrata*).*

Treatment	Na space†	ATPase activity‡
Control	0.112±0.010	0.37±0.07
DDT, 50 ppm	0.162±0.010§	0.23±0.03§

* Mean±SE for 15 gills in each group for Na space determination and 6 duplicate assays per group for ATPase assays.
† Fractional Na space after incubation at 15° for 1 hr expressed as ^{24}Na gill filament to media ratio.
‡ μmoles Pi/mg protein×hr.
§ Significantly different ($P<0.01$) from controls.

Table 4. Summary of DDT effects on osmoregulation in sea water eels.

Study	Results: Gut	Results: Gill
In vivo at 6 hr (1 ppm in water)*	7 ppm DDT Plasma osmolarity	22 ppm DDT increased 29%
In vitro (50 ppm in media)	H_2O absorption from sacs decreased 47%†	Na content of isolated gills increased 45%
Homogenate (50 ppm)	Na,K-ATPase inhibited 63%†	Na,K-ATPase inhibited 38%

* DDT solubilized with ethanol (0.1% in sea water) or N,N-dimethylformamide (0.5% in media and 5% in homogenate).
† Calculated from ref. 3.

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Table 5. Acute toxicity of Aroclor 1221 and DDT in killifish.

Compound	Initial level* in sea water	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	80	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 description of static system in text.

DDT-treated fish had increased about 30% compared to ethanol controls. Furthermore, in this table one can see that, while much of this increase was due to plasma Na, plasma K also doubled in these animals. In additional experiments with 1 ppm ^{14}C-DDT, the fish had taken up about half the radioactivity by 6 hr. The levels of radioactivity for some tissues are indicated in Table 2 and ranged from 7–22 ppm. Using isolated eel gills the fractional ^{22}Na space was found to be increased significantly (Table 3) by 50 ppm DDT. Data in Table 3 also shows that Na,K-ATPase activity was significantly inhibited (38%) by 50 ppm DDT.

Considered all together (Tables 1, 2, and 3 and summarized in Table 4), our eel data provide circumstantial evidence that disruption of osmoregulation is a primary toxic effect of DDT, *i.e.*, the DDT levels in gut and gill of fatally-dosed fish were high enough to have affected osmoregulatory Na transport under in vitro conditions. Both water absorption from gut sacs and Na content of isolated gills are directly dependent on Na pump activity and can be altered similarly by obtaining a highly specific Na,K-ATPase inhibitor. Moreover, from the published (3) dose-response curve for gut homogenate, 7 ppm DDT in mucosal tissue should still inhibit Na,K-ATPase activity

Table 6. Effects of Aroclor 1221 and DDT on serum osmolarity, Na and K in killifish.

Compound and initial level	mosmols/liter	Na^+ mEq/liter	K^+ mEq/liter
Untreated	352±2 (18)	173±2 (16)	3.6±0.2 (16)
6-Hour exposure			
Control	365±3 (6)*	171±2 (6)	4.3±0.2 (5)*
Aroclor 1221			
75 ppm	396±7 (9)†	173±3 (6)	4.4±0.3 (9)
DDT			
0.25 ppm	389±9 (8)†	173±4 (9)	4.4±0.3 (9)
24-Hour exposure			
Control	361±7 (6)	176±2 (6)	5.3±0.5 (6)*
Aroclor 1221			
25 ppm	374±7 (6)	179±4 (6)	4.5±0.3 (6)
75 ppm	407±10 (4)†	189±5 (4)†	5.0±0.4 (4)
DDT			
0.075 ppm	366±3 (7)	180±5 (5)	4.1±0.4 (5)

* Significantly different ($P < 0.05$) from untreated controls.
† Significantly different ($P < 0.05$) from ethanol-treated controls.

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by 45%, provided, of course, that tissue content on a wet weight basis is equivalent to homogenate content based on added DDT.

The data in this same curve also indicate that Mg-ATPase is less sensitive to DDT than Na,K-ATPase. Certainly, until more information is available, the present data would appear to provide a partial explanation as to why a whole body burden of less than 10 ppm DDT is often lethal to fish. It is also recognised that DDT may exert a primary toxic effect on the central nervous system; by 6 hr our fatally-dosed eels were hyperactive and easily stimulated to convulsions by touch. Such symptoms, however, could be secondary to alterations to osmolarity of plasma and cerebrospinal fluid.

Last summer, because of the chemical similarity between the insecticide DDT and the industrial PCBs (polychlorinated biphenyls) pollutants in the environment, acute toxicity and osmoregulatory effects were compared using killifish. The toxicity data, Table 5, show that, with respect to the acute lethal level in sea water, the Aroclor 1221 was less toxic than DDT by a factor of about 100. There was a "dose-response" in terms of the theoretical concentration in sea water and number of deaths for both Aroclor 1221 and DDT, but the actual dose which the fish received is unknown since the tissue levels were not measured.

The osmolarity and Na concentration of the serum (Table 6) consistently increased (toward sea water values) in fish exposed to lethal levels of either Aroclor 1221 or DDT. The Aroclor and DDT levels selected for these serum-composition studies were those that were potentially lethal to most of the fish, but low enough to have killed only some at the time (6 or 24 hr) samples were taken from those still surviving. With the higher levels tested, most of the increases in serum osmolarity and Na were statistically significant when compared to ethanol controls.

Also, serum osmolarity and K were elevated in all ethanol-treated controls ($P < 0.05$, except for 24-hr osmolarity); this elevation may have been due to the ethanol or to confinement of the fish in the experimental containers. Additional data (Table 7) show that in the extreme cases, when blood was sampled before the fish died but after they had completely lost their righting ability and remained sideways, the serum osmolarity and electrolyte concentrations were all increased well above normal. It should be noted that the osmolarity changes (Tables 6 and 7) cannot always be accounted for by changes in measured electrolytes.

Table 7. Osmolarity, Na and K in individual pooled samples of killifish serum.*

Compound and level	Exposure hr	mosmols/liter	Na^+ mEq/liter	K^+ mEq/liter
Aroclor 1221				
75 ppm	23–32	550	208	8.1
		428	216	7.3
DDT				
1 ppm	9–10	430	207	8.3
		440	210	8.9

* Each sample represents serum from about 3 fish.

Discussion

Overall, these results suggest that lethal levels of Aroclor 1221 and DDT both decrease the ability of killifish to osmoregulate to about the same degree. Also killifish and eels seem about equally sensitive to DDT. Thus, inhibition of Na,K-ATPase by appropriate levels of Aroclor 1221 was anticipated. Using a homogenate of King O'Norway (*Hemitripterus americanus*) intestinal mucosa 250 ppm of Aroclor 1221 inhibited the Na,K-ATPase by 51% and 22% in two experiments. Moreover, recently-published work by Yap et al. (6) showed that several of the Aroclor mixtures inhibited the enzyme from fish brain and kidney.

Concerning the molecular basis for Na,K-ATPase inhibition by DDT and PCBs, one may speculate that these lipophilic compounds interact with the phospholipid-activating component of this lipoprotein enzyme (7, 8). It is well known that many lipophilic compounds, e.g., cyclohexanone, inhibit the enzyme and evidence of proton interaction between DDT and a phospholipid, lecithin, recently has been reported (9). Moreover, other organochlorine insecticides, including endrin, also inhibit Na,K-ATPase from fish brain (10), and endrin has been observed to disrupt osmoregulation in both a marine and a fresh water teleost (11, 12). Thus, it seems reasonable to predict that many organochlorine

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pollutants will be found to disrupt osmoregulation in fish.

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