IMPAIRED REPRODUCTION IN THE FATHEAD MINNOW FOLLOWING CHRONIC EXPOSURE TO CONTAMINATED SEDIMENTS

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INTRODUCTION

Exposure of fish to toxic chemicals may yield effects varying from acute lethality to sublethal changes in reproduction, growth and development (Murty 1986). A sublethal effect that warrants more detailed study is the influence of environmental exposure to anthropogenic agents on reproduction and offspring growth (Weis and Weis 1989). Reproductive effects may arise in fish when some aspect of the environment alters the maternal contribution to yolk quality or quantity. With regard to anthropogenic agents, biologically important effects could arise through their direct incorporation into the yolk or through alteration in the egg yolking process. The manifestation of these subtle influences on reproductive biology could range from altered life histories of the affected generation to teratogenic effects.

There is evidence that maternal environment influences reproduction and offspring quality. Classic experiments documenting maternal effects have been conducted with cattle, rabbits and pigs (Pirchner 1983) snails, fruit flies, rye grass, and tobacco (Mather and Jinks 1982). Maternal effects are implicated in all reproductive modes, from live-bearing with extensive maternal-fetal exchange to egg or seed production. Maternal effects arise through contributions that the mother makes to her offspring's phenotype beyond her nuclear genes. Routes for these contributions are: cytoplasmic inheritance, pre-and post-natal nutrition, pre- and post-natal transmission of pathogens by feeding, imitative behavior, and interaction of sibs directly with each other or through the mother (Mather and Jinks 1982). Maternal effects may arise from non-genetic or genetic sources. For example, non-genetic effects may arise from the environment in which the mother finds herself and a genetic source could be genetically based variation in maternal nourishment of offspring. Males can influence the maternal genetic effects of their grandchildren through their daughters, despite having no impact on their own offspring through this route. "Grandfather effects" such as these have been documented in species as diverse as horses, cattle, (Pirchner 1983), snails (Boycott and Diver 1923; Sturtevant 1923; Diver et al. 1925) and mosquitofish (Reznick 1981).

Maternal environmental effects in fish on the size and number of eggs or offspring produced have been documented in several cases (e.g., Alm 1949; Reznick,1981; Cheong et al. 1984; Tanasichuk and Ware 1987). Also, many cases are known where chemicals of environmental interest induce reproductive effects (Niimi 1983; Heath 1987; Weis and Weis 1989). Such research has primarily focused on the reproductive effects of chemicals on fecundity and fertility though other, possibly subtle, effects of sublethal concentrations are likely.

The purpose of this investigation was to evaluate the relationship between reproductive effects and polychlorinated biphenyl (PCB) tissue concentrations resulting from exposure to contaminated sediments from an inland waterway. Such "residues-effects" data are infrequently (ca., 6%) reported in the literature for fish or other aquatic organisms (Dillon 1984). However, these types of data may be valuable when interpreting the biological significance of contaminant tissue concentrations in field-collected animals.

MATERIALS AND METHODS

<u>Sediments</u>

The PCB contaminated sediments were obtained from existing sediment samples previously collected from Sheboygan Harbor, Wisconsin, and stored in 15-L glass jars at 4^{0}C. Sediments were mixed to produce treatment concentrations identified as low, medium, and high which corresponded to PCB concentrations, as Aroclor 1254 equivalents, of 0.82, 14.0, and 27.0 µg/gm (dry weight), respectively.

Exposures

Adult fathead minnows (*Pimephales promelas*) were obtained from Northeastern Biologists (Rhinebeck, NY). Females averaged 25 to 40 mm in length, and males averaged 40 to 50 mm in length. Following a 30 day acclimation to all experimental conditions except sediment, fish were placed in 40 L glass aquaria containing a 2 to 4 cm layer of PCB contaminated sediment. Aquaria containing only water served as controls. The aquaria were supplied with moderate aeration and a continuous flow of aged, charcoal filtered tap water having the following mean water quality characteristics: pH 7.8, hardness 59 mg $CaCO_3$/L and 8.1 mg/L dissolved oxygen. Flow rates (80 ml/min) were set to provide three volume additions every 24 h. Temperature and flow rates were monitored daily while total dissolved solids (TSS) were monitored on a weekly basis.

During the initial experimental phase, 7 male and 21 female fish per aquarium were exposed for 5 weeks with water temperature maintained at $20^{0} \pm 1^{0}$ C and photoperiod maintained at 12-h light:12-h dark. Fish were fed 3% freeze-dried brine shrimp in the morning and 3% commercial diet (Tetra-Min) in the evening, on an estimated body weight basis. Following the initial exposure phase, water temperature was increased 1^{0}C per day to $26^{0} + 1^{0}$ C. In addition, the photoperiod was lengthened 1 h every other day to 16-h light:8-h dark to induce gametogenesis, sexual dimorphism, and reproductive activities in the test organisms. During this 10-d induction phase, spawning substrates were introduced into each of the aquarium. The use of frozen brine shrimp (3%) replaced freeze-dried brine shrimp to further induce spawning. Following induction, 1 male and 6 female fish were selected from each aquarium to evaluate treatment effects on reproduction. Fecundity and clutch size were monitored for an additional 9 weeks.

Chemical Analysis

Chemical analysis was conducted by the Tennessee Valley Authority (Chattanooga, TN). Briefly, fish were thawed, homogenized in petroleum ether, and cleaned with concentrated sulfuric acid. Water was removed during repeated extractions with sodium sulfate, and an aliquot of the homogenized fish tissue was removed for gravimetric determination of percent lipid prior to acid clean up. Extracts were run on a Hewlett-Packard gas chromatograph equipped with a 30 meter DB-5 fused capillary column and electron capture detector. All standards were purchased from Ultra Scientific (Hope, RI). The detection limit for Aroclor 1254 was 0.10 µg/g and for PCB congeners, 0.01 µg/g.

Data Analysis

Treatment effects on all parameters were analyzed by means of one-way analysis of variance. Differences were considered statistically significant at $p < 0.05$. Square root or log 10 transformations were used when data sets were not homogenous. Arc sine transformations were used for non-homogeneous data which were expressed as percentages. Mean separation for homogeneous data was achieved by means of Waller-Duncan k-ratio t test. When transformations were unsuccessful in achieving homogeneity a Proc Rank nonparametric procedure was used for mean separation (SAS Institute 1985). To facilitate statistical analysis and presentation of the PCB analytical information, data reported to be less than the detection limit were considered to be equal to the detection limit.

RESULTS

Table 1 presents the major findings of the investigation. Survival was high in all PCB-contaminated sediment treatments and ranged from 80 to 100%. Regarding reproductive effects, there was a very clear separation between the control and low treatments and the medium and high treatments, with fish reproduction in the latter treatments being almost completely inhibited. This is substantiated by examination of fecundity and frequency of egg production. The mean number of eggs produced by fish in the control, low, medium and high treatments were 5,166, 3,346, 1,172 and 97, respectively, while mean number of clutches were 22.4, 14.4, 3.2 and 0.6.

Tissue concentrations in fish from all experimental treatments were significantly different from one another after 7 and 16 week of exposure (Table 2). After 7 weeks of exposure, respective mean tissue concentrations in the control, low, medium, and high treatments were 0.10, 5.25, 13.7 and 18.4 µg/g wet weight, expressed as Aroclor 1254 equivalents. PCB residues were also determined in fish at termination of the experiment. These residues, therefore, reflect 16 weeks of continuous exposure to the experimental treatments with the last 9 weeks under conditions to induce gametogenesis and spawning activities. Mean values after 16 weeks of exposure generally reflected patterns observed after 7 weeks except that the absolute concentrations were elevated over 2 fold. Significantly different PCB concentrations in the control, low, medium, and high treatments were 0.10, 11.6, 36.0 and 47.2 µg/g wet weight, respectively.

Mean percent lipid values for all samples from this experiment are shown in Table 3. At 16 weeks of exposure, there is a significant increase in percent lipid in the medium and high treatments.

DISCUSSION

One of the objectives of this investigation was to evaluate the biological consequences of bioaccumulation. To optimize this comparison, one must have graded responses in both the consequences and the bioaccumulation components. In this investigation, both were observed.

The experimental treatments, represented by control, low, medium, and high PCB-contaminated sediments, had a significant impact on reproduction fathead minnows. This is based on the observation that both fecundity and fertility were affected by these compounds in a dose-related fashion. Interestingly, fish exposed to PCBs at medium and high concentrations had a higher percent lipid content than those not exposed or exposed to low concentrations. This brings up the interesting prospect that the energy allocation pattern of exposed fishes may have been altered from reproduction to storage. PCBs are lipophilic, suggesting that lipid stores sequester concentrated levels of these compounds. Thus, even if environmental concentrations were to decrease, when and if these stores are metabolized, they could release high levels of PCBs into the organism. One characteristic of PCB activity is estrogenicity (Hansen 1987). This property makes it likely that these compounds influence reproductive function. However, in addition to reproductive and hormonal effects, PCBs have a multiplicity of other toxicological effects including neurotoxicity, immunotoxicity, hepatotoxicity, as well as mutagenic and carcinogenic effects (Safe 1984). Furthermore, commercial PCB products, such as Aroclor 1254, are mixtures of individual biphenyl molecules; or congeners. There are 209 theoretically possible congeners and, recently, much attention has been given to their individual toxicological effects. For example, Dillon et al. (1989, 1990) examined the effects of individual PCB congeners on survival, growth, and reproduction in the freshwater cladoceran, *Daphnia magna* (IUPAC numbers 52, 77, 101, 118, 138, 153, 180) and freshwater fish, *Pimephales promelas* (IUPAC numbers 52, 101, 138, 153, 180). These species accumulated substantial amounts of each congener examined. However, there were little to no detectable effects on survival, growth and reproduction. These investigators indicate that because the concept of PCB toxicity has been developed primarily through technical findings with mammals, caution should be exercised when applying those concepts to fish and other aquatic organisms.

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Table 1. Survival, fecundity and frequency of egg production in fathead minnows exposed to PCB-contaminated sediment treatments

Treatment	Survival (%) male	Survival (%) female	Fecundity (total eggs)	Frequency (total clutches)
Control	100	100	5,166 ± 1,152 A	22.4 ± 3.3 A
Low	100	100	3,346 ± 417 A	14.4 ± 2.8 A
Medium	80	95	1,172 ± 1,114 B	3.2 ± 3.0 B
High	80	90	97 ± 9 B	0.6 ± 0.6 B

Values are reported as mean ± SE. Values within columns followed by the same letters are not significantly different ($p < 0.05$).

Table 2. Tissue concentrations of PCB in fathead minnows exposed for 7 and 16 weeks to PCB-contaminated sediments

	Aroclor 1254 (μg/g wet weight)	
Treatment	7 Weeks of exposure	16 Weeks of exposure
Control	0.10 ± 0.0 A	0.10 ± 0.0 A
Low	5.25 ± 0.28 B	11.6 ± 1.32 B
Medium	13.7 ± 0.97 C	36.0 ± 2.13 C
High	18.4 ± 0.66 D	47.2 ± 3.09 D

Values are reported as mean ± SE. Values within columns followed by the same letters are not significantly different ($p < 0.05$).

Table 3. Percent lipid content of fathead minnows exposed for 7 and 16 weeks to PCB-contaminated sediments

Treatment	7 Weeks of exposure	16 Weeks of exposure
Control	4.5 ± 0.45 A	4.8 ± 0.75 A
Low	4.8 ± 0.25 A	5.7 ± 0.74 AB
Medium	4.9 ± 0.23 A	7.6 ± 0.93 B
High	4.6 ± 0.16 A	7.8 ± 0.60 B

Values are reported as mean ± SE. Values within columns followed by the same letters are not significantly different ($p < 0.05$).