Bull. Environm. Contam. Toxicol. 25, 234-240 (1980)

Differences in the Effects of Fuel Oil, an Oil Dispersant, and Three Polychlorinated Biphenyls on Fin Regeneration in the Gulf Coast Killifish, *Fundulus grandis*

Sue W. Fingerman
Department of Biology, Tulane University, New Orleans, LA 70118

Several environmental pollutants have been found to inhibit growth in animals (FLICK et al. 1965, REHFELD et al. 1971, WEIS and WEIS 1975, TURK and HIETMAN 1976, SZARO et al. 1978, MILLER et al. 1978, ALBERS 1979, HOFFMAN 1979). As a result of experiments performed in this laboratory on the long range effects of low levels of environmental pollutants on molting and limb regeneration in the fiddler crab, Uca pugilator (FINGERMAN and FINGERMAN 1977, 1979a,b) and because animals in nature are rarely exposed to a single pollutant, a series of experiments was conducted to determine the effects, if any, of a single exposure to several pollutants, singly and in combination, on fin regeneration in the Gulf Coast killifish Fundulus grandis. The pollutants investigated were a fuel oil, an oil dispersant, and three polychlorinated biphenyls (PCBs).

MATERIALS AND METHODS

Adult female Fundulus grandis purchased from bait dealers in Louisiana were used. The fish were maintained in all-glass aquaria in 50% artifical sea water (Instant Ocean, Aquarium Systems, Inc.) under static conditions; the aquaria were aerated and kept at a constant temperature, 28°C, and on a 12 hour light-12 hour dark schedule. The fish were not fed during an experiment. The water in the aquaria was changed twice weekly.

The lower half of the caudal fin, about 1 mm from the base of the fin rays, was removed with a scapel (WEIS and WEIS 1975). The fish were intubated with a fuel oil (Shell Oil Company), an oil dispersant (BP 1100X, British Petroleum North America, Inc.), the three PCBs, Aroclor 1242 (Monsanto Lot No. G266K), Aroclor 1254 (Monsanto Lot No. H116A), Aroclor 1268 (Monsanto Lot No. G266M), or combinations. Dosages for fish receiving the fuel oil or dispersant were calculated as follows: weight of fish in grams/ 80 = dosage in ml. Dosages for fish receiving a PCB:

0007-4861/80/0025-0234 $01.40

weight of fish in grams/40 = dosage in ml. The PCBs were first dissolved in acetone and ultimately diluted 1:1000 in artifical sea water to provide the desired final concentration of 0.0008% of PCB in sea water with 0.1% acetone.

The regenerating part of the fin was measured 7 days after removal and every 7 days thereafter for 28 days. A dissecting microscope fitted with an ocular micrometer was used to measure regeneration. To compare the rates of regeneration of different fish the R value of BLISS (1956) which was devised originally for use in studies of limb regeneration in crustaceans was adapted for use in these studies of fin regeneration.

$$R \text{ value} = \frac{\text{length of regenerate in mm}}{\text{total length of the fish in mm}} \times 100$$

The mean R value for each 7 day period was calculated. Student's t test was used to determine the statistical significance of the difference between means.

EXPERIMENTS AND RESULTS

A preliminary experiment was conducted to determine whether 0.1% acetone alone could affect regeneration in fish. One group of fish was intubated with 0.1% acetone in sea water while another group was given sea water alone. There was no statistically significant difference between the R values of the group receiving sea water with acetone and of the fish receiving sea water alone. Consequently, the control groups in both sets of experiments that will be described below were intubated with sea water containing 0.1% acetone, the same concentration as in the sea water with the PCBs.

In the initial experiment begun April 11, 1978, and repeated beginning May 10, 1978, female Fundulus grandis were intubated with a single dose of Aroclor 1242, Aroclor 1254, Aroclor 1268, fuel oil, Aroclor 1242 + fuel oil, Aroclor 1254 + fuel oil or Aroclor 1268 + fuel oil. As previously stated, a group of control fish were intubated with sea water plus acetone. Three fish were used in each group in the first experiment and 4 per group in the second. The lower half of the caudal fin was removed at the time the fish were intubated. The results of the two experiments were similar and were averaged for statistical analysis.

After 7 days the R value of the control group was not statistically different from the R values of the experimental groups (Table I). However, after 14 days the average R value of the control group was 2.86, significantly larger than that of the 1268 +

fuel oil group which averaged 0.77 ($p < 0.001$), the 1242 + fuel oil group which averaged 1.66 ($p < 0.05$), the 1254 + fuel oil group which averaged 1.19 ($p < 0.01$), and of the fuel oil group which averaged 0.95 ($p < 0.01$). The R values of the 1242, 1254, and 1268 groups were not statistically different from those of the controls. The mean R value of the controls at 21 days was 5.10, statistically different from the 1268 + fuel oil group which averaged 2.17 ($p < 0.001$), 1254 + fuel oil group which averaged 1.89 ($p < 0.02$), and the fuel oil group with 1.77 ($p < 0.02$). The 1242, 1254, 1268, and 1254 + fuel oil groups were not significantly different from the controls. After 28 days the control group averaged 6.55. The 1268 group was for the first time significantly different from the controls, averaging 5.15 ($p < 0.01$); the 1268 + fuel oil averaged 3.84 ($p < 0.05$), the 1242 + fuel oil averaged 3.07 ($p < 0.05$), 1254 + fuel oil averaged 3.37 ($p < 0.01$), and the fuel oil group averaged 2.25 ($p < 0.01$). The 1242 and 1254 groups were not significantly different from the controls after 28 days.

The second set of experiments was a slight variation of the first in that the Aroclors (1242 and 1254) that produced no significant effect by themselves in the first series of experiments were not used whereas the oil dispersant BP 1100X which was not used in the first set of experiments was used in the second set, singly and in combination with Aroclor 1268 and fuel oil. In the experiments begun November 22, 1978, and repeated starting October 29, 1979, and December 10, 1979, female Fundulus grandis were intubated with Aroclor 1268, Aroclor 1268 + fuel oil, Aroclor 1268 + BP 1100X, fuel oil + BP 1100X, BP 1100X, or fuel oil. Controls were intubated with sea water and acetone. The other procedures were the same as in the first set of experiments.

After 7 days the amount of regeneration that occurred in the 1268 group was surprisingly significantly greater with a mean R value of 1.60 ($p < 0.05$) than the control group which had a mean of 1.28 (Table II). But the 1268 group was not significantly different from the control group during the rest of the experiment. The 1268 + fuel oil group was significantly different from the controls after 7 and 14 days, the R values for the fish which received 1268 + fuel oil averaged 1.65 ($p < 0.02$) and 4.20 ($p < 0.02$), respectively, while the controls averaged 1.28 and 3.55, respectively. Three of the groups, 1268 + BP 1100X, BP 1100X, and fuel oil, were not statistically significant from the controls during the 28 days that this experiment ran. The BP 1100X + fuel

TABLE I

R values (mean ± standard error) of the regenerating caudal fins of control specimens of Fundulus grandis and of specimens exposed to Aroclor 1242, Aroclor 1242 + fuel oil, Aroclor 1254, Aroclor 1254 + fuel oil, Aroclor 1268, Aroclor 1268 + fuel oil, and fuel oil in the spring.

	Days			
	7	14	21	28
Control No.	0.59±0.17 N=5	2.85±0.30 N=4	5.20±0.34 N=3	6.55±0.09 N=3
Aroclor 1268 No., p	0.59±0.02 N=6, N.S.	1.84±0.35 N=4, N.S.	3.71±0.46 N=3, N.S.	5.15±0.14 N=2,0.01
Aroclor 1268 + fuel oil No., p	0.31±0.09 N=6, N.S.	0.77±0.13 N=5,0.001	2.17±0.28 N=5,0.001	3.84±0.93 N=3,0.05
Aroclor 1242 No., p	0.82±0.14 N=7,N.S.	2.58±0.22 N=7,N.S.	4.94±0.30 N=7, N.S.	6.70±0.70 N=3,N.S.
Aroclor 1242 + fuel oil No.,p	0.63±0.03 N=4, N.S.	1.66±0.37 N=4, 0.05	1.89±0.83 N=3,0.02	3.07±1.32 N=3,0.05
Aroclor 1254 No., p	0.46±0.09 N=6, N.S.	2.52±0.39 N=6,N.S.	4.76±0.52 N=4, N.S.	6.90±0.66 N=3, N.S.
Aroclor 1254 + fuel oil No., p	0.37±0.06 N=5,N.S.	1.19±0.26 N=4,0.01	2.97±1.02 N=3,N.S.	3.37±1.06 N=2,0.01
Fuel oil No., p	0.47±0.05 N=7,N.S.	0.95±0.29 N=4,0.01	1.77±0.75 N=4,0.02	2.25±0.59 N=3,0.001

TABLE II

R values (mean ± standard error) of the regenerating caudal fin of control specimens of Fundulus grandis and of specimens exposed to Aroclor 1268, Aroclor 1268 + fuel oil, Aroclor 1268 + BP 1100X, BP1100X, BP 1100X + fuel oil, and fuel oil in the fall.

	Days			
	7	14	21	28
Control No.	1.28±0.10 N=33	3.55±0.20 N=29	5.02±0.37 N=23	5.44±0.45 N=19
Aroclor 1268 No.,p	1.6±0.12 N=21,0.05	3.54±0.24 N=15,N.S.	5.35±0.42 N=13,N.S.	6.13±0.81 N=10,N.S.
Aroclor 1268 + fuel oil No., p	1.65±0.09 N=22,0.02	4.20±0.12 N=20,0.02	5.62±0.40 N=15,N.S.	6.30±0.76 N=8,N.S.
Aroclor 1268 + BP 1100X No.,p	1.20±0.07 N=21,N.S.	3.10±0.17 N=20,N.S.	4.82±0.25 N=15,N.S.	5.31±0.20 N=14,N.S.
BP 1100X + fuel oil No.,p	0.96±0.09 N=25,0.05	2.61±0.16 N=23,0.001	4.05±0.23 N=19,0.05	5.08±0.29 N=17,N.S.
BP 1100X No.,p	1.22±0.08 N=18,N.S.	3.04±0.17 N=14,N.S.	4.29±0.21 N=14,N.S.	4.62±0.26 N=11,N.S.
Fuel oil No.,p	1.24±0.13 N=18,N.S.	3.28±0.19 N=17,N.S.	4.94±0.30 N=15,N.S.	5.41±0.32 N=12,N.S.

oil group was statistically significantly different from the controls for the first 21 days of the experiment, averaging 0.96 ($p < 0.05$), 2.61 ($p < 0.001$), and 4.15 ($p < 0.05$), while the controls were 1.28, 3.55, and 5.02, respectively.

DISCUSSION

Several investigations have revealed the inhibitory effects of PCBs, oil dispersant or oils on growth. FLICK et al. (1965), REHFELD et al. (1971), and TURK and TIETMAN (1976) found growth was retarded in chickens fed PCBs. NEBEKER et al. (1975) conducted experiments to determine safe levels of Aroclor 1242, 1248, and 1254 for the fathead minnow, Pimephales promelas, and Aroclor 1248 for the flagfish, Jordanella floridae. The growth of young fathead minnows was retarded when they were exposed to Aroclors 1242 and 1248, though their lengths were not significantly different from the controls; but the opposite was found when the fathead minnows were exposed to Aroclor 1254, the mean weights of the fish not being significantly different from the controls while growth in length was delayed. But when the flagfish was exposed to Aroclor 1248 for 40 days their mean weight was only 15% that of the controls. WEIS and WEIS (1975) found retarded fin regeneration in specimens of Fundulus heteroclitus, a fish closely related to the one used in the present investigation, when exposed to several insecticides, including DDT. It is interesting to note that in the present investigation by the end of the experiments conducted in the spring (Table I), Aroclor 1268 by itself had a significant inhibitory effect, but in the fall this PCB showed no such effect (Table II). Also, in the spring the fuel oil used alone had a significant inhibitory effect on fin regeneration but in the fall the fuel oil by itself had no significant effect. MILLER et al. (1978) found that a single, small oral dose of Kuwait or South Louisiana crude oil caused cessation of growth in herring gull chicks. ALBERS (1979) found that ducklings hatched from eggs exposed to a 5:1 crude oil/Corexit 9527 dispersant mixture had lower mean weights than ducklings from eggs exposed to either crude oil or Corexit 9527 alone. HOFFMAN (1979), studying the embryotoxic effects of crude oil with nickel and vanadium, found that external applications of crude oil alone caused reduced growth in mallards. In the present investigation regeneration was significantly less in the fish exposed to BP 1100X + fuel oil, but in neither the BP 1100X group nor in the fuel oil group was there any significant difference from the controls. It is also interesting when comparing the fall data (Table II) with the spring data (Table I) that in the fall there was significant effects on regeneration during the first 14 days of the experiment in the 1268 and 1268 + fuel oil groups, while in the spring the significant difference appeared later. The most obvious differences between the two sets of data being (a) the fact

that at one time of year the fuel oil by itself did not affect regeneration, while at another time of year it had a significantly inhibitory effect, and (b) the fact that in the 1268 + fuel oil group regeneration was significantly inhibited at one time of year and significantly greater at another time of year.

The results of the present investigation point up the need to consider not only the effects of a single pollutant present in the environment, but the effects of that particular pollutant in combination with the many others present and the effects of these combinations at different times of the year. The fact that exposure to 1268 + fuel oil in the spring caused less regeneration while exposure in the fall resulted in the opposite effect, greater regeneration, and that the fuel oil by itself significantly reduced the regeneration in the spring while in the fall it caused no significant difference, clearly indicates the complexity of our environmental problems and the need for additional research into the problems.

REFERENCES

ALBERS, P.H.: Bull. Environ. Contam. Toxicol. 23, 661 (1979).

BLISS, D.E.: In Bertil Hanstrom, Zoological papers in Honour of his Sixty-fifth Birthday, November 20, 1956, Wingstrand, K.G. (Ed.) pp.56 (1956).

FINGERMAN, S.W. and M. FINGERMAN: Bull. Environ. Contam. Toxicol. 18, 138 (1977).

FINGERMAN, S.W. and M. FINGERMAN: Vie Milieu 29, 69 (1979a).

FINGERMAN, S.W. and M. FINGERMAN: Bull. Environ. Contam. Toxicol. 21, 352 (1979b).

FLICK, D.F., R.G. O'DELL, and V.A. CHILDS: Poultry Sci. 44, 1460 (1965).

HOFFMAN, D.J.: Bull. Environ. Contam. Toxicol. 23, 203 (1979).

MILLER, D.S., D.P. PEAKALL, and W.B. KINTER: Science 199, 315 (1978).

NEBEKER, A.V., F.A. PUGLISI, and D.L. DEFOE: Trans. Amer. Fish. Soc. (3), 562 (1974).

REHFELD, B.M., R.L. BRADLEY, JR., and M.L. SUNDE: Poultry Sci. 50, 1090 (1971).

SZARO, R.O., M.P. DIETER, and G.H. HEINZ: Environ. Res. 17, 426 (1978).

TURK, D.E. and K.G. HIETMAN: Poultry Sci. 55, 672 (1976).

WEIS, J.S. and P. WEIS: Trans. Amer. Fish. Soc. (1), 135 (1975).