OPTICAL MALFORMATIONS INDUCED BY INSECTICIDES IN EMBRYOS OF THE ATLANTIC SILVERSIDE, *MENIDIA MENIDIA*

Since the banning of DDT from use in the United States, other insecticides such as malathion, parathion, and Sevin[1] (carbaryl) have come into greater use. Though not persistent like DDT, these insecticides, like DDT, find their way into aquatic ecosystems and thus into the spawning grounds of aquatic organisms. Various insecticides have been shown to cause developmental abnormalities. Malathion, for example, has been shown to cause skeletal malformations in birds (McLaughlin et al. 1963; Walker 1967; Greenberg and LaHam 1969), mammals (Tanimura et al. 1967), and reptiles (Mitchell and Yntema 1973).

The experiments described herein were designed to study the effects of DDT, malathion, and Sevin on the development of the Atlantic silverside, *Menidia menidia*. Since previous studies had all indicated that sensitivity decreases with embryonic age, we initiated our treatment early in development.

Materials and Methods

Adult *M. menidia*, from the vicinity of Montauk, N.Y., were collected by a seine during June and July. Eggs and sperm were obtained by stripping the fish, as described by Costello et al. (1957:228-233). The fertilized eggs were separated into small clumps and, after being washed, were placed randomly in glass finger bowls in 100 ml of Millipore-filtered seawater (salinity 30‰) and incubated at 20°C. The insecticides malathion (95% analytical reagent, Supelco Inc., Bellefonte, Pa.), DDT (*p,p'*-DDT, 72% technical grade, Montrose Chemical Co., Torrance, Calif., recrystallized from ethanol to yield 98% *p,p'*-DDT), and Sevin (99.2% carbaryl, Union Carbide Corp., New York, N.Y.) were introduced as acetone solutions into experimental dishes during either early cleavage (2-4 cell stage) or late cleavage (about 100 cells—see Costello et al. 1957, fig. 104), at concentrations of 10 to 500 parts per billion (ppb). Control dishes received an equivalent amount of acetone (10 μl). The solutions were not changed; thus we were studying the effect of a single application of the chemicals (the concentration of which undoubtedly decreased over time due to adsorption). Development was followed with reference to the descriptions of Costello et al. (1957). At appropriate times, eggs were examined to see the percentage which had successfully completed gastrulation and, later, the percentage which had successfully initiated heartbeat. In the first two experiments hatching rates were noted and only the newly hatched fry were examined for malformations. Since they appeared normal, in the subsequent experiments embryos were examined for malformations with considerably more success. Some embryos were preserved in glutaraldehyde, dechorionated, sectioned, and stained with hematoxylin and eosin.

A repeat experiment was performed in the following summer using the same procedures.

Results

In the first experiment, eggs were treated at the late cleavage stage with malathion at 10 and 100 ppb and Sevin at 25 and 100 ppb. There were over 200 eggs in each dish. Percents of successful axis formation and heartbeat initiation were lower than controls in most treated groups (Table 1) but did not always show a dose-related effect. Hatching commenced 14 days after fertilization and continued for 6 days, at which time the experiment was terminated. No difference was noted in hatching times in the various groups and no abnormalities were observed in the fry, although some dead ones were seen in each group.

In the second experiment, eggs at the 2-4 cell stage were exposed to DDT at 25 and 100 ppb and to malathion at 10 and 100 ppb. There were again about 200 eggs in each dish. As in the previous experiment (Table 1) treated groups had lower rates of axis formation and of heartbeat initiation than controls. Hatching commenced 14 days after fertilization and continued for 6 days, at which time the experiment was terminated. No difference was noted in hatching times in the various groups and no abnormalities were noted in the fry, although, as before, some dead ones were noted in each group.

In the third experiment, eggs at the late cleavage stage were exposed to DDT at 10, 25, and 100 ppb, malathion at 10, 100, and 500 ppb, and Sevin at 25, 100, and 500 ppb. There were about 50 eggs in each dish. When checked for axis formation and heartbeat initiation, the treated eggs were again lower than controls. Embryos were care-

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 1. — Insecticide effects on percentage of axis formation, heartbeat, optic abnormalities, and hatching. Concentrations in parts per billion (ppb).

Item	Control	DDT (ppb)			Malathion (ppb)						Sevin (ppb)			
		10	25	100	10	25	100	500	1,000	2,500	10	25	100	500
Experiment 1 (late cleavage):														
Axis formation	54				23		19					36	48	
Heartbeat	46				22		13					35	48	
Hatch	21				19		6					21	27	
Experiment 2 (2-4 cell stage):														
Axis formation	41		27	9	28		21							
Heartbeat	41		25	5	23		21							
Hatch	28		14	2	11		7							
Experiment 3 (late cleavage):														
Axis formation	17	12	13	10	17		13	15				16	7	13
Heartbeat	17	12	11	6	13		9	13				16	7	9
Optic anomalies	0	50	50	60	40		60	33				40	57	25
Experiment 4 (late cleavage):														
Axis formation	53	45	30	21	37	13	10				30	24		
Heartbeat	53	43	30	20	34	13	6				30	20		
Optic anomalies	1	11	9	15	9	22	30				17	11		
Experiment 5 (late cleavage):														
Axis formation	96	81	82	29					83	65	65	50	50	
Heartbeat	96	70	82	6					83	32	62	50	50	
Optic anomalies	0	1	0	50					4	12	6	12	30	

fully examined for developmental abnormalities, and various optic malformations were discovered in the insecticide-treated embryos. These took the form of unilateral and bilateral microphthalmia (reduced size of eyes), unilateral and bilateral anophthalmia (absence of eyes), and cyclopia (a single median eye) (Figure 1). Severely retarded embryos were also noted. Percentages of those with successful axis formation which showed optical abnormalities were quite high in all treated groups, while none were observed in the control group. Abnormal embryos were fixed prior to hatching. (It was assumed that they would die prior to hatching since no abnormal fry had been found in the previous experiments.) At hatching, which commenced 15 days after fertilization and continued for 7 days, one fish with scoliosis was noted in 10 ppb malathion.

In the fourth experiment, eggs were again exposed at the late cleavage stage to DDT at 10, 25, and 100 ppb, malathion at 10, 25, and 100 ppb, and Sevin at 10 and 25 ppb. There were about 200 eggs in each dish. When checked for axis formation and heartbeat, treated groups were lower

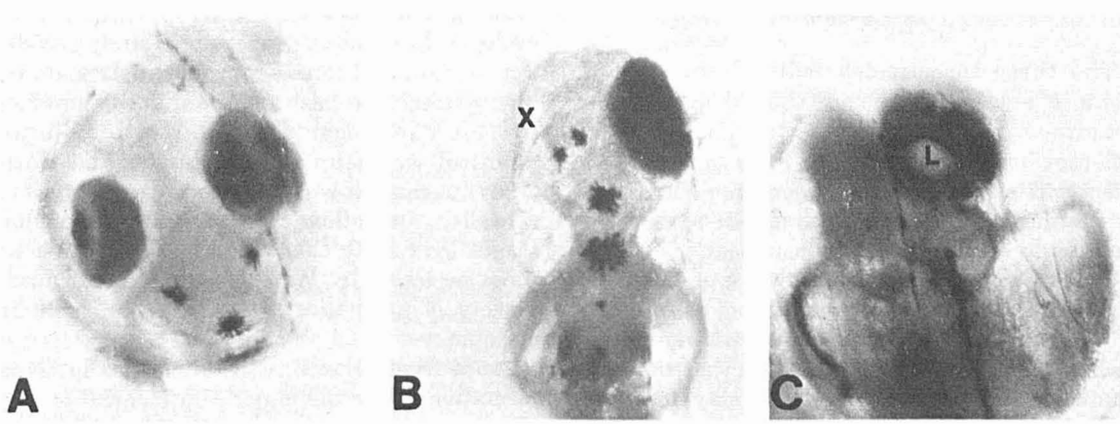

FIGURE 1.—Photomicrographs of whole, fixed, 2-wk-old *Menidia menidia* embryos at approximately 20×. A is a control embryo, while B is a 10 ppb Sevin-treated embryo with unilateral anophthalmia (the site of the undeveloped eye is marked by X), and C is a cyclopic embryo from a 10 ppb malathion-treated batch (transmitted light illuminates the single lens at L).

than controls. At this time, and for several days after, abnormal embryos were noted. These included the severely retarded embryos and the optical abnormalities noted earlier. Only one control embryo showed slight microphthalmia. Hatching commenced after 11 days and continued for 9 days, at which time the experiment was terminated. After hatching, lordotic fry were seen in the 10 ppb malathion, 10 ppb Sevin, and 25 ppb DDT groups. These skeletal abnormalities were quite rare, however.

Eye diameters of hatched fry were measured with an ocular micrometer to see if there were slight reductions in optic size in the apparently normal specimens, but no difference between experimental and control fry was seen.

The fifth experiment was performed the following summer using about 100 eggs per dish. Eggs were exposed at late cleavage to DDT at 10, 25, and 100 ppb, Sevin at 10, 25, and 100 ppb, and malathion at 1 and 2.5 ppm. Treated groups were again lower than controls in rate of axis formation and heartbeat initiation. Abnormal embryos were seen in most treated groups (Table 1) and all embryos which exhibited optic malformations also showed retardation, stunting of growth, sparse body pigment, and abnormal cardiac development in which the heart remained a very thin, feebly beating tube without differentiation of the chambers. There were also embryos with this syndrome in which the eyes appeared normal. Hatching commenced after 12 days, and several fry with scoliosis were seen in the malathion dishes.

Discussion

The three insecticides reduced survival of *Menidia* embryos, although this reduction was not always correlated with the dose and varied in different batches of eggs. The main embryotoxic effect was at early stages, preventing successful axis formation. Of those which formed axes, most went on to establishment of heartbeat.

Notable optic malformations were observed in embryos exposed to DDT, malathion, and Sevin. These three insecticides are quite different from each other chemically, and the fact that they all produced similar malformations may indicate that this species has a propensity toward this type of malformation and various agents can invoke them. This propensity is supported by the presence of one control embryo with slight microphthalmia in one eye. McEwan et al. (1949) likewise concluded that the jewelfish, *Hemichromis bimaculata*, had a tendency to vary abnormally in certain directions and that an abnormal environment accentuated this tendency. The most common optic abnormalities seen in our fish were unilateral anophthalmia and microphthalmia. True cases of cyclopia were rare, though several embryos showed partial convergence of the eye cups, with optic cups directed somewhat ventrally rather than laterally.

Stockard (1907) produced cyclopia in *Fundulus* embryos by treatment with $MgCl_2$. In another study (1910) he produced cyclopean, anophthalmic, and monophthalmic *Fundulus* embryos after treatment with alcohol, results similar to those in the present study.

Histological examination of our material revealed a case in which the optic cup had partly formed, but appeared to be facing inward rather than outward and had lost its connection to the brain. No lens was present in this specimen. Smithberg (1962) found that tolbutamide caused eye malformations in the medaka, *Oryzias latipes*. However, these malformations involved degeneration of the eye cup after the lens had been formed, and lenses were present in all the abnormal embryos. These malformations were accompanied by circulatory defects, which he considered responsible for the eye defects.

Retardation of development was seen by Battle and Hisaoka (1952) in their studies of effects of ethyl carbamate (urethan) on embryos of the zebrafish, *Brachydanio rerio*. Some of their embryos also exhibited optical malformations including anophthalmia, microphthalmia, and cyclopia. In Hisaoka's subsequent study (1958) of 2-acetylaminofluorene on zebrafish embryos, microphthalmia was one abnormality produced by this carcinogen. The antibiotic chloramphenicol was found by Anderson and Battle (1967) to cause a variety of teratogenic effects in zebrafish, including cyclopia and intermediate stages leading to this condition. Colchicine was likewise found by Waterman (1940) to cause a variety of anomalies in the medaka, including cyclopia.

Aside from general retardation, the optic malformations were the major teratological effect of the insecticides on *Menidia* the first year. Skeletal malformations were also noted but they were relatively rare. In the following year, a variety of malformations in addition to the optic abnormalities were produced. This difference is perplexing, and is probably due to genetic differences among individuals of this species in susceptibility to the chemicals. This is more understandable when it is realized that relatively few females can supply all the eggs needed for an entire experiment. Such variability in response makes this species a poor one to use in teratological studies.

Effects were seen at dosages as low as 10 ppb. These are levels far lower than those which produced noticeable effects in *Fundulus heteroclitus* embryos (Weis and Weis 1974) in which it was necessary to increase the dosage to parts per million. This may be due to differential permeability of the chorions of the two species and/or to a higher general resistance of *Fundulus* which is generally considered a hardier fish than *Menidia*. The dose levels which affected *Menidia* are levels near those which have been found temporarily, in solution or suspension, in natural areas (Finley et al. 1970; Kennedy and Walsh 1970). Therefore these adverse effects could occur during the development of fish embryos in nature.

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