# Developmental and Behavioral Effects of Embryonic Exposure to DE-71 in *Fundulus heteroclitus*

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## INTRODUCTION

While most organohalogens are currently decreasing in environmental concentrations, polybrominated diphenyl ether (PBDE) levels are rapidly increasing. PBDEs are added to electronics, plastics, textiles, and foam cushioning to reduce the likelihood and severity of fires. However, these chemicals have been shown to be highly persistent and mobile in the environment, found even in the Artic and the deep sea.[1] Mounting concern over the safety of these compounds has prompted the European Union as well as the State of California to ban one class of PBDEs, the penta brominated diphenyl ethers (PeBDEs).

Much remains to be learned about the toxicity PeBDEs and their metabolites. Several animal studies indicate that PeBDEs may pose a developmental hazard, with the most sensitive endpoints being thyroid hormone disruption, neurodevelopmental deficits, and neurobehavioral toxicity.[2,3] While several studies have documented the presence of PeBDEs in fish[1] very few fish developmental toxicity studies have been published. The goals of this preliminary study were to explore various dose ranges and embryonic exposure regimes, characterize the effect on development in an environmentally relevant fish model, and test for latent behavioral effects.

## MATERIALS AND METHODS

Animal model. *Fundulus heteroclitus* or killifish, is a common estuarine minnow with an important role in tidal ecosystems on the Atlantic coast of the United States. The killifish is a widely used developmental and ecotoxicological model with a transparent chorion to allow for developmental observation. They are sexually dimorphic and can be manually spawned and produce large numbers of eggs. Animals were caught at King's Creek, Virginia. They were maintained in the laboratory in 25ppt artificial seawater at 25°C, in a recirculating water system. Adult fish were fed flake food four times daily. Larval fish were fed live Artemia once a day.

Chemicals. The commercial PeBDE mixture DE-71 (lot 7550OK20A) was manufactured by Great Lakes Chemical Corporation (West Lafayette, IN) and was supplied as a generous gift from Kevin Crofton, USEPA. DE-71 was dissolved in DMSO.

Exposure. Ten embryos per group were dosed in 20ml scintillation vials (1 embryo per vial) with 10ml of DE-71 and 21 µg /L ethoxyresorufin in 20ppt artificial seawater. The DMSO concentration in all vials was 0.0027%. At 7 days post fertilization (dpf),

embryos were transferred to vials with clean artificial seawater. Three dosing regimes have been explored. The first dosing regime included doses of 0, 1, 10, and 100 µg /L DE-71. The second was a low dose (0, 0.1. 0.5, and 1ug/L) exposure. The third dosing regime involved replacing the dosing solution (0, 0.1, 1, and 10 µg /L) on day 3 and day 5 dpf, thus receiving three administrations of the dosing solution. All doses began on the day of fertilization and ended at 7 dpf.

In Ovo EROD and Deformities. At 7 dpf, embryos were placed into clean seawater and assayed for in Ovo ethoxy-resourufin-O-deethylase (EROD) (adapted[4]). At 10 dpf embryos were examined for deformities under 50x magnification.

Hatching. In the days following the deformity score, water was changed every other day. At 14 dpf, embryos were placed on a shaker for 15 minutes at 170 rpm to stimulate hatching. This was repeated until 25 dpf when successful hatching was considered completed.

Larval swim test. At 5 days post hatch (dph) a larval swim test was performed (adapted[5]). After a 1 minute habituation period, fish activity levels were recorded as the number of times the fish crossed a line in a 25mm circular grid during a 60 second period. Fish were then agitated by stirring a metal bar in the vial four times in 15-second intervals. Line crossing activity was recorded for the following 60 seconds.

Predation Assay. Each larva was placed in a Petri dish with 5 *Artemia* and allowed 5 minutes to catch prey[6]. Predation rates were calculated by dividing the number of *Artemia* caught by the time taken. This assay was performed on 3 and 8 dph larvae.

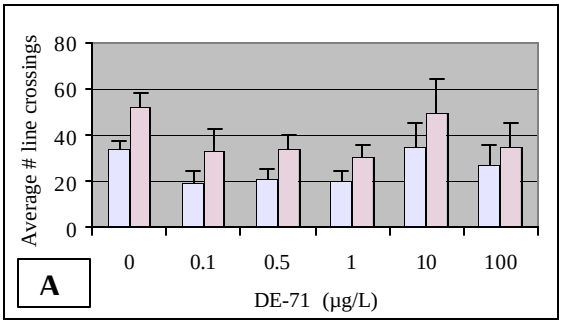
Swim maze. A fish water maze was used to assess spatial discrimination learning in killifish. The maze consisted of a three-chambered fish tank, used to differentiate response latency from choice accuracy[7]. Fish from the first dosing regime were assessed at 3 months post hatch.

RESULTS & DISCUSSION

Lack of CYP1A induction and deformities. In general, PeBDEs have been found to be weak to moderate inducers of the metabolic enzyme cytochrome P4501A (CYP1A), as assayed by metabolism of EROD[8]. In this study, all three trials showed no significant difference in in-Ovo EROD induction, nor were any deformities noted (data not shown).

Hatching Delay. The day of 50% hatch was delayed by 1- 4.5 days in the DE-71 exposed embryos (see Table 1). The greatest delay was observed in the third (multiple) dosing regime.

Larval Swim Test. At low doses, a decrease in activity was observed; at higher doses, the effect appears to swing back towards control levels (see Figure 1a). An increase in activity level (hyperactivity) was observed in the third multiple-dosing regime (see Figure 1b). However, these trends were not significant and require further examination.



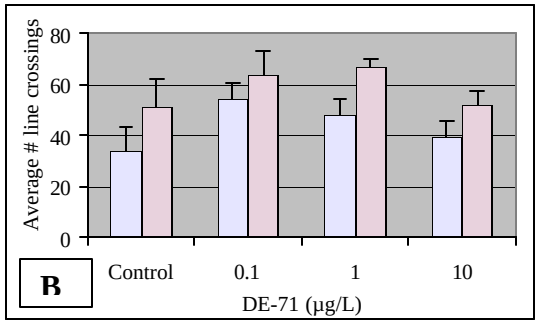
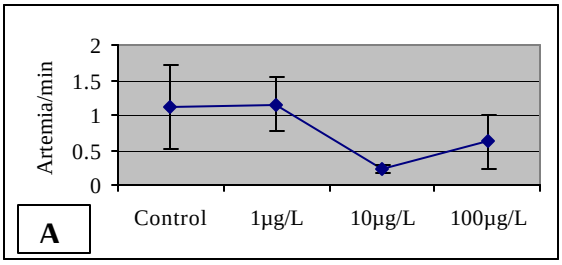




**Figure 1A: Larval Swim Test.** The average number of line crossings in 5 dph larvae exposed to varying doses of DE-71 over two experiments. The dotted bars are swimming activity of habituated fish; the hatch bars are swimming activity of agitated fish. The observed trend is a depression of activity in the lower doses and a return to control levels in the highest doses.

**Figure 1B: Larval Swim Test.** The average number of line crossings in 5 dph larvae exposed to varying doses of DE-71 that were refreshed three times during embryonic development. The trend shows an inverted U-shaped response, with the lower doses having a greater effect than the higher dose.

Predation Test. At 8 dph, the 10 µg /L group showed a marked decrease in predation rate (Artemia/minute) (see Figure 2A). At 3 dph, no difference was seen in predation rate among any groups (data not shown). At 8 dph, the third dosing regime (multiple exposures) showed a decrease in predation rate in the lowest two doses (see Figure 2b).



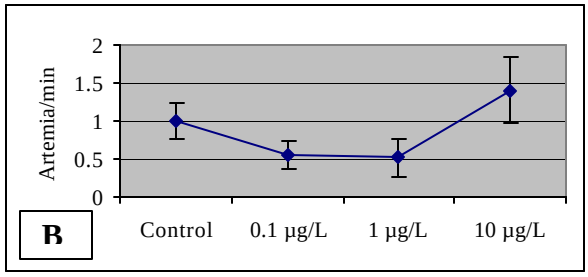




**Figure 2A: Predation rate of the first dosing regime.** A decrease in the 10 µg /L and a slight decrease in the 100 µg /L exposure groups was observed.

**Figure 2B: Predation rate of the Multiple dosing regime.** A decrease in the 0.1 µg /L and 1 µg /L dose groups was observed.

Swim maze. Fishes were successful at learning the maze. The 1 µg /L treatment group had the least success in learning the correct v. wrong chamber choice, although this was not statistically significant. The 10 µg /L group showed lower choice latency (seconds taken to choose a chamber) than controls, a result which is suggestive of hyperactivity. This effect was significant (p-value = 0.026), the dose effect was shown to be significant as a quadratic contrast (p-value = 0.0332).

CONCLUSIONS

While not conclusive, this study suggests a nonmonotonic dose response to the PeBDE mixture DE-71. Future studies will include even lower and more environmentally

relevant concentrations, and will also analyze contaminant body burden in order to compare with findings of PeBDE levels in wild-caught fish.

Hatching delay (between 2-4.5 days) was the most dramatic effect noted. Early life-stage exposure to DE-71 in these dosing regimes produced subtle behavioral effects. Fish whose activity level had been altered (both hypo and hyperactive) also performed worse in the predation test (see Table 2). Furthermore, this study suggests the learning ability of fish exposed to DE-71 could be affected. While the fish maze study was only statistically significant in a few parameters, when taken with trends in other behavioral data, these preliminary data are suggestive that some fish are exhibiting some behavioral effects 3 months after cessation of exposure.

| **Dose (µg/L DE-71)** | **50% Hatching (days after controls)** | **Swim test (activity)** | **Predation rate** | **Swim maze performance** |
|---|---|---|---|---|
| 0 | 0 | | | |
| 0.1 | +2 | Decrease | N/A | N/A |
| 0.5 | +1.5 | Decrease | N/A | N/A |
| 1 | +2 | Significant Decrease (p = 0.0139) | Decrease | Poor learning |
| 10 | N/A | | Decrease | Significant Decreased Latency (p=0.026) |
| 100 | N/A | | Slight decrease | |
| 0.1 (3x) | + 1 day | Increase | Decrease | N/A |
| 1 (3x) | +4.25 | Increase | | N/A |
| 10 (3x) | +4.5 | | | N/A |

**Table 1: Compiled Trends Observed in DE-71 treated embryos.** (3x) Indicates the third dosing regime in which the dosing solution was administered three times. N/A indicates data not available. Statistically significant data points are noted and p-values are given.

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