Pergamon

Environmental Toxicology and Chemistry, Vol. 14, No. 1, pp. 153–156, 1995

Printed in the USA
0730-7268/95 $9.50 + .00

0730-7268(94)00158-8

Short Communication

EFFECTS OF EMBRYONIC EXPOSURE TO METHYLMERCURY ON LARVAL PREY-CAPTURE ABILITY IN THE MUMMICHOG, *FUNDULUS HETEROCLITUS*

JUDITH S. WEIS*† and PEDDRICK WEIS‡
†Department of Biological Sciences, Rutgers University, Newark, New Jersey 07102
‡Department of Anatomy, Cell Biology & Injury Science, New Jersey Medical School, Newark, New Jersey 07103

(*Received* 11 *January* 1994; *Accepted* 6 *June* 1994)

Abstract—Embryos of the mummichog (*Fundulus heteroclitus*) were exposed to 2, 5, or 10 μg/L methylmercury (meHg) throughout development; these are concentrations below those which cause teratological effects in this species. After hatching, larvae were maintained in clean seawater and tested for prey-capture ability, using *Artemia salina* nauplii. Larvae that had been exposed to 10 μg/L methylmercury (and in two out of three trials, 5 μg/L) initially exhibited slower prey-capture ability than did the other groups. This is an indication of a subtle functional impairment due to the toxicant ("behavioral teratology"). However, the effect was transitory, and by about 1 week after hatching the prey capture of these larvae equalled that of the controls and the other treated groups. Growth of these larvae was also comparable to that of controls. The exposure may have caused retardation of neurological development, which was subsequently compensated for, and therefore no long-lasting effects were produced. In the field, however, embryos exposed to toxicants would probably continue to be exposed as larvae, and might not have the opportunity to recover from the deleterious effects, but rather might have them augmented.

Keywords—Methylmercury Behavioral teratology Fish larvae Prey capture *Fundulus heteroclitus*

INTRODUCTION

Exposure to pollutants can alter normal behavior, and toxicological modifications of specific behaviors may affect an organism's ability to deal with the environment. Neurotoxic chemicals can produce behavioral changes that can alter crucial aspects of ecology. Feeding is one behavioral response with obvious importance to survival. Impaired prey capture in laboratory experiments has been noted in fish after exposure to sublethal concentrations of various pollutants [1–4]. Little et al. [4] noted that the frequency of strikes was less sensitive than prey capture. Henry and Atchison [5] urged that behavioral tests be developed with clear ecological relevance and field verification.

Experimental exposures of adult mummichogs (*Fundulus heteroclitus*) to mercury indicated that 1 week of exposure could lead to reduced prey-capture ability [6]. In the field, a population of this species living in an environment contaminated with mercury and other pollutants was also found to have reduced prey-capture ability [7] as well as reduced condition and growth. The impaired prey-capture ability could be responsible for the reduced growth.

Atchison et al. [8] reviewed the literature on effects of metals on fish behavior and noted that behavior is a much more sensitive response than those used in standard bioassays. They noted that all studies done to date were on juvenile or adult fish, and that no studies had been reported on effects of metals on feeding behavior during the critical stage at which fish switch from yolk to reliance on exogenous food sources. As fish larvae have a limited ability to withstand starvation, the normal development of feeding behavior is critical to survival.

Behavioral development in fish larvae occurs in association with the development of the sensory and locomotor systems that gradually refine the ability to detect and respond to prey. Improvements in visual acuity should result in increased feeding success. Miller et al. [9] studied the ontogenetic improvements in behavior and histological measures of visual acuity and found that reaction of larvae to prey improved more slowly than the anatomical measures would indicate, indicating that while initial limitations may be visual, later limitations are more likely to be behavioral. Thus, along with the morphological aspects of development, learning can increase the ability to capture prey. This development is also accompanied by growth, which provides greater muscular power for attacks. The selectivity of larvae for food also improves with age and is influenced by prey abundance [10].

The study of developmental defects caused by environmental contaminants has been much more focused on mammals than on aquatic organisms. In mammalian teratology it has become clear that functional impairment is a more subtle response to toxicants than is the production of gross anatomical abnormalities. A considerable amount of attention is being devoted to "behavioral teratology," that is, behavioral abnormalities in anatomically normal animals after embryonic exposure to toxicants. Rodent studies have shown that prenatal exposure to lead can alter long-term memory processes [11]. Humans exposed prenatally to mercury in

*To whom correspondence may be addressed.

the Minamata or Iraq incidents suffered from severe brain damage, mental retardation, and/or psychomotor retardation [12].

There has been little attention paid to this aspect of developmental responses of fish to environmental pollutants. In one study [13], rainbow trout, exposed during late organogenesis to benzo[*a*]pyrene, exhibited a decreased tendency to orient themselves upstream, a response critical for salmonid survival.

The following study was undertaken to ascertain whether embryonic exposure to methylmercury (meHg) at levels below those that cause morphological abnormalities would affect prey-capture ability of mummichog (*Fundulus heteroclitus*) larvae.

METHODS

Adult specimens of *F. heteroclitus* were collected with minnow traps from East Hampton, New York, during June and July 1993. Eggs and sperm were obtained by stripping the fish. Fertilized eggs were placed in glass fingerbowls with filtered seawater (27‰). Any eggs that did not cleave were discarded. Methylmercuric chloride (ICN Pharmaceuticals, Plainview, NY) was added from a 0.1 mg/ml stock solution to expose groups of embryos to 0, 2, 5, or 10 μg/L meHg. (Previous work [14] had shown that these concentrations are below those causing malformations—about 20 μg/L.) Three replicate dishes were used for each exposure. Concentrations were nominal, but solutions were changed daily until hatching, which occurs about 2 weeks after fertilization. Embryos were maintained in an incubator at 22°C and a 14:10-h light:dark cycle. After hatching, larvae were maintained in clean filtered seawater in fingerbowls in the same incubator and were fed *Artemia salina* nauplii daily after the initial prey-capture test. They were tested staring at 3 d of age, and at regular intervals thereafter for prey-capture ability. (Pilot studies had shown that larvae were not ready to feed until 3 d after hatching.) The exact days of testing varied among the different trials so as to get a more complete picture of the development of this behavior. Testing always occurred at 1000 to 1200 h.

The test consisted of placing five newly hatched *Artemia* nauplii in 3 ml seawater in a depression slide in which the depression was 33 mm in diameter, then adding one fish larva. (The newly hatched larvae are about 5 mm long, total length.) The time of capture of each *Artemia* was noted over a 5-min period. At least 10 mummichog larvae from each group were tested each time. A number of measures of prey capture were considered for analysis: the time to first capture, the time between the first and second captures, the number of prey caught in 1 min, and the number caught in 5 min. The lengths of the fish at 1 month were measured in the second trial.

Data were analyzed by one-way ANOVA, and, when the data were not normally distributed (as happened on occasion), they were log-transformed prior to the analysis and again tested for normality. ANOVA was followed by Bonferroni *t* tests, and significance was set at the 0.05 level.

RESULTS

Three-d-old larvae, which had not encountered food previously, often remained quite still when placed in the test chamber. When a brine shrimp came into its 2- to 3-mm field of vision and attracted its attention, the fish usually responded by eating the brine shrimp. It was quite variable as to whether or not a brine shrimp came into the fish's field of vision. The measurements of time to the first and second captures were therefore highly variable, ranging from a few seconds to "infinity" if none were captured during the time period, as was sometimes the case. Thus, these measures were considered unsatisfactory.

After encountering the first prey, most fish became more active in terms of searching for other prey and could lunge at brine shrimp about 1 cm away. Some fish, however, remained in the same spot. Some exhibited what appeared to be a "panic" reaction, moving rapidly in an uncoordinated way, and ignoring any prey encountered during that time. For the 3-d-olds in the second and third trials, the number of prey caught in 1 min was usually low in all groups, often being zero. The older larvae generally were more active and effective predators, and all groups consumed all prey within the 5-min period. Therefore, the most reliable measures for statistical analysis were the number captured in 5 min for the 3-d-olds (except in trial 1), and the number eaten in 1 min for older larvae. By 11 d, some fish were seen to pick at nonmoving particles (*Artemia* chorions accidentally introduced into the chamber), not just the active brine shrimp.

The first trial was with larvae from eggs fertilized in mid-June and exposed as embryos to either 5 or 10 μg/L meHg. ANOVA had 2 *d.f.* between, 30 *d.f.* within. The groups that had been exposed to the meHg showed considerable impairment of prey-capture ability on day 3: The number caught in 1 min was 0.5 ± 0.9 for the 10 μg/L group, 1.4 ± 0.9 for the 5 μg/L group, and 2.7 ± 1.8 (SD) for controls (Fig. 1). Bonferroni *t* tests showed two groups: the controls and 5 μg/L group, and the 5 and 10 μg/L group. This impairment was seen also in the other measurements, although only the data on number of *Artemia* captured are presented. By day 5, however, the exposed larvae were no longer significantly slower than the controls.

The second trial was with larvae from eggs fertilized in early July, and exposed to 2, 5, or 10 μg/L meHg as embryos. On day 3, the number captured in 1 min was very low in all groups, so the day-3 data presented are on the number captured in 5 min rather than 1 min. The groups exposed to 5 and 10 μg/L were slower at capturing prey than were controls (0.1 ± 0.3 for the 10 μg/L group, 0.3 ± 0.7 for the 5 μg/L group vs. 2.2 ± 1.0 for controls in 5 min) (Fig. 2). ANOVA had 3 *d.f.* between, 36 *d.f.* within. Bonferroni *t* tests showed the controls and 2 μg/L group to be significantly different from the 5 and 10 μg/L groups. At 6 d these larvae remained significantly impaired relative to controls (1.6 ± 1.3 for the 10 μg/L group, 2.5 ± 1.6 for the 5 μg/L group, and 4.4 ± 0.8 for controls in 1 min), but by day 11 the difference had disappeared. When tested again on days 15 and 19, they were again not different from controls.

After 1 month, when the larvae were preserved and their lengths measured, there were no significant differences among the various groups. Controls averaged 9.83 ± 0.57 mm, the 2 μg/L meHg group averaged 10.75 ± 0.35 mm, the 5 μg/L meHg group averaged 10.25 ± 0.28 mm, and the 10 μg/L meHg group averaged 9.21 ± 0.70 mm.

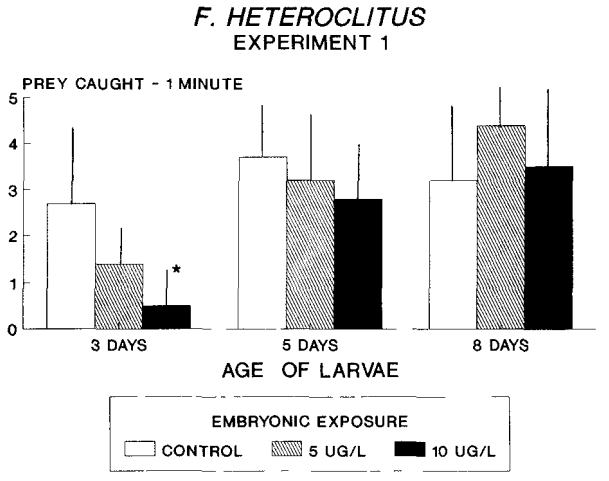

Fig. 1. Capture of *Artemia* (mean + SD) by larval mummichogs of different ages after embryonic exposure to methylmercury—experiment 1. *Significantly different from controls at 0.05 level.

The third trial was with larvae from eggs fertilized in late July and exposed to 2, 5, or 10 μg/L meHg as embryos. Again, on day 3 after hatching, the 10 μg/L group was significantly slower than the others (number captured in 5 min, 2.5 ± 1.7 vs. 4.3 ± 1.4 for controls). ANOVA had 3 *d.f.* between, 36 *d.f.* within. The 5 μg/L group was not different from controls in this trial. On day 7 this difference was again seen (number captured in 1 min 2.2 ± 1.9 vs. 4.3 ± 0.8). However, by day 10, on which only the controls and the 10 μg/L group were tested, there was no difference between them.

DISCUSSION

In general, the prey-capture rate improved with larval age. This is similar to what has been reported with other species [15] and is probably due to increased development of the nervous system and to previous experience [9]. An impairment in initial prey-capture ability was seen in larvae exposed to 5 or 10 μg/L meHg as embryos. This is evidence for behavioral teratology. However, this impairment was transitory, and by about a week after hatching, the exposed larvae were comparable to controls.

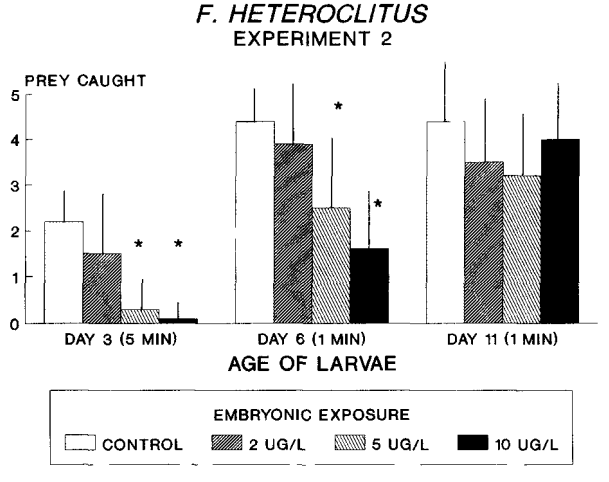

Fig. 2. Capture of *Artemia* (mean + SD) by larval mummichogs of different ages after embryonic exposure to methylmercury—experiment 2.

Some variation appeared in the three trials in how long the effect lasted and whether it was seen in groups exposed to 5 μg/L. Variation is not surprising since these are wild-caught, not inbred, fish. There was also variation in prey capture of 3-d-olds; in trials 2 and 3, many of the fish in all groups captured no prey in 1 min, making for poor comparisons. Therefore, 5-min data rather than 1-min data are used for those trials.

As the effect was transient, the neurotoxic mechanism of meHg may have been a retardation of neurological development or a depression of neurochemical processes rather than a pathological effect. If development was retarded, compensation apparently took place. If neurochemistry was affected, it might involve the neurotransmitter serotonin. Adults from the population exposed to contaminants and known to be poor predators [7] have significantly less brain serotonin and 5-hydroxyindoleacetic acid, a serotonin metabolite [16], than fish from a reference area.

Prenatal methylmercury exposure in mammals can have permanent effects, causing abnormal neuronal migration and differentiation, and damage to microtubules in neurons and astrocytes [17]. However, the fish nervous system has extensive regenerative capacity and is much more labile than that of mammals, so that recovery from a pathological insult could be possible in young fish.

There were no permanent effects of the early prey-capture deficit, and growth was not affected. These larvae were maintained in clean water and had abundant food. In the "real world" in which food resources are not as abundant and as easily obtained, the behavioral deficit might have had more long-lasting impacts. Our maintenance feeding consisted of providing a high density of food into a confined container. The prey was *Artemia*, which has a jerky, conspicuous pattern of movement that may make it easier to detect than many types of zooplankton found in the estuary [18].

Furthermore, in a polluted environment, the fish, which are nonmigratory, would be exposed to contaminants in food and water as larvae, and the effects would probably be augmented by this continued exposure. After hatching, the chorion, which can serve as a barrier to reduce chemical accumulation in the embryo, is gone, and the uptake and effects of contaminants may be greater [19]. It is likely that the impaired prey capture of the adult mummichogs from the polluted area [7] is produced largely by post-hatching exposures to the toxicants in their environment.

Functional deficiencies noted in mammals after embryonic exposures can be permanent or temporary. Transitory neurobehavioral effects of embryonic lead exposure have been observed. There is evidence in children that prenatal exposure to lead can reduce cognitive functioning, but that this effect diminishes over time [20,21]. We have demonstrated that a transitory functional deficiency can be brought about by embryonic exposure to a neurotoxic chemical, methylmercury, in a fish species, as well.

Acknowledgement—The authors are grateful to the staff of the Marine Science Center at the Southampton Campus of Long Island University for their hospitality. We are also grateful to Rebecca Carroll for technical assistance.

REFERENCES

1. **Cairns, J.** and **J. Loos.** 1967. Changed feeding rate of *Brachydanio rerio* (Hamilton-Buchanan) resulting from exposure to sublethal concentrations of zinc, potassium dichromate, and alkyl benzene sulfonate detergent. *Proc. Penn. Acad. Sci.* **40**:47–52.
2. **Nyman, H.G.** 1981. Sublethal effects of lead (Pb) on size selective predation by fish—applications on the ecosystem level. *Verh. Internat. Verein. Limnol.* **21**:1126–1130.
3. **Morgan, M.J.** and **J.W. Kiceniuk.** 1990. Effect of fenitrothion on the foraging behavior of juvenile Atlantic salmon. *Environ. Toxicol. Chem.* **9**:489–495.
4. **Little, E.E., R.D. Archeski, B. Flerov** and **V. Kozlovskaya.** 1990. Behavioral indicators of sublethal toxicity in rainbow trout. *Arch. Environ. Contam. Toxicol.* **19**:380–385.
5. **Henry, M.** and **G.J. Atchison.** 1991. Metal effects on fish behavior: Advances in determining the ecological significance of responses. In M.C. Newman and A.W. McIntosh, eds., *Metal Ecotoxicology: Concepts and Applications*, Lewis, Chelsea, MI, pp. 131–143.
6. **Weis, J.S.** and **A.A. Khan.** 1990. Effects of mercury on the feeding behavior of the mummichog, *Fundulus heteroclitus*, from a polluted habitat. *Mar. Environ. Res.* **30**:243–249.
7. **Weis, J.S.** and **A.A. Khan.** 1991. Reduction in prey-capture ability and condition of mummichogs from a polluted habitat. *Trans. Am. Fish. Soc.* **120**:127–129.
8. **Atchison, G.J., M.G. Henry** and **M. Sandheinrich.** 1987. Effects of metals on fish behavior: A review. *Environ. Biol. Fishes* **18**:11–25.
9. **Miller, T.J., L.B. Crowder** and **J.A. Rice.** 1993. Ontogenetic changes in behavioural and histological measures of visual acuity in three species of fish. *Environ. Biol. Fishes* **37**:1–8.
10. **Pryor, V.K.** and **C.E. Epifanio.** 1993. Prey selection by larval weakfish (*Cynoscion regalis*): The effects of prey size, speed, and abundance. *Mar. Biol.* **116**:31–37.
11. **Munoz, C., K. Gerbe, H. Lillienthal** and **G. Winneke.** 1988. Significance of hippocampal dysfunction in low level lead exposure in rats. *Neurotoxicol. Teratol.* **10**:245–253.
12. **Clarkson, T.W.** 1992. Mercury: Major issues in environmental health. *Environ. Health Perspect.* **100**:31–38.
13. **Ostrander, G., J.J. Anderson, J.P. Fisher, M.L. Landolt** and **R.M. Kocan.** 1990. Decreased performance of rainbow trout, *Oncorynchus mykiss*, emergence behaviors following embryonic exposure to benzo[*a*]pyrene. *Fish. Bull.* **88**:551–555.
14. **Weis, P.** and **J.S. Weis.** 1977. Methylmercury teratogenesis in the killifish, *Fundulus heteroclitus. Teratology* **16**:317–326.
15. **Connaughton, V.P.** and **C. Epifanio.** 1993. Influence of previous experience on the feeding habits of larval weakfish *Cynoscion regalis. Mar. Ecol. Prog. Ser.* **101**:237–241.
16. **Smith, G., A.T. Khan, J.S. Weis** and **P. Weis.** Behavior and brain chemistry correlates in mummichogs (*Fundulus heteroclitus*) from polluted and unpolluted environments. *Mar. Environ. Res.* (in press).
17. **Choi, B.H.** 1983. Effects of prenatal methylmercury poisoning upon growth and development of fetal nervous system. In T.W. Clarkson, G.F. Nordberg and P.R. Sager, eds., *Reproductive and Developmental Toxicity of Metals.* Plenum, New York, NY, pp. 473–495.
18. **Buskey, E.J., C. Coulter** and **S. Strom.** 1993. Locomotory patterns of microzooplankton: Potential effects on food selectivity of larval fish. *Bull. Mar. Sci.* **53**:29–43.
19. **Weis, J.S.** and **P. Weis.** 1989. Effects of environmental pollutants on early fish development. *Rev. Aquat. Sci.* **1**:45–73.
20. **Baghurst, P.A., E.F. Robinson, A.J. McMichael, G.V. Vimpani, N.R. Wigg** and **R.J. Roberts.** 1987. The Port Pirie cohort study: Lead effects on pregnancy outcome and early childhood development. *Neurotoxicology* **8**:395–402.
21. **Dietrich, K.N., P.A. Succop** and **R.L. Bornschein.** 1990. Lead exposure and neurobehavioral development in later infancy. *Environ. Health Perspect.* **89**:13–19.