Published in final edited form as:

Neurotoxicology. 2016 March ; 53: 93–101. doi:10.1016/j.neuro.2015.12.018.

Effects of embryonic exposure to polychlorinated biphenyls (PCBs) on anxiety-related behaviors in larval zebrafish

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Abstract

The zebrafish (*Danio rerio*) is an excellent model system for assessing the effects of toxicant exposure on behavior and neurodevelopment. In the present study, we examined the effects of sub-chronic embryonic exposure to polychlorinated biphenyls (PCBs), a ubiquitous anthropogenic pollutant, on anxiety-related behaviors. We found that exposure to the PCB mixture, Aroclor (A) 1254, from 2 to 26 hours post-fertilization (hpf) induced two statistically significant behavioral defects in larvae at 7 days post-fertilization (dpf). First, during 135 min of free swimming, larvae that had been exposed to 2 ppm, 5 ppm or 10 ppm A1254 exhibited enhanced thigmotaxis (edge preference) relative to control larvae. Second, during the immediately ensuing 15-min visual startle assay, the 5 ppm and 10 ppm PCB-exposed larvae reacted differently to a visual threat, a red 'bouncing' disk, relative to control larvae. These results are consistent with the anxiogenic and attention-disrupting effects of PCB exposure documented in children, monkeys and rodents and merit further investigation.

Keywords

attention; behavior; polychlorinated biphenyls; startle; thigmotaxis; zebrafish

1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants found in soil, sediment and air as well as in marine and freshwater ecosystems (Alava et al., 2012; Li et al., 2009; Safe, 1994; Tanabe, 1985). Although their production has been banned in the United States for more than three decades and more globally since 2001, PCBs have a very long half-life, are extremely lipophilic and are poorly metabolized leading to their bioaccumulation in animal and human tissues and their biomagnification up the food chain (Jensen, 1987; Porte and Albaigés, 1994; Van Oostdam et al., 2005). High levels of PCBs have been found in the fat deposits of humans and marine mammals with diets consisting

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primarily of fish and fish-eating mammals (Bernhoft et al., 1997; Dewailly et al., 1993; Hickie et al., 2007; Mos et al., 2010; Ross et al., 2004; Wolkers et al., 2007). Dewailly et al. (1996) found PCB levels in breast milk of 2.9 mg/kg for women in Arctic Quebec, five times higher than for southern Quebec women. Wickizer et al. (1981) reported PCB levels ranging from trace amounts to 5.1 parts-per-million (ppm) in the breast milk of nursing mothers residing in Michigan. Ross et al. (2000) found PCB loads in excess of 250 ppm in male transient orcas feeding on seals.

Maternal consumption of PCB-contaminated fish is a major route of human fetal and neonatal exposure as PCBs are readily transferred to the fetus through the placenta and to the nursing infant in breast milk (Jacobson et al., 1984). The detrimental effects of such exposures have been well documented (Boucher et al., 2009; Darvill et al., 2000; Faroon and Ruiz, 2015; Feeley and Brouwer, 2000; Jacobson et al., 1985; Ribas-Fitó et al., 2001; Rogan et al., 1986; Schantz et al., 2003; Stewart et al., 2000; Verner et al., 2010; Winneke, 2011). Prenatal PCB exposure has been correlated with poor habituation scores, abnormal reflexes, psychomotor deficits, hypotonia, and changes in autonomic function (Huisman et al., 1995a,b; Koopman-Esseboom et al., 1996; Sagiv et al., 2008; Stewart et al., 2000). Cognitive effects have been shown to persist past infancy and into childhood (Grandjean and Landrigan, 2006; Jacobson and Jacobson, 1996; Vreugdenhil et al., 2002) with several studies reporting links between PCB exposure and attention problems, hyperactivity, and impulsivity (Boucher et al., 2012; Jacobson and Jacobson, 1996, 1997; Patandin et al., 1999; Sagiv et al., 2010; Stewart et al., 2000; Verner et al., 2015).

Research in nonhuman animals exposed perinatally to PCBs has produced similar results (Eubig et al., 2010; Tilson et al., 1990). Monkey and rodent studies have demonstrated PCB-induced hyperactivity, perseverative behavior, impaired learning and elevated anxiety-related behavior including increased thigmotaxis and social avoidance (Corey et al., 1996; Eriksson and Fredriksson, 1996; Levin et al., 1988; Orito et al., 2007; Rice, 1999; Rice and Hayward, 1997; Schantz et al., 1989, 1991, 1995; Sugawara et al., 2006). Results from some studies (e.g., Rice, 2000) are in agreement with evidence that exposure to PCBs may contribute to attention deficit hyperactivity disorder (ADHD). These findings from human and animal studies underscore the importance of understanding the effect of embryonic exposure to PCBs on neurodevelopment and behavior.

The zebrafish (*Danio rerio*) is an excellent model system for studying toxicological effects on behavior (Bailey et al., 2013; de Esch et al., 2012; He et al., 2014; Liu et al., 2012; Padilla et al., 2012; Reif et al., 2015; Richendrfer et al., 2014; Sipes et al., 2011; Sloman and McNeil, 2012; Truong et al., 2014). Eggs are fertilized externally and in large numbers, providing an opportunity for immediate large-scale embryonic exposure to toxicants in multiple concentrations and mixtures. Early development is rapid, synchronous and well-characterized (Kimmel et al., 1995). Larvae hatch from the chorion at 2–3 days post fertilization (dpf) and are free swimming following swim bladder inflation at 4–5 dpf. Within a week of fertilization, zebrafish larvae exhibit a range of locomotor, foraging, predatory and defensive behaviors (Budick and O'Malley, 2000; Colwill and Creton, 2011b; Spence et al., 2008; Wolman and Granato, 2012). Moreover, larvae at this stage are still small enough to be imaged in multiwell plates. The development of high-throughput

automated imaging systems has facilitated rapid screening of the behavior of large numbers of subjects to capitalize on these attributes of the developing zebrafish (Berghmans et al., 2007; Bruni et al., 2014; Delvecchio et al., 2011; Gerlai, 2010; Kokel et al., 2010; Lessman, 2011; Mathias et al., 2012; Muto et al., 2005; Raldúa and Piña, 2014; Rihel and Schier, 2012; Rihel et al., 2010).

Although the developing zebrafish has been used extensively to examine the effects of pesticides and pharmaceuticals on neurodevelopment and behavior, relatively few studies have taken advantage of this model system to examine PCB exposure effects. Chronic exposure to the commercial PCB mixture Aroclor (A) 1254 and to individual congeners may induce significant developmental defects including cardiac, craniofacial, skeletal and retinal abnormalities in zebrafish embryos and larvae (Billsson et al., 1998; Grimes et al., 2008; Ju et al., 2012; Li et al., 2014; Olsson et al., 1999; Orn et al., 1998; Sisman et al., 2007; Wang et al., 2012). Continuous exposure to A1254 from immediately after fertilization until testing at 7dpf has also been found to disrupt performance on an optomotor assay consistent with impaired visual acuity (Zhang et al., 2015). Finally, offspring of adult zebrafish that had been exposed to a PCB mixture of 13 congeners for 8 months were found to be hyperactive at 5 dpf (Péan et al., 2013).

Recently, our laboratory reported that exposure to 5 ppm and 10 ppm of A1254 during early-stage embryonic development (2 to 26 hours post-fertilization, hpf) altered anxiety-related behaviors in 7 dpf zebrafish larvae on a visual avoidance assay (Lovato et al., 2016). Briefly, in one experiment, the PCB-exposed larvae were less likely than the control larvae to avoid a red ‘bouncing’ disk, a stimulus that is perceived as a visual threat (Pelkowski et al., 2011), that was presented intermittently for two hours. In a second experiment, the PCB-exposed larvae displayed a stronger edge preference (thigmotaxis), a commonly used index of anxiety (Ahmad and Richardson, 2013; Champagne et al., 2010; Colwill and Creton, 2011a; Maximino et al., 2010; Schnörr et al., 2011). In the present study, we modified our visual avoidance assay to examine the effects of early embryonic PCB exposure on free swimming (phase 1) and on visual startle behavior (phase 2) in 7 dpf zebrafish larvae. Our goals were to explore further the effects of PCB exposure on anxiety-related behaviors and to devise a visual startle assay suitable for video recording and more precise measurement of PCB exposure effects on avoidance behavior.

2. Materials and methods

2.1 Embryo collection

Details of our breeding population of adult male and female wild type zebrafish, their housing and maintenance schedule, and our protocol for embryo collection were the same as those described in Lovato et al. (2016). Briefly, mixed sex adults were housed in 20 gallon tanks and maintained on a 14 h light/10 h dark cycle. They were fed a combination of frozen or fresh brine shrimp and flake fish food once or twice per day. Embryos were collected for ninety minutes following light onset in shallow trays placed in the bottom of the tanks. All protocols involving animals were approved by the Institutional Animal Care and Use Committee of Brown University (Providence, RI) prior to the initiation of experimentation.

2.2 PCB Exposure

Details of our PCB exposure and embryo rearing protocols were identical to those described in Lovato et al. (2016). Briefly, individual stock solutions of 2, 5 and 10 mg/ml of Aroclor (A) 1254 (Ultra Scientific, Kingston, RI) in dimethyl sulfoxide (DMSO) were diluted (matching the 0.1% DMSO control) to final concentrations of 2, 5, and 10 ppm in egg water (60mg/l Instant Ocean in deionized water and 0.25 mg/l methylene blue). Embryos at the 4 cell stage with intact chorions were statically exposed for 24 hours at a density of approximately 25 embryos per 50 mL petri dish (Corning no. 430591) and incubated at 28.5°C. Egg water (EW) and DMSO in EW at a final concentration of 0.1% were used as the two controls. A1254 is a commercial PCB mixture consisting mainly of non-coplanar, ortho-substituted congeners. Its congener profile is similar to the congeners found in human tissues (Angulo et al., 1999; Hansen, 1999; Kodavanti et al., 2011; Yang and Lein, 2010) indicating its suitability for testing PCB exposure effects in a model system.

Following treatment exposures, all embryos were transferred to microfuge tubes and triple-rinsed in egg water. They were then placed in 1L plastic breeding tanks (Aquatic Habitats) containing approximately 500mL of egg water and incubated at 28.5°C until they reached 7dpf. During this period, any dead larvae and particulates were removed and egg water in each tank was replaced to maintain water quality. The developing larvae were examined for malformations such as pericardial and yolk sac edemas, curved body axis and sideways position. Larvae exhibiting such defects were not used for behavior assessment. Food supplements were not provided because developing zebrafish larvae absorb nutrition from their yolk sac through 7 dpf (Jardine and Litvak, 2003).

2.3 Image collection

Details of the imaging system and preparation of the 6-well plates with agarose rings (Corning Costar no. 3506) have been described previously (Pelkowski et al., 2011; Lovato et al., 2016). Four 6-well plates with one larva per well were placed on the LCD screen (1366 × 768 pixel resolution and a brightness of 220 cd/m²) of an inverted laptop on the bottom shelf of a tall cabinet. A plastic diffuser (Pendaflex 52345) was placed between the multiwell plates and the screen to avoid moiré patterns. Larvae were imaged from above by an 18 megapixel Canon EOS Rebel T2i digital camera with an EF-S 55–250mm f/4.0–5.6 IS zoom lens with image quality = 5184 × 3456 pixels using Canon's remote capture software. Acquired images were compressed as 0.6MB JPEGs and stored on a standard desktop computer (Dell OptiPlex).

2.4 Behavioral testing

Larvae were tested at 7 dpf. In phase 1 (free swimming period), they were exposed to a uniform white background with no visual stimuli (Suppl. Fig. 1A) for 135 min; in phase 2 (VSA), they were exposed to the uniform white background for 5 min (pretest) followed by a 5 min period (test) with a red 'bouncing' disk (Suppl. Fig. 1B) followed by another 5 min period (posttest) of the uniform white background. There was no delay between phases 1 and 2. Images were captured every 6 s for 150 minutes. A red disk (RGB values were 255, 0, 0) with a 1.35 cm diameter was programmed in Microsoft PowerPoint to travel back and forth in a straight line across the upper half of each well at a rate of 1.65 cm/s. Over three

replications, a total of 72 larvae at each PCB concentration (2, 5, and 10 ppm) and a total of 48 larvae in each control condition (EW and DMSO) were tested.

2.5 Image analysis and quantification of behavior

Images were imported into ImageJ (<http://rsb.info.nih.gov/ij/index.html>) and an automatic macro was used to split color channels to remove the red disk, subtract the background, apply a threshold, and identify larvae on the basis of particle size (Suppl. Fig. 1C–1F). The X,Y coordinates of each larva's centroid obtained from Image J were imported into a customized Microsoft Excel template and compared to the X,Y coordinates of the midpoint of the larva's well to determine if the larva was (1) on the same side of the well as the 'bouncing' disk or on the opposite side (avoidance) and (2) in the center of the well or on the edge of the well (thigmotaxis). Pseudo-avoidance scores were determined for periods with no visual stimuli by similarly calculating percent observations on which the larva was located on the side of the well away from the side where the 'bouncing' disk was presented in the VSA test period. The X,Y coordinates of the larvae's centroids in pairs of consecutive images were used to quantify overall activity (swim speed) in mm/min and instances of inactivity (still) defined as a displacement of less than 0.3 mm in a 6 second interval. All measurements of larval location were taken in the horizontal plane.

2.6 Statistical analyses

The data from the free swimming period (phase 1) were analyzed in 15 min blocks using a mixed factors analysis of variance (ANOVA) with Blocks (9 levels) as a repeated measure and PCB (4 levels) as the between subjects factor (SPSS v. 22.0). The data from the 5-min pretest, test and posttest periods comprising the VSA (phase 2) were analyzed using one way ANOVAs. The data from the two control groups (EW and DMSO) were combined because there were no statistically significant differences between these groups on the behavioral measures. Dunnett's tests (two-tailed) were used to make post-hoc comparisons between each PCB-treated group and the combined control group.

3. Results

3.1 Effects of PCB-exposure on free swimming (phase 1)

Embryonic PCB-exposure increased thigmotaxis (Fig. 1 and Suppl. Fig. 2). There was a significant main effect of PCB, $F(3,308) = 4.9$, $p < .01$, but no significant Blocks effect, $F(8,2464) = 1.5$, and no significant PCB x Blocks interaction, $F(24, 2464) = 1.2$. Mean percent thigmotaxis was higher in all three PCB-exposed groups relative to the control larvae ($p = .035$ for 2 ppm, $p = .006$ for 5 ppm, and $p = .003$ for 10 ppm).

Embryonic PCB-exposure did not affect mean swim speed or mean percent still (Fig. 2A and 2B). Neither the PCB x Blocks interactions ($F_s < 1$) nor the main effects of PCB ($F_s < 1$) was significant. However, the main effect of Blocks was significant for both measures. Mean swim speed declined across the free swimming period, $F(8,2464) = 35.2$, $p < .001$, whereas mean percent still increased, $F(8,2464) = 16.1$, $p < .001$.

The pseudo-avoidance measure confirmed that neither the PCB-exposed nor the control larvae had a bias for either side of the well during phase 1 with no 'bouncing' disk present. There were no significant main effects of PCB ($F < 1$) or Blocks, $F(8, 2464) = 1.2$, $p > .10$, and no significant PCB x Blocks interaction ($F < 1$). Mean percent pseudo-avoidance scores $\pm$ SEM were 54.1 ± 2.7 , 50.2 ± 2.7 , 52.5 ± 2.7 and 50.5 ± 2.3 for 2 ppm, 5 ppm, 10 ppm and controls, respectively.

3.2 Effects of PCB-exposure on the VSA (phase 2)

Embryonic PCB-exposure affected response to the visual startle stimulus (Fig. 3). Analysis of the mean displacement in mm/min for the 6-sec interval between the last image of the pretest period and the first image of the test period revealed a significant main effect of PCB, $F(3, 308) = 6.1$, $p < .001$. There was a significant reduction in mean swim speed for the 5 ppm ($p < .001$) and the 10 ppm ($p < .05$) exposed groups relative to the control larvae. Mean swim speed was also lower in the 2 ppm larvae relative to the control larvae but this difference was not statistically significant ($p > .10$).

The pretest, test and posttest data from the VSA are shown separately for avoidance (Fig. 4A), thigmotaxis (Fig. 4B), swim speed (Fig. 4C) and still (Fig. 4D). Both PCB-exposed larvae and control larvae showed significant changes in behavior with the visual stimulus present (test) than without the visual stimulus (pretest and posttest). Statistically significant differences were evaluated using repeated measures ANOVAs to compare behavior in the test period with behavior in the pre- and post-tests for each group separately. To test for PCB-exposure effects per se, one way ANOVAs were used to compare the groups within each of the three periods (pretest, test and posttest).

Larval behavior was affected by the presence of the 'bouncing' disk in all groups. Repeated measures ANOVAs revealed that, for three measures (mean percent avoidance, mean swim speed and mean percent still), there were statistically significant differences between the test period and the pretest ($ps < .01$) and posttest ($ps < .01$) periods for the control and each of the PCB-exposed groups. Mean percent thigmotaxis was also significantly greater in the test than in the pretest ($p < .001$) or posttest ($p = .003$) periods for the control larvae and in the test than in the pretest ($ps = .021$) period for the PCB-exposed larvae. However, mean percent thigmotaxis was not significantly greater in the test than the posttest period for the 2 ppm and 10 ppm PCB-exposed larvae ($ps > .10$) although it was for the 5 ppm larvae ($p = .018$).

Analysis of the pretest data revealed no significant PCB effect for either activity measure (still and swim speed) or pseudo-avoidance (all $F_s < 1$). As expected, there was a significant main effect of PCB for thigmotaxis, $F(3, 308) = 3.1$, $p < .05$. Consistent with the phase 1 data, mean percent thigmotaxis was numerically greater for all three PCB-exposed groups relative to the control larvae. However, not all differences reached statistical significance ($p = .057$ for 2 ppm; $p = .022$ for 5 ppm; and $p = .101$ for 10 ppm).

Analysis of the test data revealed no significant PCB effect for either activity measure (still and swim speed) or thigmotaxis (all $F_s < 1$). The main effect of PCB approached statistical significance for mean percent avoidance, $F(3, 308) = 2.2$, $p = .085$, as the control larvae exhibited numerically higher avoidance scores than the PCB-exposed larvae.

Analysis of the posttest data revealed no significant PCB effect for pseudo-avoidance, thigmotaxis or swim speed, $F_{(3,308)} < 1.8$, $p > .10$. The main effect of PCB approached statistical significance for percent still, $F_{(3,308)} = 2.2$, $p = .085$, as the control larvae exhibited numerically higher percent still scores than the PCB-exposed larvae.

4. Discussion

Sub-chronic embryonic PCB-exposure influenced the behavioral phenotype of 7 dpf zebrafish larvae in two ways. First, PCB-exposed larvae showed enhanced thigmotaxis (edge preference) throughout the free swimming period (phase 1). Second, PCB-exposed larvae showed an altered response to the moving visual stimulus in the VSA (phase 2) as measured by a decrease in swim speed (mm/min) between the last image in the 5-min pretest and the first image in the 5-min test with the moving disk. In addition, spontaneous activity levels but not thigmotaxis changed systematically throughout phase 1; for both PCB-exposed and control larvae, mean swim speed gradually declined and mean percent still gradually increased.

4.1 PCB exposure and anxiogenic behavior

Prenatal and postnatal PCB exposures have been shown to increase anxiety-related behaviors in rodents (Eriksson and Fredricksson, 1996; Orito et al., 2007) and to elevate corticosterone levels in rats (Miller et al., 1993). Our novel finding of potentiated thigmotaxis in PCB-treated larvae in an extended free swimming period (phase 1) is in line with these reports of an anxiogenic effect of PCB-exposure and partially aligns with results of Lovato et al. (2016). However, our VSA data do little to strengthen an anxiogenic account of PCB-exposure effects. Thigmotaxis was not significantly elevated in the PCB-treated relative to the control larvae during presentation of the ‘bouncing’ disk; there was no evidence for a relative increase in freezing (percent still); and mean percent still posttest scores suggest that the control larvae were slower to recover from the effects of the visual threat than the PCB-exposed larvae. To be sure, a ceiling effect may have obscured detection of a significant PCB-exposure effect for thigmotaxis but this does not provide a plausible explanation for the pattern of results with freezing (percent still).

The possibility that heightened thigmotaxis may arise from a PCB-induced visual defect rather than increased anxiety seems unlikely. Although continuous exposure to 1 ppm A1254 has been shown to induce significant structural and functional retinal defects at 7 dpf (Wang et al., 2012; Zhang et al., 2015), it is clear from our VSA data that, with a short PCB-exposure window, larvae could see the ‘bouncing’ disk. They showed changes in mean swim speed and mean percent still that were indistinguishable from those shown by the controls suggesting that their ability to see the disk’s movement was similar to that of the control larvae. Further research is needed to understand the nature of the enhanced thigmotaxic effect observed in the PCB-exposed larvae.

4.2 PCB exposure and cognition

Various symptoms of ADHD and executive function deficits have been linked to perinatal exposure to PCBs (Jacobson and Jacobson, 2003; Sagiv et al., 2010; Stewart et al., 2005;

Vreugdenhil et al., 2004). In a comprehensive review, Boucher et al. (2009) concluded that prenatal PCB exposure was associated with impairments in executive functions and deficits in processing speed, verbal abilities and visual recognition memory. More recently, Neugebauer et al. (2015) have reported that prenatal PCB exposure is associated with increased inattention in line with results from Sagiv et al. (2008). Our novel finding that mean swim speed between the last pretest image on the white background and the first test image with the red 'bouncing' disk was significantly reduced in the 5 ppm and 10 ppm PCB-exposed larvae relative to the control larvae is perhaps more consistent with an attention deficit than a systemic high dose hypoactivity effect as no other significant differences on swim speed were found. The PCB-exposed larvae may have been slower to notice the 'bouncing' disk or slower to process the stimulus. A limitation of the 6-s time-point sampling method we used is that it does not allow further refinement of these hypotheses or exclusion of other possibilities such as slower swim initiation in stationary larvae.

Although not statistically significant, we did find evidence that avoidance of the 'bouncing' disk was deficient in the 5 ppm and 10 ppm PCB-exposed larvae. This finding echoes a similar but significant avoidance defect at these concentrations reported by Lovato et al. (2016) using a related assay. In both assays, the avoidance response requires that the larva keeps track of the path of the 'bouncing' disk and swims to the other side of the well. Elements of successful avoidance likely include executive function processes such as planning, response inhibition and decision-making which are all impaired by prenatal PCB exposure. Continuous video recording of the VSA would help characterize the source of this avoidance deficit by providing precise information about stimulus detection, reaction time, swim speed and the path of the avoidance response. Moreover, it would help clarify the contribution that differences in thigmotaxis in the present study might have made in attenuating the avoidance deficit.

4.3 Spontaneous activity

We observed comparable systematic changes in spontaneous activity over the course of the free swimming period in PCB-exposed and control larvae; mean swim speed (mm/min) gradually declined and mean percent still gradually increased. Such behavioral changes are commonly associated with habituation and sensitization processes, respectively. The present study, however, was not designed to demonstrate such processes and a simple motor fatigue explanation of these activity changes cannot be ruled out. Nevertheless, given the literature indicating an association between PCB-exposure and cognitive impairments including deficits in visual recognition memory, this aspect of our findings warrants further investigation using an appropriate design to assess learning (O'Neale et al., 2014; Rescorla and Holland, 1976; Roberts et al., 2011; Wolman et al., 2011). It is also worth noting that the activity changes in phase 1 occurred over a period of time that substantially exceeds the short acclimation periods typically used to establish a stable baseline in zebrafish larvae. Clearly, such changes in baseline activity have important implications for studies using quasi-experimental AB designs.

We also found no evidence of PCB-induced hyperactivity. Both mean swim speed and mean percent still were similar between the PCB-exposed and control larvae in phase 1 over a range of response values. Although animal studies have established a strong link between prenatal PCB exposure and hyperactivity (Tilson et al., 1990), expression of hyperactivity effects may sometimes be delayed (Kuriyama and Chahoud, 2004). Thus, it is possible that hyperactivity might be detected in late stage larval or juvenile zebrafish exposed to PCBs embryonically. Alternatively, PCB exposure may have induced qualitative differences in swim trajectories such as erratic swim movements which have been observed in adult zebrafish under stress (Barcellos et al., 2007; Cachat et al., 2010, 2011; Egan et al., 2009; Levin et al., 2007; Stewart et al., 2011) that could not be detected with 6-s time point sampling. Further research is needed to assess these possibilities.

4.4 Assay development

Time-point sampling is widely recognized as an efficient method for accurately representing behavioral states and frequently occurring behaviors (Martin and Bateson, 2007). In a previous study, we estimated that 75% of the swim speed of 7 dpf wild type larvae is measured by 6-s time-point sampling; we also reported that percent edge preference is nearly identical for 6-s and 60-s time-point sampling rates and that avoidance behavior is highly variable (Pelkowski et al., 2011). In the present study and in previous work from our laboratory (e.g., Colwill and Creton, 2011b; Lovato et al., 2016; O'Neale et al. 2014), 6-s time point sampling has revealed several statistically significant effects. However, time-point sampling is not without its limitations (Fontaine et al., 2008; Martin and Bateson, 2007). The nascent technology of automated continuous tracking promises to yield computational methodology suitable for profiling subtle differences in swim trajectories between control and PCB-exposed larvae, for precisely quantifying relationships between behavior and external cues such as the 'bouncing' disk in our VSA, and for generating duration information about behavior (Fontaine et al., 2008).

4.5 Conclusions

Exposure to the PCB mixture A1254 from 2 hpf to 26 hpf influenced the phenotype of 7 dpf zebrafish larvae in two ways. Thigmotaxis was significantly elevated throughout the free swimming period in the PCB-exposed larvae relative to the controls. There was also a significant decrease in swim speed during the transition from pretest to testing with the 'bouncing' disk in the larvae exposed to the two highest PCB concentrations. These behavioral changes are consistent with the anxiogenic and attention-disrupting effects of PCB exposure observed in children, monkeys and rodents and warrant further exploration. Such findings underscore the neurodevelopmental risks of fetal and neonatal exposure to PCBs in humans, the need for model systems to study windows of susceptibility and underlying mechanisms, and the importance of continued monitoring of PCB levels in the environment and in the diet of high-trophic level organisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute of Environmental Health Sciences (R03 ES017755) and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (R01 HD060647). Robbert Creton also received funding from the National Institute of Environmental Health Sciences (P42 ES013660). We thank Emma Welch for contributions to this project and two anonymous reviewers for helpful comments.

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Highlights

- Effect of Aroclor 1254 on free swimming and visual startle was evaluated in zebrafish larvae.
- PCB-exposure from 2–26 hours post-fertilization induced two behavioral defects at one week.
- Thigmotaxis was elevated during free swimming in the 2, 5 and 10 ppm exposed larvae.
- Transitional swim speed (from Pre to Test) was reduced in the 5 and 10 ppm exposed larvae.
- Continued monitoring of PCB levels in the environment is strongly recommended.

Fig. 1.

Effect of embryonic PCB exposure on edge preference at 7 dpf. Mean ($\pm$ SEM) percent thigmotaxis in the 135-min period of free swimming on a uniform white background with no visual stimulus (phase 1) shown separately for the combined controls (EW and DMSO) and the three PCB-exposure concentrations (2 ppm, 5 ppm and 10 ppm). Asterisks denote statistically significant differences between the control and the PCB exposed larvae ($p = .035$ for 2 ppm, $p = .006$ for 5 ppm, and $p = .003$ for 10 ppm, Dunnett's two tailed tests).

Fig. 2.

Effect of embryonic PCB exposure on activity measures at 7 dpf. A) Mean ($\pm$ SEM) swim speed (mm/min); B) mean ($\pm$ SEM) percent still. For both panels, data are plotted in 15-min blocks for the 135-min period of free swimming on a uniform white background with no visual stimulus (phase 1) and shown separately for the three PCB-exposure concentrations (2 ppm, 5ppm and 10 ppm) and the combined controls (EW and DMSO).

Fig. 3.

Effect of embryonic PCB exposure on transitional swim speed at 7 dpf. Mean ($\pm$ SEM) swim speed (mm/min) between the last pretest image and the first test image in the VSA shown separately for the controls (EW and DMSO combined) and the three PCB-exposure concentrations (2 ppm, 5 ppm and 10 ppm). Asterisks denote statistically significant differences between the control and the PCB exposed larvae ($p < .001$ for 5 ppm and $p < .05$ for 10 ppm, Dunnett's two tailed tests).

Fig. 4. Effect of embryonic PCB exposure on VSA behavior at 7 dpf. A) Mean ($\pm$ SEM) percent avoidance; B) mean ($\pm$ SEM) percent thigmotaxis; C) mean ($\pm$ SEM) swim speed (mm/min); D) mean ($\pm$ SEM) percent still. For all panels, data are shown separately for each of the three 5-min periods corresponding to pretest (Pre), test with the red ‘bouncing’ disk (Test) and posttest (Post) and for the combined controls (EW and DMSO) and the three PCB-exposure concentrations (2 ppm, 5 ppm and 10 ppm). Asterisks denote statistically significant differences between the control and the PCB exposed larvae ($p < .05$ for 5 ppm, Dunnett’s two tailed test). The letter ‘a’ above a bar indicates a significant difference between that mean and the corresponding mean in the test with the red ‘bouncing’ disk (Test, $ps < .05$, repeated measures ANOVAs).