Table 2

Reversal of the Inhibition of L-asparagine Synthetase from L5178Y/AR by Extracts of Dogfish, *Squalus acanthias* and Skate, *Raja ocellata* with Various Counter Agents

Counter Agent	% Reversal of the Inhibition of L-Asparagine Synthetase of L5178Y/AR			
	Dogfish Extracts		Skate Extracts	
[M]	Aqueous Liver Extract	Ethanolic Liver Extract	Plasma	Pancreas
	(78)		(72)	(68)
EGTA [4 mM]	0	0	21	9
NH_4Cl [1 M]	0	0	2	30
Cetylpyridium chloride [140 mM]	0	0	0	4
Dithiothreitol [4 mM]	0	11	1	9
Diisopropylfluorphosphate [7 mM]	0	0	8	1

Legend: Numbers in parenthesis are the % inhibition of L-asparagine synthetase of L5178Y/AR by tissue extracts in the absence of counter agents.

The possibility also was considered that fish L-asparagine synthetase was a thermolabile enzyme so that the 37° incubation temperature was giving rise to spurious underestimation of its activity. Precedent for such a pitfall exists (Drug Metab. Disposition 2: 546, 1974). Incubations therefore were carried out at 12°, 25°, and 37° for standard (30 min.) as well as protracted (3 hr) periods. Under these conditions, the specific activity of L-asparagine synthetase in dogfish uterus, pancreas, spleen, kidney and liver was not significantly different than previously reported (MDIBL Bull. 12: 68, 1972).

In view of the potency of the inhibition produced by skate tissue extracts, it is possible that the unidentified factors present in these extracts could mask or moderate the synthesis of L-asparagine *in vivo* — provided that the putative piscine synthetase is functionaaly analogous to the mammalian enzyme. Perhaps the generalized increase in inhibitory metallic elements in skate tissues compared to dogfish tissues could account for the greater net potency of inhibition of L-asparagine synthetase in the former case. Studies to examine these points are in progress.

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Effects of Xenobiotic Compounds on Development of the Embryo of *Fundulus heteroclitus*

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Having demonstrated that embryos of the teleost *Fundulus heteroclitus* serve as good test systems for the effects of DDT on one aspect of the ecosystem (Crawford, R.B. and Guarino, A.M. Arch. Environ. Contam. Tox., in press) we determined that other compounds should be tested. Therefore nine xenobiotic compounds were analyzed for their effects on development in *Fundulus* embryos. The compounds selected were pesticides and other enviromental contaminants.

Eggs were incubated in Stender dishes at 16° Incubation in xenobiotic-containing media was begun min. post-fertilization, using 10 ml of media per dish an 30 to 40 eggs per dish. Solutions were changed daily fo the first 10 days and every 3 days thereafter. Observation of development were made with the dissecting micro scope with staging performed according to criteria o Oppenheimer.

All compounds tested were in 50% filtered sea wate at concentrations of 10, 1, 0.1 and 0.01 ppm. Solution were prepared according to the solubility of th xenobiotic. For example, diquat and paraquat, being quit water soluble, were dissolved directly in 50% sea water A group of less soluble compounds (malathion, aroclor parathion, pentachlorophenal, and 2,3,4-T) were dis solved in acetone. Ten μl of each stock acetone solutio was added to 100 ml of 50% sea water to make the tes solutions. In the cases of the very insoluble compounds (aldrin and sevin), they were first dissolved in acetone Emulphor (polyethoxylated castor oil), an emulsifying agent, was added at the ratio of 1:9. Then 10 μl of this stock solution was added to 100 ml of 50% sea water.

The results of these egg incubations are summarized in Table 1. All were found to cause abnormal develop ment. Malathion, parathion, and pentachlorophenal at 1(ppm prevented normal development beyond the blastula In the case of malathion and parathion, approximately 50% formed a keel which developed and differentiated abnormally and then died. Even in the cases where keel did not form, cell differentiation occurred. Melanophores xanthophores, and guanophores were formed, blood islands appeared and masses of contracting muscle could be seen. These effects were much like those reported previously when specific inhibitors of RNA or protein synthesis were introduced to the incubation media a various times after fertilization. Pentachlorophenol (which can uncouple oxidative phosphorylation) mimiced the effect of anaerobic media or cyanide by completely inhibiting gastrulation.

Table 1

Xenobiotics Effects on Fundulus Development[a]

Xenobiotic	ppm	Inhibition of Gastrulation	Abnormal Keel Formation	Diminished Pigmentation	Development Slowed	No Hatching	Abnormal Fry
Malathion	0.01	–	–	100	100	–	68
	0.1	–	–	100	100	–	100
	1.0	–	–	100	100	100	–
	10.0	50	56	–	–	100	–
Aroclor	0.01	–	–	–	–	–	–
	0.1	–	–	100	100	–	–
	1.0	–	–	100	100	–	–
	10.0	–	–	100	100	–	70
Aldrin	0.01	–	2	50	100	5	–
	0.1	–	–	50	100	26	–
	1.0	–	7	50	100	23	–
	10.0	–	–	50	100	21	100
Diquat	0.01	–	–	–	–	–	100
	0.1	–	–	50	55	–	100
	1.0	–	–	65	35	–	100
	10.0	–	–	77	77	–	100
Parathion	0.01	–	–	–	–	–	–
	0.1	–	–	–	–	–	100
	1.0	–	–	–	–	–	100
	10.0	54	46	–	–	100	–
Pentachlorophenol	0.01	–	–	–	–	–	100
	0.1	–	–	–	–	–	100
	1.0[b]	–	–	–	–	–	–
	10.0	100	–	–	–	–	–
Sevin	0.01	–	–	17	17	–	100
	0.1	–	–	27	27	–	100
	1.0	–	–	36	36	–	100
	10.0[c]	–	–	–	–	100	–

[a]Incubations, observations and descriptions of effects are in text. Numbers given are percent of embryos showing the abnormality.

[b]PCP at 1.0 ppm caused complete developmental arrest at stage 25, 10th day of incubation.

[c]Sevin at 10.0 ppm prevented heart beat and circulation and subsequent development.

[d]Paraquat had no effect at all concentrations tested except for slightly lethargic fry which survived the experimental period.

[e]2,3,4–T had no effect at all concentrations.

Although upon incubation in many of these nobiotic compounds development through hatching pears to be normal (although often delayed), the sulting fry may exhibit a pattern suggesting severe urotoxicity. These abnormal fry appear lethargic and e usually rigid and arched. They cannot swim in a aight line and they are unable to right themselves. on stimulation by touch or be vibrations from the nch they exhibit what appear to be convulsive tremors. ey usually die within a day or two of hatching. It is not expected that some of the most toxic effects would be anifested during that time when the yolk nutrients are ing rapidly depleted and the incorporated xenobiotic mpound becomes distributed to critical organ sites.

An interesting effect of Sevin (10 ppm) should be ted. These embryos failed to initiate heart beat and circulation when they had reached the appropriate stage. Embryogenesis was generally arrested at this stage, although some further changes occurred including continued development of the heart. Similar effects of Sevin on the heart has been reported using *Fundulus* (Weis, P., and Weis, J., Teratology, 10, 263-1974).

It should be emphasized that, for proper analysis of toxicity of these compounds on developing systems, the behavior of the hatched fry must be observed. With this in mind, it appears that the teleost embryo can be a useful system for monitoring the toxicity of xenobiotic compounds.